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Ichthyological Research
The Ichthyological Society of Japan

ISSN 1341-8998
Volume 60
Number 4

Ichthyol Res (2013) 60:334-342
DOI 10.1007/s10228-013-0355-x

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FULL PAPER

Cost-benefit of feeding on anthropogenic organic matter: lipid changes in a detritivorous fish (*Prochilodus lineatus*)

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Received: 21 February 2013 / Revised: 6 May 2013 / Accepted: 3 June 2013 / Published online: 9 July 2013

Abstract The lipid composition of muscle and liver of detritivorous fish *Prochilodus lineatus*, settling particles and sediments from reference and polluted areas of the Paraná-Rio de la Plata basin were analyzed to evaluate the impact of feeding on contaminated detritus. Overall, muscular lipids were highly variable (14 ± 13 % wet mass, ww) and increased with body mass trough a rapid triglyceride accumulation [82 ± 7.9 % neutral lipids (NL) for fish weighing less than 1 kg to 99 ± 0.51 % NL for fish weighing more than 4 kg] with a parallel decrease of free fatty acid (13 ± 6.0 to 0.11 ± 0.23 % NL). Liver lipids were more uniform (6.0 ± 2.1 % ww) and were dominated by triglycerides (40 ± 21 % NL) and free fatty acids (34 ± 19 % NL). Compared with fish from reference areas in the North, polluted fish from Buenos Aires presented higher muscular lipid contents (24 ± 13 vs. 3.9 ± 3.1 % ww) and triglyceride abundance (98 ± 3.5 vs. 84 ± 9.7 % NL), and enlarged livers (Hepatosomatic index 1.4 ± 0.4 vs. 0.7 ± 0.2) enriched with esterified cholesterol (20 ± 9.1 vs. 11 ± 9.9 % NL). These differences were consistent with the higher proportions of lipids, enriched with free fatty acids and triglycerides, in stomach contents, settling particles and sediments from Buenos Aires relative to the North of the basin. The change in *Prochilodus lineatus* diet from organic-poor vegetal detritus in the north to polluted but abundant and fresh anthropogenic matter at Buenos Aires resulted in multiple alterations of lipid metabolism.

Keywords Lipid composition · Detritus · Pollution · Rio de la Plata · *Prochilodus*

Introduction

Although frequently overlooked, detritivory is the prevailing pathway of energy flow and material cycling in most ecosystems, determining trophic structure, biodiversity, and system productivity (Moore et al. 2004). This is particularly significant in neotropical turbid hydrographic systems, where detritivorous fish of the genus *Prochilodus* (Prochilodontidae, Characiform) play a key ecological role, controlling the ecosystem metabolism through regulation of benthic matter settling, microbial community composition, and particulate organic carbon flow (Taylor et al. 2006). In the Rio de la Plata basin, the second largest of South America (>3 million km^2, 500–800 km^3 of freshwater per year) with a huge sedimentary load (90 × 10^6 tons/year), the ichthyomass is dominated by *Prochilodus lineatus* (Valenciennes), popularly known as "*sábalo*" which constitutes the target of the principal freshwater

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Electronic supplementary material The online version of this article (doi:10.1007/s10228-013-0355-x) contains supplementary material, which is available to authorized users.

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fishery and is the main prey item for large predatory fish (Sverlij et al. 1993). The sábalo has many noteworthy morpho-physiological adaptations to detritivory, including a sucker-like mouth, filtering ridges, three-dimensional gillrakers, cardiac (food reservoir) and pyloric stomachs (grinder), and increased assimilation surface in the intestine through numerous pyloric caeca and mucosal folds (Bowen 1983). *Prochilodus lineatus* has also remarkable migratory habits, moving hundreds of kilometers in a flood-controlled trophic (downstream)-reproductive (upstream) migration (Sverlij et al. 1993).

Sewage-derived organic matter has been recognized as an important energy subsidy for aquatic food webs leading to trophic structure alterations and fish production increase (deBruyn et al. 2003). In this context, the presence of a dominant and specialized detritivorous fish feeding on anthropogenic detritus in polluted areas poses a critical situation. Buenos Aires city concentrates one-third of the total argentine population and most of its industrial capacity, severely impacting the metropolitan area of the Río de la Plata, which is polluted with persistent organic pollutants (POPs), hydrocarbons, and heavy metals discharged by sewers (ca. 2 million m^3 day^{-1}) and industrial effluents (Colombo et al. 2005a, b, 2006; Tatone et al. 2009). *Prochilodus lineatus* collected in this area contains high levels of sewage markers such as linear alkyl benzenes (used as intermediate in detergent production) and coprostanol (derived from microbial reduction of cholesterol in human gut), hydrocarbons, and POPs in muscle reflecting selective feeding on sewage-derived organic matter (Colombo et al. 2007a, b, 2011). Furthermore, several morphometrical and biochemical alterations in this fish have been related to consumption of sewage-derived organic matter (Speranza and Colombo 2009). The quali–quantitative study of lipid composition is particularly important in polluted environments since they regulate the degree of bioaccumulation of hydrophobic contaminants and serve as a protective storage depot against toxic effects in vital organs (Gobas and Mackay 1987; Geyer et al. 1994) leading to the survival of the fattest hypothesis (Lassitier and Hallam 1990). On the other hand, persistent organic pollutants can alter lipid composition, disrupting several catabolic and anabolic pathways at different levels such as gene expression, enzyme function, and endocrine signaling (Addison 1982; Fletcher et al. 2005; Sato et al. 2008).

The objective of this paper was to evaluate the changes in the lipid composition of *P. lineatus* (muscle and liver) and its diet (stomach contents, settling particles and sediments) from polluted and reference areas to understand the alterations of lipid metabolism related to the ingestion of anthropogenic organic matter and their possible adaptive significance.

Materials and methods

Sampling. Fish (*Prochilodus lineatus*) were collected every 3 months between 2006 and 2011, near the main sewer outfall of Buenos Aires in the Rio de la Plata (BA, Fig. 1). During the same period, fish were also collected from four southern stations in the Río de la Plata (south Rio de la Plata, sRLP), at 12 sites along the Paraná River 200–1,000 km away from Buenos Aires (Paraná, PAR), and from four northern sites located >1,000 km from Buenos Aires along the Paraná and Paraguay Rivers (North, N). These samples were collected during the season with highest fish abundance: spring for Rio de la Plata fish and winter for Parana and North fish. A total of 481 adult individuals (BZ: 160, sRLP: 122, PAR: 134, N: 65) were collected with gillnets by local fisherman. After measuring the standard length and total body mass, fish were opened by the ventral midline and livers were weighed and collected in plastic flasks as well as the contents of the cardiac stomach, which constitute a reservoir chamber without digestive function (Bowen 1983). A portion of dorsal muscle was excised and wrapped with aluminum foil. Samples were immediately frozen in dry ice, transported to the laboratory, and stored at –20 °C until analysis.

In addition, superficial sediments and settling particles were collected at selected locations (Fig. 1). Sediments were collected using a stainless-steel Van-Veen grab

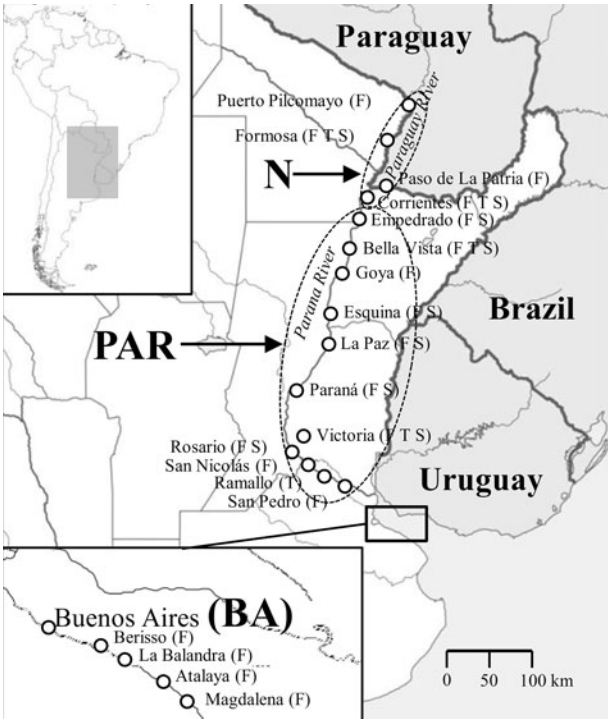

Fig. 1 Sampling stations of fish (*F*), sediments (*S*) and settling particles (*T*) in the Rio de la Plata Basin. *BA* Buenos Aires, *sRLP* south Rio de la Plata, *PAR* Parana, *N* North

sampler and settling particles were collected deploying fixed 10-cm diameter sediment traps at 1.5 m from the surface during 12–36 h. Sediments and trap material were transported refrigerated to the laboratory.

Biochemical analyses. Lipids were extracted from muscle and liver samples with chloroform: methanol (2:1 v/v) in a tissue homogenizer (Folch et al. 1957) and from sediments, settling particles and stomach contents with acetone-dichloromethane-petroleum ether (1:2:2) and sonication. After gravimetrical determination of lipid content, the extracts were stored in chloroform at −20 °C.

Neutral lipid classes were analyzed by thin-layer chromatography (TLC) using 250 micron silica gel GHL plates (Analtech) and hexane/diethyl ether/acetic acid (80:30:2) as mobile phase. This solvent system effectively separated diglycerides [Retardation factor (Rf) 0.21 ± 0.023], cholesterol (Rf 0.26 ± 0.021), free fatty acids (Rf 0.52 ± 0.030), triglycerides (Rf 0.72 ± 0.027), and cholesterol esters (Rf 0.82 ± 0.018). The plates were charred with 50 % sulphuric acid at 150 °C, scanned, and analyzed using SigmaGel software (Jandel Scientific). Quantification was accomplished using response factors obtained from spot areas of standard lipids mixtures (diolein, cholesterol, oleic acid, triolein, and cholesterol-oleate; Sigma-Aldrich) run in parallel with the samples. Neutral lipid proportion was calculated as the sum of individual lipid class contributions. Lipid composition analyses were reproducible to ± 5–13 % (assay by quintuplicate in randomly selected samples). Linear adjust (R^2) of 5-point standard calibration curves ranged between 0.90 and 0.97. To identify other lipid classes present in stomach contents, settling particles and sediments, lipid extracts were separated by preparative TLC and the spots were identified after revealing the plate in iodine atmosphere. The spots were then scrapped into glass tubes and saponified with 1 M KOH in 90 % methanol (3 h at 60 °C, Kates 1986). Unsaponifiable lipids were extracted with Hexane/ethylether (4:1) and analyzed by TLC with a mobile phase according its polarity: chloroform/methanol/water/acetic acid (25:15:4:2) for $Rf < 0.2$; hexane/diethylether/acetic acid (80:30:2) for Rf between 0.2 and 0.7; and Hexane/ethylether (96:4) for $Rf > 0.7$ (Ackman 1991). Standards of sterols (stigmasterol, dihydrocholesterol), fatty alcohols (palmityl, oleyl, and stearyl alcohol), ketones and aliphatic hydrocarbons were run in parallel with the extracts for identification.

Statistical analysis. Statistical analysis was carried out using XLSTAT (Addinsoft SARL, Paris, France). Data were expressed as mean ± standard deviation. A *t* test was used to perform comparisons between two means as well as to evaluate the correlation coefficients significance. For comparisons between multiple samples, analysis of variance (ANOVA) and the Tukey test were used. A significance level of $p < 0.05$ was employed, except otherwise indicated. The analysis correlated variables and their regression slopes was performed by analysis of covariance (ANCOVA).

Results

As morphometric characteristics, *Prochilodus lineatus* standard length (L) averaged 44 ± 5.6 cm (range 30–60 cm) and body mass (M) 2,321 ± 1,096 g (range 500–5,927 g). The condition index ($CI = M_T\ L^{-3}$) averaged 2.6 ± 0.52 (range 1.2–4.3) and showed clear geographical variations; principally between Buenos Aires and North (3.0 ± 0.47 vs. 2.3 ± 0.42, respectively; $p < 0.0001$); the differences between Buenos Aires and Paraná (2.4 ± 0.46) or south Rio de la Plata (2.6 ± 0.35) were less pronounced but still highly significant ($p < 0.0001$).

Muscle. Figure 2 and Electronic Supplemental Material (ESM) Table S1 summarize total lipid content and neutral lipid class composition of *P. lineatus* muscle. In general, total lipids were highly variable (range 0.20–56; mean 14 ± 13 % wet mass) and they were correlated to body mass ($r = 0.76$; $p < 0.0001$; Fig. 3). Muscle lipids were dominated by neutral lipids (87 ± 15 % of total lipids), which were mainly constituted by triglycerides (92 ± 9.1 %), followed by small proportions of free fatty acids (5.5 ± 7.1 %) and cholesterol (1.8 ± 1.8 %), and almost negligible proportions of diglycerides and cholesterol esters (0.52 ± 0.62 and 0.091 ± 0.27 %, respectively).

Neutral lipid class composition showed marked changes with body mass and muscle lipid content (Fig. 2). The triglyceride proportion fitted a positive logarithmic relationship ($r = 0.62$; $p < 0.001$) with body mass, increasing from 82 ± 7.9 % in fishes weighing less than 1 kg to 99 ± 0.48 % in fish weighing more than 4 kg. The increase of triglyceride proportion with lipid content was even more marked fitting also a logarithmic curve ($r = 0.89$; $p < 0.0001$, Fig. 4) from 70 ± 7.4 %, in fish with less than 1 % of lipids to 99 ± 0.82 % when they reached more than 10 % of muscular fat. As triglycerides increased, the contribution of other lipid classes decreased sharply from a total 32 ± 6.8 % in fish with less than 1 % lipids, to 1.2 ± 0.63 % in fish with more than 10 % of lipids. The lower end of this trend was represented by three small juvenile fish from North (body mass 27 ± 8.1 g; total muscle lipids 0.73 ± 0.40 %) which contained only 44 ± 23 % triglycerides, and 44 ± 19 % free fatty acids, 9.9 ± 3.8 % cholesterol, 1.0 ± 0.54 % cholesterol esters, and 0.77 ± 0.24 % diglycerides.

Muscle lipid content and composition of *P. lineatus* exhibited marked geographical differences which maximized between Buenos Aires and North fish. *P. lineatus* from Buenos Aires stood out by their very high fat content

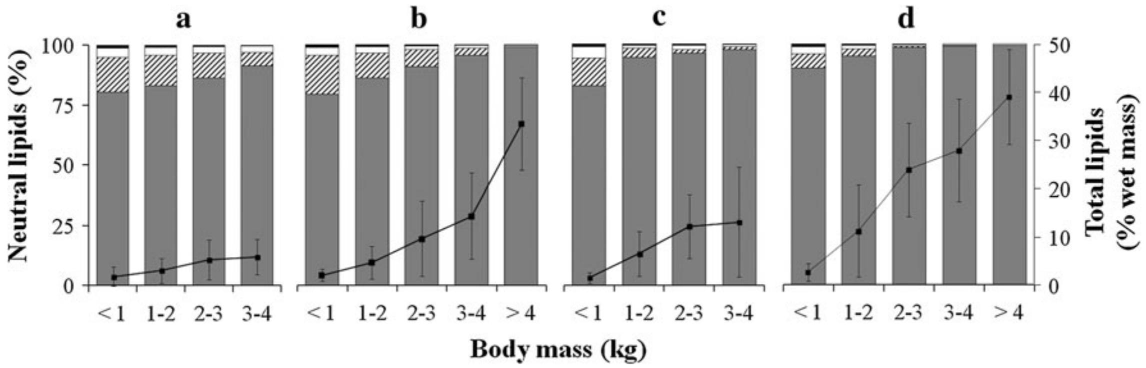

Fig. 2 Neutral lipid composition (*bars*, *left axis*) and total lipid content (*lines*, *right axis*) of muscle with body mass in *Prochilodus lineatus* from **a** North, **b** Paraná, **c** south Rio de la Plata and **d** Buenos Aires. *Dark gray* triglycerides, *crosshatched* free fatty acids, *white* cholesterol, *black* diglycerides, *gray* cholesterol esters

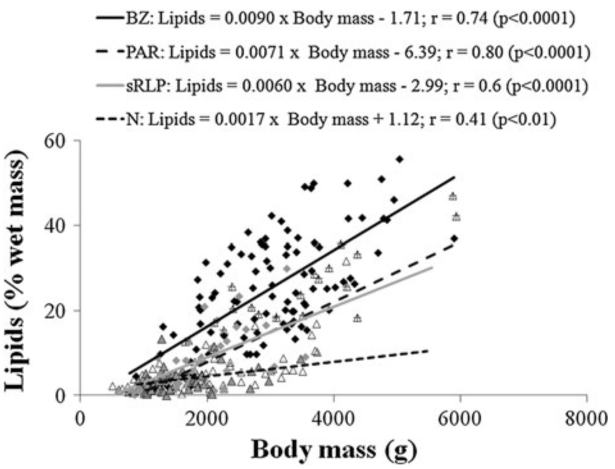

Fig. 3 Relationship of muscular lipid content with body mass of *Prochilodus lineatus* collected in North (*gray triangle*), Paraná (*white triangle*), south Rio de la Plata (*gray diamond*) and Buenos Aires (*black diamond*). Migratory specimens from Buenos Aires specimens are indicated by a cross (+)

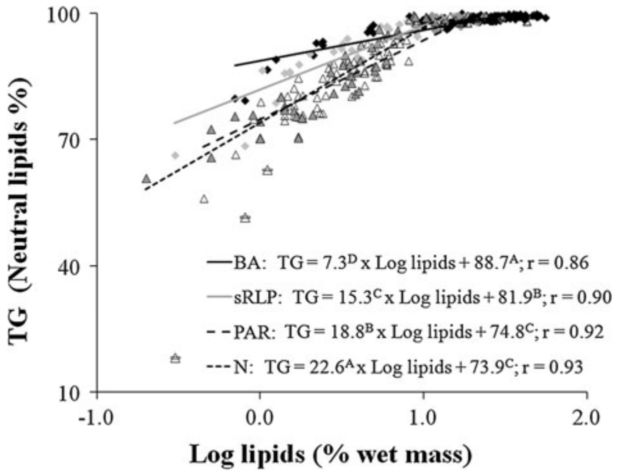

Fig. 4 Logarithmic muscular lipid content and triglycerides (*TG*) proportion of *Prochilodus lineatus* collected in North (*gray triangle*), Paraná (*white triangle*), south Rio de la Plata (*gray diamond*) and Buenos Aires (*black diamond*), with North juvenile fish indicated (*white triangle with bar*). Values with different superscript letters indicate significant differences ($p < 0.05$) in slope or elevation between sampling station

(24 ± 13 % wet mass), which was six times higher in average than in the North (3.9 ± 3.1 %; $p < 0.0001$), whereas Parana and south Rio de la Plata presented intermediate values but still significantly lower than Buenos Aires (9.7 ± 10 and 7.9 ± 6.9 % ww, respectively; $p < 0.01$). The rate of increase of lipids with body mass showed also significant differences between sites as indicated the slope comparison of these relationships (Fig. 3). Buenos Aires fish presented a four times higher slope relative to North ($b = 0.0090$ vs. 0.0017, $p < 0.0005$) with intermediate values in Paraná and Río de la Plata ($b = 0.0071$ and 0.0060), whose slopes differed significantly both from Buenos Aires ($p < 0.05$) and from North ($p < 0.01$). Previous work in the same cohort of fish allowed to identify 21 individuals from Paraná [marked with a cross sign (+) in Fig. 2] as migratory specimens belonging to Buenos Aires based on their high CI (2.8 ± 0.52), lipid content (25 ± 9.1), and also pollutant levels (Speranza et al. 2012). The exclusion of these specimens from the Paraná regression resulted in a significant reduction of the slope ($b = 0.0035$, $p < 0.005$), not significantly different to that of North fish ($p = 0.337$).

Superimposed on the difference in terms of lipid content, there was a clear spatial difference in lipid class composition, basically an enhanced triglyceride accumulation in Buenos Aires, specially marked in fish with less than 10 % lipids. The triglyceride-log lipid content relationships (Fig. 4) were tested by ANCOVA, which control the variance related to body mass and lipid contents. Fish from Buenos Aires had a significantly lower slope and a higher intercept ($p < 0.0001$) than those from North, denoting a higher triglyceride accumulation in the smallest

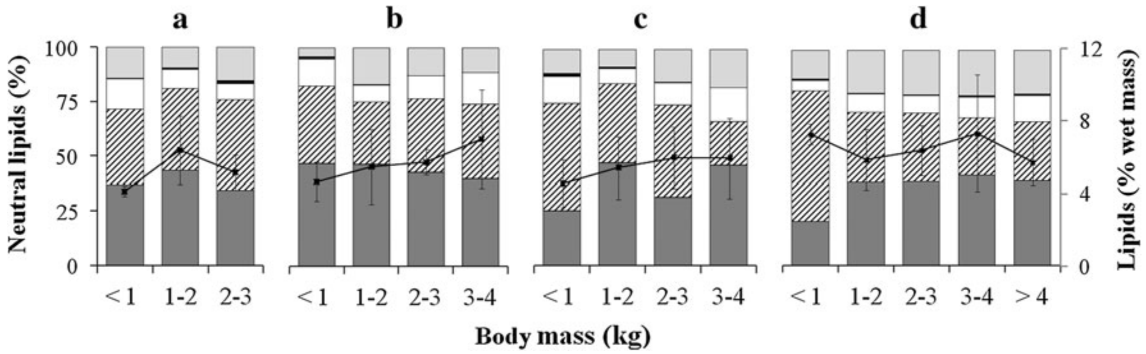

Fig. 5 Neutral lipid composition (*bars*, *left axis*) and total lipid content (*lines*, *right axis*) variation of *Prochilodus lineatus* liver with body mass for **a** North, **b** Paraná, **c** south Rio de la Plata and **d** Buenos Aires specimens. *Dark gray* triglycerides, *crosshatched* free fatty acids, *white* cholesterol, *black* diglycerides, *gray* cholesterol esters

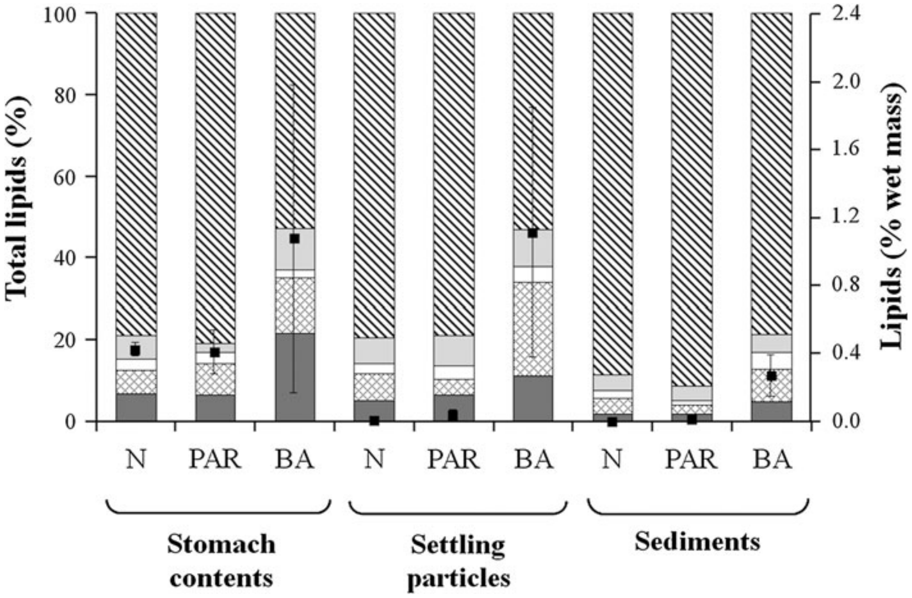

Fig. 6 Neutral lipid class composition (*bars*, *left axis*) and total lipid content (*lines*, *right axis*) of *Prochilodus lineatus* stomach content, settling particles and sediments from Buenos Aires (*BA*) and Paraná (*PAR*). *Dark gray* triglycerides, *gridded* free fatty acids, *white* cholesterol, *gray* cholesterol esters, *crosshatched* non-characterized lipids

specimens. Fish from south Rio de la Plata and Paraná had an intermediate regression, with slopes significantly different from the other sites ($p < 0.01$).

Liver. *Prochilodus lineatus* liver mass averaged 19 ± 11 g and the hepato-somatic index (HSI = liver/body mass × 100) ranged between 0.28 and 3.2 (1.2 ± 0.45). Total lipid content and neutral lipid class composition of the liver is summarized in Fig. 5 and ESM Table S2. In general, liver had a more uniform lipid content with respect to muscle (range 1.0–17, mean 6.0 ± 2.1 % wet mass) without significant relationships to any morphometric variable. The neutral lipid fraction was smaller than that in muscle (55 ± 18 % vs. 87 ± 15 % total lipids) and presented a more balanced composition dominated by triglycerides (40 ± 19 %) and free fatty acids (34 ± 18 %), followed by esterified (16 ± 11 %) and free cholesterol (9.2 ± 5.5 %) and minor amounts of diglycerides (0.74 ± 0.91 %). Unlike muscle, the neutral lipid composition of liver did not show any correlation with its lipid content.

Liver showed less pronounced spatial differences relative to muscle. However, the HSI indicated a clear contrast between Buenos Aires with the highest values and North with the lowest (1.4 ± 0.40 vs. 0.66 ± 0.21, respectively; $p < 0.0001$), and intermediate but still significantly different ($p < 0.0001$) values for Paraná (0.85 ± 0.27) and south Rio de la Plata (1.1 ± 0.36). The other parameter displaying spatial difference was the higher cholesterol ester proportion in liver of Buenos Aires fish relative to south Rio de la Plata, Paraná and North (20 ± 9.1 vs. 12 ± 9.6, 11 ± 8.2 and 11 ± 9.9 %, respectively; $p < 0.001$).

Stomach contents, settling particles and sediments. The lipid content and lipid classes' composition of *P. lineatus* stomach contents, settling particles and sediments is presented in Fig. 6 and ESM Table S3. In general, total lipid contents of stomach contents and settling particles were comparable and significantly higher than that of sediments (0.67 ± 0.62 and 0.78 ± 0.79 vs. 0.11 ± 0.15 %ww, respectively; $p < 0.05$). In contrast to muscle and liver, where the lipid classes quantified accounted for virtually all neutral lipids (>99 %) and a dominant fraction of total lipids (55–87 %), in stomach contents, settling particles and sediments they represented only 14–39 % total lipids. The non-characterized fraction of lipids was significantly higher in bottom sediments respect to settling particles and stomach contents (86 ± 7.7 vs. 61 ± 16 and 67 ± 15 % total lipids, respectively; $p < 0.005$). Related to this difference, the lipid composition also showed contrasting differences, especially in sediments. Triglycerides were significantly higher in stomach contents and settling particles relative to sediments (13 ± 8.9 and 9.5 ± 3.2 vs. 3.0 ± 1.8 % total lipids; $p < 0.005$), while free fatty acids and cholesterol esters decreased from settling particles (18 ± 11 and 8.3 ± 3.3 % total lipids), stomach contents (9.3 ± 4.9 and 6.1 ± 5.1 % total lipids) to sediments (4.9 ± 3.2 and 4.0 ± 1.7 % total lipids). Cholesterol proportion was rather homogeneous in three compartments (3.6 ± 2.9, 2.5 ± 1.3 and 2.3 ± 2.1 %, respectively). Diglycerides were not detected in any compartment.

In order to identify the non-characterized lipids, a detailed TLC analysis (see "Materials and methods") was performed in selected samples. All of them were unsaponifiable (non-acylated) lipids, identified as plant pigments (Rf 0.020–0.13), fatty alcohols (Rf 0.31–0.39), sterols other than cholesterol (Rf 0.23–0.28), ketones (Rf 0.84–0.88), and aliphatic hydrocarbons (Rf 0.86–0.96). Pigments were detected almost exclusively in stomach contents from Parana.

Within these general variations, both the lipid content and composition of all three compartments showed significant spatial differences, basically higher contents and proportions of characterized lipids at Buenos Aires. Total lipids in settling particles and sediments from Buenos Aires (1.1 ± 0.74 and 0.27 ± 0.12 % ww) were 1–2 orders of magnitude higher compared with North (0.013 ± 0.0063 and 0.0087 ± 0.0039 % ww; $p < 0.01$) and Paraná (0.041 ± 0.028 and 0.014 ± 0.021 % ww; $p < 0.05$). This total lipid difference was not significant for stomach contents (Buenos Aires: 1.1 ± 0.91, Paraná: 0.41 ± 0.13, North: 0.43 ± 0.04 % ww). Conversely, the non-characterized lipids were much lower in Buenos Aires for all three compartments (Fig. 6; ESM Table S3). The lipid composition also displayed significant differences between Buenos Aires and the other sites, basically a higher proportion of free fatty acids, triglycerides, and cholesterol esters in stomach contents (14 ± 6.1, 22 ± 5.8 and 10 ± 4 0.2 % in Buenos Aires vs. 7.7 ± 2.1, 6.3 ± 4.1 and 2.2 ± 0.80 % in Paraná and 5.8 ± 0.47, 6.6 ± 4.0 and 5.9 ± 3.3 %, respectively, in North; $p < 0.05$), free fatty acid and triglycerides in settling particles (23 ± 8.5–11 ± 2.4 % in Buenos Aires vs. 3.8 ± 1.1–6.4 ± 0.69 % in Paraná and 6.5 ± 1.4–5.2 ± 2 0.3 % in North; $p < 0.005$), and cholesterol, free fatty acid, and triglycerides in sediments (3.9 ± 2.8, 8.0 ± 2.9 and 4.9 ± 1.4 % in Buenos Aires vs. 1.0 ± 0.14, 2.3 ± 0.50 and 1.8 ± 0.6 % in Paraná and 1.7 ± 0.83, 4.0 ± 1.6 and 1.7 ± 0.12 % in North, $p < 0.05$).

Discussion

Prochilodus lineatus has long been recognized for its large fat depots which were exploited for oil production (Brenner 1952). The muscular lipid depots increased with body mass, reflecting the replacement of water by lipids as fish grows (Speranza and Colombo 2009), and they were dominated by neutral lipids (87 ± 15 % of total lipids), mostly triglycerides, in a proportion similar to that observed in other *Prochilodus* species from Brazil (*Prochilodus scrofa* 88 ± 2.1 %, Maia et al. 1994; *Prochilodus cearensis* 75 ± 5.1 %, Maia et al. 1999). The relative abundance of triglycerides increased with the abundance of total lipids reflecting the change of metabolic activity from active growth to an accumulative adult phase. In juvenile and smallest specimens triglyceride proportions were low (32 %), similar to free fatty acids, but as fish grew and lipid content increased, triglycerides rapidly dominated the lipid composition (ca. 90 % in fish with > 10 % of lipid content), reflecting the importance of muscle as energy storage in adult specimens.

Superimposed on this general pattern, the clear geographical differences observed suggested some biochemical alterations in *P. lineatus* feeding anthropogenic organic matter at Buenos Aires. The exceptionally high muscular lipid contents of this fish (24 ± 13 %), with its high CI (3.0 ± 0.47), were in clear contrast with the low or moderate lipid contents of North, Paraná and south Rio de la Plata fish, which were comparable to previous reports for the Upper Paraná (3.7 %, Matsuhita and de Souza Matsuhita and Souza 1994); middle Paraná (5.6–14 %, Bayo and Maitre 1983) and Rio de la Plata (7.1–11 %, Brenner 1953). The higher CI and lipid accumulation rates at Buenos Aires, indicated by the significantly higher lipid-body mass slopes, suggested that sewage-derived organic matter was a cost-efficient food resource allowing a higher body mass gain through an enhanced lipogenesis. Besides the abundant dietary inputs of fresh anthropogenic matter as the main factor leading to this metabolic alteration, a possible interference of the high contaminant load at Buenos Aires in lipid metabolism cannot be ruled out, since enhanced lipid accumulation, triglyceride deposition, and body mass gain have been observed in fish exposed to organic pollutants (Addison 1982; Geyer et al. 1994; Adeyemi et al. 2011; Marit and Weber 2011). The "obesogen" character of organic pollutants has been effectively demonstrated in rats and fish (Grun and Blumberg 2009; Janesick and Blumberg 2011; Meador et al. 2011). This in turn facilitates the bioaccumulation of hydrophobic pollutants in low turnover lipid depots increasing fish tolerance to POPs (Lassitier and Hallam 1990) and permitting the exploitation of highly polluted, but very abundant sewage-derived organic matter as food resource.

The geographical variation observed in lipid composition of *P. lineatus* was also related to their migratory behavior. Fish from North, with low lipid contents mixed in the middle Paraná with fatty, upstream-migrant specimens from Buenos Aires whose extensive visceral fat prevented a substantial reduction of muscular lipids during the migratory effort (Speranza and Colombo 2009). The mixing of both fish stocks was evidenced by the significant reduction of the lipid-body mass slope when migratory individuals (outliers) were excluded from regression. Previous analyses in the same cohort of fish showed that migratory individuals from Buenos Aires also differentiate from North fish by their much higher pollutant loads, i.e., 8-times higher for PCBs (16 ± 7.2 vs. 2.2 ± 3.5 μg g^{-1} dry mass, $p < 0{,}05$), 6-times higher for aliphatic hydrocarbons (227 ± 114 vs. 41 ± 52 μg g^{-1}, $p < 0{,}05$), 5-times higher for organochlorine pesticides and linear alkylbenzenes (3.6 ± 3.2 vs. 0.67 ± 0.75 and 42 ± 24 vs. 8.8 ± 21 μg g^{-1}, respectively, $p < 0{,}05$), and they could be effectively discriminated by chemometric analysis of biochemical and pollutant data (Speranza et al. 2012).

The lower and more homogeneous lipid contents of liver relative to muscle was also reflected in the lipid composition of this organ which was more balanced, with higher proportions of free fatty acids, cholesterol and cholesterol esters reflecting the importance of this organ in lipid metabolism. Consistent with the spatial differences observed in muscle, the higher cholesterol ester proportion in livers from Buenos Aires fish could be related to the already mentioned dietary differences (higher dietary lipid or carbohydrate intake; MacDonald 1966) and also to pollutant interference, since cholesterol metabolism is linked to the regulatory mechanisms controlling the expression of enzymes involved in detoxification process (Rezen et al. 2011). Laboratory experiments of rats exposed to organochlorines indicated a consistent increase of cholesterol synthesis in the liver (Kohli et al. 1979; Yagi and Itokawa 1980; Nagaoka et al. 1990), a reduction of its catabolism, and an impairment of its biliary excretion (Nagaoka et al. 1990; Fletcher et al. 2005; Lu et al. 2011). The excess cholesterol is normally esterified to a fatty acid previous to its storage and exportation to peripheral tissues in lipoproteins (Tocher 2003). Xenobiotics could also interfere at this stage as has been noted in rats exposed to PCBs which showed an increase in the activity of lecithin-cholesterol acyltransferase (LCAT), the enzyme involved in cholesterol esterification (Nakagawa et al. 1986). This would explain the accumulation of cholesterol esters in liver of exposed organisms as observed in rats exposed to dioxins (Albro et al. 1978), in fish exposed to pesticides (Lal and Singh 1987a, b), and in this work, in fish feeding highly contaminated settling material from Metropolitan Buenos Aires.

The HSI is commonly used to assess the overall metabolic condition of fish and could be influenced by both nutritional factors and pollution (Van der Oost et al. 2003). Higher nutrient availability could result in increased HSI, normally due to increased carbohydrate (glycogen) and lipid deposition (Peres and Oliva-Teles 2002, Hamre et al. 2003, Benedito-Palos et al. 2008), whereas HSI increase in exposed animals is basically due to pollutant interference (Dethloff and Schmitt 2000). In fact, the HSI is widely used as xenobiotic exposure index in fish since it is affected by the enhancement of the detoxifying metabolism in liver (Van der Oost et al. 2003). A more specific link between the alteration of lipid metabolism in the liver and the organ enlargement has been observed in rats exposed to organics (Holmberg et al. 1972; Chu et al. 2005; Nishiumi et al. 2008; Arzuaga et al. 2009). In this context, the higher HSI index of Buenos Aires *P. lineatus* (HSI: 1.4 ± 0.40 vs. <1 in the other fish, $p < 0.005$) might have had some connection to its higher nutritional status but the livers did not show significant differences in carbohydrate and lipid contents relative to North fish (Speranza and Colombo 2009). Moreover, the hepatomegaly observed in Buenos Aires fish has been also observed in *P. lineatus* exposed to urban-industrial effluents (Almeida et al. 2005), thus supporting the interpretation of a xenobiotic-related interference as the driving factor.

The neutral lipid composition of stomach contents, settling particles and sediments was very complex but still provided useful information about *P. lineatus* nutritional sources. The similarity of lipid contents and composition between settling particles and stomach contents supported the interpretation that *P. lineatus* feeds preferentially on the fresh, recently deposited flocculent organic material, but not on consolidated sediments whose higher proportions of non-characterized lipids reflected an advanced degradation. This was also supported by the similarity in terms of PCB, linear alkylbenzene, and hydrocarbon profiles between settling particles and *P. lineatus* muscle (Colombo et al. 2007a, b, c).

The clear geographical differences observed in the lipid composition of all three compartments, i.e. higher proportion of lipids, rich fatty acid and triglyceride contents, and lower non-characterized fraction at Buenos Aires relative to Paraná and North, were consistent with the higher muscular triglyceride accumulation in *P. lineatus* from this site and reflected the impact of fresh anthropogenic organic discharges in this area.

This geographical difference in organic matter sources was so significant that it has been even noted previously for bulk parameters such as ignition loss which was significantly higher (36 %) in stomach contents of Rio de La Plata *P. lineatus* relative to Parana specimens (Villar et al. 2001). As expected for more reactive, better characterized lipid fractions, fatty acids and triglycerides showed even more marked contrasts between stomach contents of Buenos Aires relative to North fish (100 % and ca. 200 % higher). Overall, these results reflected the impact of anthropogenic discharges on the availability of fresh organic matter for *P. lineatus,* enriched in lipids and contaminants which are coabsorbed (Kuksis 1986; Van Veld 1990).

In summary, *P. lineatus* found a rich, cost-efficient yet highly contaminated food resource in the anthropogenic organic matter settled close to major sewers in Buenos Aires in opposition to organic-poor vegetal detritus in the North. This resulted in multiple metabolic alterations in Buenos Aires fish, i.e., higher accumulation of lipids and triglycerides in the muscle, and enlarged livers with higher proportions of cholesterol esters.

Acknowledgments This work was carried out with Research Grants to J.C. Colombo from the Argentinean Agency for Scientific Research Promotion (ANPCyT) and from the National University of La Plata (UNLP). E.D. Speranza is member of the Research Career of the Argentinean National Research Council (CONICET). L.M. Tatone and N. Cappelletti are postdoctoral fellows of the CONICET. J.C. Colombo 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 where it was performed.

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