**Sterols biomarkers settling material and sediments from contrasting areas of the Rio de la Plata basin**

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**Abstract:**

To complete

Keywords: Sterols, Sewage markers, Settling material, Rio de la Plata.

**Introduction**

To complete.

Moreira 2013:

Thesedimentsthatreachtheestuarycome

mainly fromtheParanáRiverandfromthedrainageofanumber

of smalltributariesalongtheArgentineancoast.Theserivers,carry

high amountsofnutrients,suspendedparticulateanddissolved

organicmattertotheestuaryand,therefore,totheadjacentshelf

waters.TheamountofsedimentstransportedbytheRDPhasbeen

estimatedinmorethan160milliontonsy1 (Simionatoetal.,2011b). As aconsequence,itisoneofthemostturbidestuariesintheworld,

with extremeconcentrationsmorethan400gm3 (Framiñan

and Brown,1996).

**Materials and methods**

The sampling strategy included sites with contrasting anthropogenic impact: the heavily polluted Buenos Aires metropolitan area of the Rio de la Plata estuary, near the main sewer outfall (BA, 34°43.329'S - 58°10.301'O) and a more pristine site ︡~200 km upstream on the Uruguay River, in the Ñandubaysal bay (N, 33°05.270'S - 58°21.374'W; Fig.1). Sampling campaigns were carried out seasonally from 2007 to 2014. Settling material were collected in pre-weighed polypropylene conical Falcon tubes coupled to a fixed 10cm diameter cylindrical sediment trap deployed at 1.5m during 1-3 days (BA) or 30-60 days (N). Superficial sediments were collected using a stainless steel Hydro-Bios Van-Veen grab sampler. Samples were immediately refrigerated and transported to the laboratory. Tubes containing the settling material were centrifuged and weighed after discarding supernatant water. Water content was determined gravimetrically. Total particle flux was computed as:

Sedimentation rate was calculated as:

The average settling material density (2.65 g.cm-3) was taken from previous work in the same sampling sites.

The discharge of the Uruguay River was calculated as the turbinated plus compensation flow discharged daily by the Salto Grande Dam, located upstream N station and averaged for each sediment trap deployment period (wholesale electricity market administration company: [www.cammesa.com](http://www.cammesa.com)). The discharge of the Rio de la Plata estuary was assumed as the sum of the corresponding monthly discharges of the Uruguay River and the Parana River, measured at its main channels (Paraná Guazú and Paraná de las Palmas; Base de Datos Hidrológica Integrada, [bdhi.hidricosargentina.gov.ar](http://www.bdhi.hidricosargentina.gov.ar); Menendez, 2002).

Lipids were extracted ultrasonically with acetone:dichloromethane:petroleum ether (1:2:2). The extract was dried over anhydrous sodium sulfate and lipid content was determined gravimetrically. Deuterated sterols (deuterocholesterol and deuterositosterol, Steraloids, Inc., Newport, RI, steraloids.com) were added as internal standards. In order to avoid the interference of fatty acids, lipids were saponified with 1M KOH in methanol and non-saponifiable compounds were extracted with petroleum ether – diethyl ether (4:1 v/v, Christie, 1989). The extracts were concentrated under a nitrogen stream and derivatized with N,O-Bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA:TMCS, 10:1v/v; AppliChem GmbH, Darmstadt, Germany, [www.applichem.com](http://www.applichem.com); Sigma-Aldrich, St. Louis, MO, USA, [www.sigmaaldrich.com](http://www.sigmaaldrich.com)) for 3 hours at 60ºC. The resulting trimethylsilyl derivatives were concentrated to dryness under nitrogen and resuspended in toluene prior analysis.



**Fig. 1.** Sampling stations of settling material and sediments in the metropolitan coastal area of the Rio de la Plata estuary, near Buenos Aires main sewer (BA) and at a northern site in the Uruguay River (N).

Samples were analyzed using a Perkin Elmer Clarus 500 GC-MS (Perkin Elmer, Waltham, MA, USA; [www.perkinelmer.com](http://www.perkinelmer.com)) fitted with a Quadrex 007-5MS capillary column (60 m, 0.32 mm i.d., 0.25 μm; Quadrex Corp., Bethany, CT, USA., [quadrexcorp.com](http://www.quadrexcorp.com)) Helium was used as a carrier gas with a flow rate of 1.2 mL/min and the temperature of injector was set at 250ºC (split-splitless mode). The oven temperature program was started at 100 ºC, followed by heating to 225 ºC at 15 ºC /min and to 300 ºC at 3 ºC /min with a final holding time of 10 min. The transfer line temperature was set at 200 ºC and the analytes were ionized by 70eV electron impact at 180 ºC. The mass spectrometer was simultaneously operated in scan mode (from 60 to 600 amu) and selective ion monitoring. Data were acquired and processed with TurboMass 5.1 software (Perkin Elmer).

Steroids with their trivial and IUPAC names, molecular weight, retention times and mass-to-charge ratios (m/z) used for quantification and confirmation are presented in Table 1. Compounds were identified by comparison with authentic standards of 14 steroids (Steraloids, Sigma-Aldrich), literature data and interpretation of mass spectrometric fragmentation patterns. Quantification was performed using a 4-points calibration curve (0,2-50 μg ml-1) with the available standards (Table 1). Peak areas were corrected according internal standard recoveries. Commercially standards were not available for some compounds (cholestenol, campestanol, 24-Ethylcoprostanol and γ-Sitosterol) which were quantified based on response factors of structurally related sterols.

**Table 1.** Names, formula, molecular weight (MW), retention time (Rt) and mass of ions used for quantification (target ion) and identification (confirmatory ions) of sterols and steroids (coprostane and coprostanone) analysed in this work.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Trivial name** | **IUPAC Name** | **Formula** | **MW** | **Rt** | **Target ion** | **Confirmatory ions** | |
| Coprostane | 5β -Cholestane | C27H48 | 372.67 | 30.80 | 217 | 357 | 372 |
| Coprostanol | (3β,5β)-Cholestan-3-ol | C27H48O | 388.67 | 35.57 | 370 | 355 | 215 |
| Epicoprostanol | (3α,5β)-Cholestan-3-ol | C27H48O | 388.67 | 36.08 | 370 | 215 | 355 |
| Cholestanol \* | (3β,5α)-Cholestan-3-ol | C27H48O | 388.67 | 36.16 | 215 | 355 | 370 |
| Coprostanone | (5α)-Cholestan-3-one | C27H46O | 386.65 | 37.13 | 386 | 231 | 370 |
| Deuterocholesterol | (3β)-Cholest-5-en-3-ol-25,26,26,26,27,27,27-D7 | C27H46O | 393.70 | 37.31 | 129 | 336 | 375 |
| Cholesterol | (3β)-Cholest-5-en-3-ol | C27H46O | 386.65 | 37.48 | 329 | 129 | 368 |
| Dehydrocholesterol | (3β)-Cholesta-5,7-dien-3-ol | C27H44O | 384.64 | 37.73 | 215 | 445 | 355 |
| Brassicasterol | (3β,22E)-Ergosta-5,22-dien-3-ol | C28H46O | 398.66 | 38.19 | 456 | 129 | 366 |
| Desmosterol | (3β)-Cholesta-5,24-dien-3-ol | C27H44O | 384.64 | 38.36 | 129 | 343 | 253 |
| Ergosterol | (3β)-Ergosta-5,7,22-trien-3-ol | C28H44O | 396.65 | 39.17 | 343 | 337 | 468 |
| Campestanol \* | (3β,5α,24R)-Ergostan-3-ol | C28H50O | 402.70 | 39.75 | 343 | 129 | 384 |
| Campesterol | (3β,24R)-Ergost-5-en-3-ol | C28H48O | 400.68 | 39.92 | 343 | 129 | 382 |
| 24-Ethylcoprostanol \* | (3β,5β,24S)-Stigmastan-3-ol | C29H52O | 416.72 | 40.19 | 398 | 215 | 383 |
| Stigmasterol | (3β,22E)-Stigmasta-5,22-dien-3-ol | C29H48O | 412.69 | 40.55 | 129 | 255 | 484 |
| γ-Sitosterol \* | (3β,24S)-Stigmast-5-en-3-ol | C29H50O | 414.71 | 41.82 | 129 | 473 | 488 |
| Deutero-β-Sitosterol | (3β)-Stigmast-5-en-3-ol-25,26,26,26,27,27,27 -D7 | C29H50O | 421.75 | 42.00 | 129 | 364 | 403 |
| β-Sitosterol | (3β)-Stigmast-5-en-3-ol | C29H50O | 414.71 | 42.20 | 129 | 488 | 473 |
| Stigmastanol | (3β)-Stigmastan-3-ol | C29H52O | 416.72 | 42.59 | 215 | 473 | 488 |

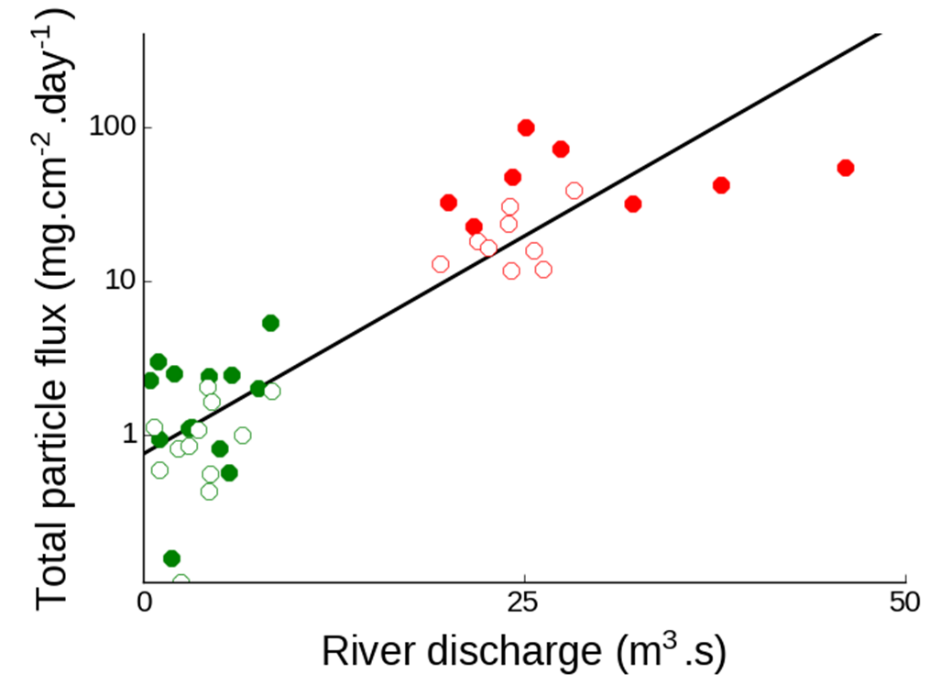
\*: Sterols for which no authentic standard was available.

The limit of detection (LOD) of each steroid was estimated by calculating the signal to-noise ratio (S/N) of triplicate standard solutions in the range of 0,2-50 μg ml-1 . LODs values averaged……., ranging from () to (). Reproducibility was assessed by the relative standard deviation (RSD) of triplicate analysis of the same samples in different batches, and averaged 11±3.8 The method was highly linear in the range of concentrations of calibration curves (R2>0.99 for all steroids with available authentic standards). Recoveries of deuterated internal standards averaged 96±1.7 Individual recoveries, evaluated by analysis of spiked samples ranged from 82±15% (Ergosterol) to 110±19% (Desmosterol). Detailed information on method performance is provided in S1.

Statistical analysis was carried on with Python scripting language, using SciPy, NumPy, MatPlotlib and pandas libraries. Multivariate analyses was executed in *R* language, using ggplot2 and ggbiplot packages. Data were expressed as mean ± SD. Relative standard deviation (RSD: [data – mean].100.SD-1) was used to assess parameter variability. To avoid division by zero errors, the ratios between individual sterols were calculated as: %sterol-A.(%sterol-A + %sterol-B)-1. The accumulation efficiency of sterols from settling material to sediments was estimated as the relationship between the annual vertical flux of the sterol and its corresponding one-year inventory in sediments (sterol concentration in sediment \* annual mineral flux). Student’s 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 temporal variation in sterol vertical flux.

**Results**

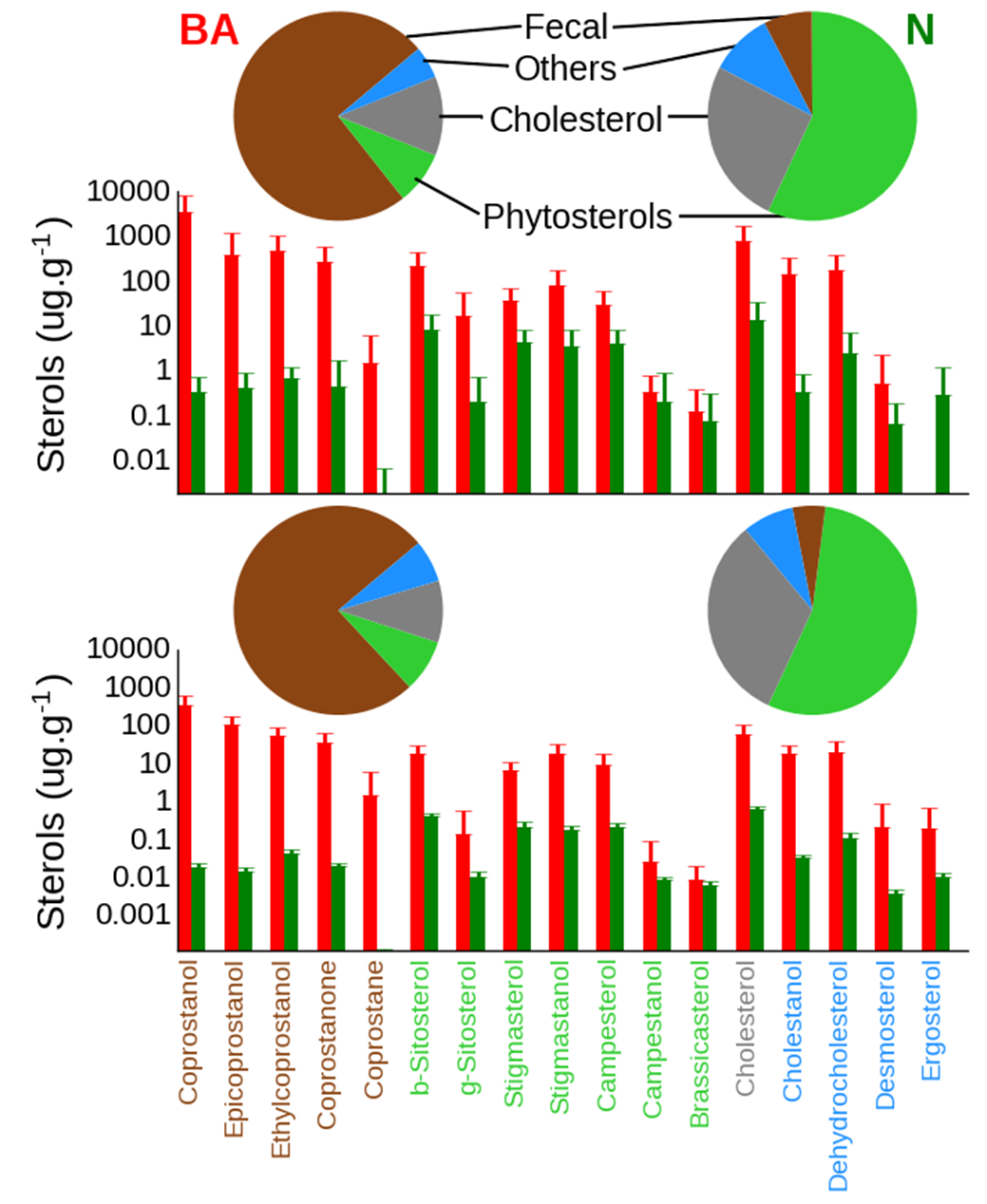
Total particle flux presented clear spatial variation, with average values 23 times higher at BA than at N (34±24 mg.cm2.day-1vs. 1.5±1.1 mg.cm2.day-1, respectively). Sedimentation rate exhibited a 9-times difference between BA and N (4.7±3.3 cm.year-1 vs. 0.54±0.42 cm.year-1). The settling material flux was largely dependent on river discharge which ranged 19465-46088m3.s-1 at BA and 420-8410 m3.s-1 at N, fitting an exponential curve (R2 = 0.78, *p*<0.0001; Fig. 2).



**Fig. 2.** Relationship between river discharge and total particle flux at North (green circles) and Buenos Aires (red circles) sites during warm (September to March, solid circles) and cold months (April to August, empty circles).

The total sterol concentration in settling material was highly variable (RSD: 113%) and exhibited marked geographical variation, with a three orders-of-magnitude difference between BA and N (7140±7905 vs. 41±47 μg.g-1 dry weight). Total sterols in sediments were 10-22 times lower than in settling material and were less variable (RSD: 10-61%) and also presented a three orders-of-magnitude difference between BA and N (708±454 vs. 1.9±0.18 μg.g-1).

Sterol composition showed contrasting differences between BA and N, both in settling material and sediments (Fig. 3, S2). At BA, the sterol composition of settling material was dominated by faecal sterols (75±5.4% of total sterols), mostly coprostanol (52±11%), followed by cholesterol (12±2.9%) and phytosterols (8.3±3.6%), mainly represented by β–sitosterol (4.4±1.9%). At N, phytosterols were the main constituents, making up 57±13%, basically through β–sitosterol (19±5.4%), stigmasterol (15±7.9%) and campesterol (13±11%), followed by cholesterol (26±12%) and only 7.5±7.0% of faecal sterols, mainly ethylcoprostanol (3.9±4.7%) and, to a lesser extent, coprostanol (1.3±1.3%). The sediment sterol profile, on a percentage basis, was very similar to that of settling material, except for minor differences at BA: a higher epicoprostanol, stigmastanol and cholestanol proportions (16±4.5, 2.6±1.5 and 2.8±1.1%, respectively, *p*<0.05) and less cholesterol (9.6±3.9%, *p*<0.05).



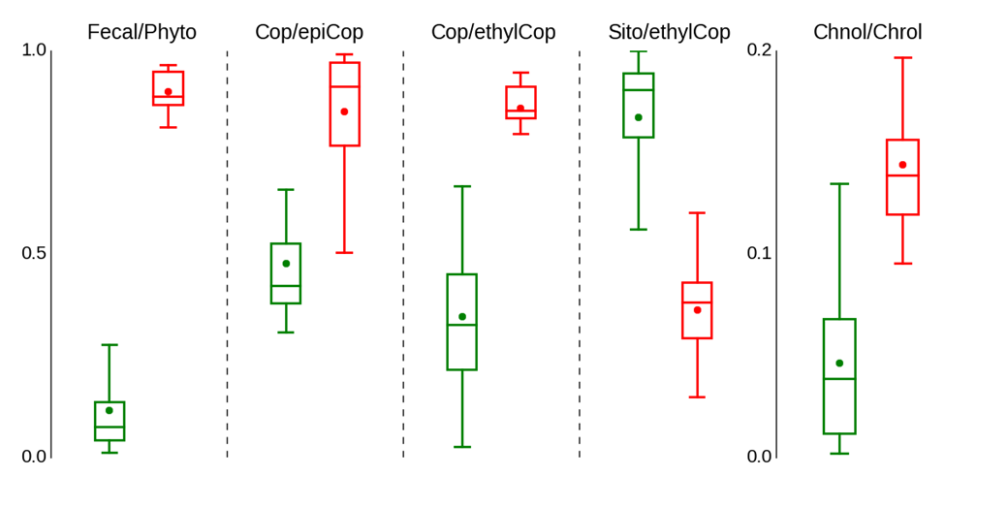
**Fig. 3.** Sterol composition of settling material (top panel) and sediments (bottom panel) at Buenos Aires (BA, red, left pie chart) and North (N, dark green, right pie chart). Pie charts show proportions of cholesterol, fecal sterols, phytosterols and other sterols. Bar graphs show the individual sterols concentrations, in a dry weight basis (note the logarithmic scale). Colours of sterols labels correspond to the pie charts grouping (brown for faecal sterols, green for phytosterols, grey for cholesterol and blue for the remaining sterols analysed in this study).

Vertical flux of total sterol was highly variable and averaged 116±168 mg.cm-2.year-1 at BA, with coprostanol accounting up to 60% of it (69±108 mg.cm-2.year-1, Table 2). At N, vertical flux of sterols were four orders of magnitude lower, 29±44 μg.cm-2.year-1 and cholesterol and β–sitosterol were the sterols with the highest fluxes. The accumulation efficiency, used to evaluate the preservation of sterol from settling material in superficial sediments, was 2-7 times higher at BA compared with N. The general pattern of accumulation efficiency of individual sterols was rather similar for both sampling sites. Epicoprostanol had the highest accumulation efficiency (BA: 40, N: 5.9), followed by phytosterols (BA: 9.8-14, N: 2.9-3.4), cholestanol (BA:10, N: 6.1) and coprostanone (BA: 10, N: 3.7). Cholesterol was the lest preserved sterol (BA: 4.6, N: 1.6).

**Table 2.** Accumulation efficiency of sterols from settling material in superficial sediments (%) and vertical flux (expressed between parentheses as mg.cm-2.year-1 for BA and as μg.cm-2.year-1 for N). Minor sterols (<1% of total sterols) were excluded from calculations.

|  |  |  |
| --- | --- | --- |
|  | **Site** | |
| **Compound** | **BA** | **N** |
| Coprostanol | 6.5  (69±108) | 2.2  (0.30±0.42) |
| Epicoprostanol | 40  (3.6±4.1) | 5.9  (0.36±0.55) |
| 24-Ethylcoprostanol | 7.6  (9.8±15) | 2.5  (0.45±0.54) |
| Coprostanone | 10  (5.8±9.4) | 3.7  (0.49±1.4) |
| β-Sitosterol | 9.8  (4.2±7.5) | 2.9  (6.6±9.9) |
| Stigmasterol | 10  (0.40±0.36) | 3.3  (3.2±4.3) |
| Stigmastanol | 14  (1.3±1.6) | 3.1  (2.8±4.2) |
| Campesterol | 12  (0.42±0.57) | 3.4  (3.3±4.4) |
| Cholesterol | 4.6  (16±26) | 1.6  (9.7±19) |
| Cholestanol | 10  (2.0±4.3) | 6.1  (0.30±0.68) |
| Dehydrocholesterol | 6.2  (3.2±4.6) | 1.7  (1.8±4.6) |
| **Total** | **7.4**  **(116±168)** | **3.6**  **(29±44)** |

In order to discriminate different organic matter sources and to assess different pathways of sterol degradation in settling material, several sterol ratios were evaluated (Fig. 4). All the ratios presented highly significant differences between BA and N (*p*<0.0001). The ratio between faecal sterols and phytosterols was much higher at BA than at N (0.90±0.044 vs 0.12±0.10). The β–sitosterol/24-ethylcoprostanol index, also used to evaluate the contribution of faecal and plant sterols (Nash et al., 2005) was higher at N (0.36±0.15 vs. 0.84±0.17). The coprostanol/epicoprostanol and the cholestanol/cholesterol ratios, used to assess the degradation of the sterol signal (Fattore et al., 1996, Chalaux et al., 1995), were higher at BA (0.85±0.15 vs 0.48±0.15 and 0.14±0.036 vs 0.046±0.041, respectively). The relationship between coprostanol and 24-ethylcoprostanol, useful to distinguish between different faecal soures of sterols (Leeming et al., 1996) was higher at BA (0.86±0.064 vs 0.35±0.19).



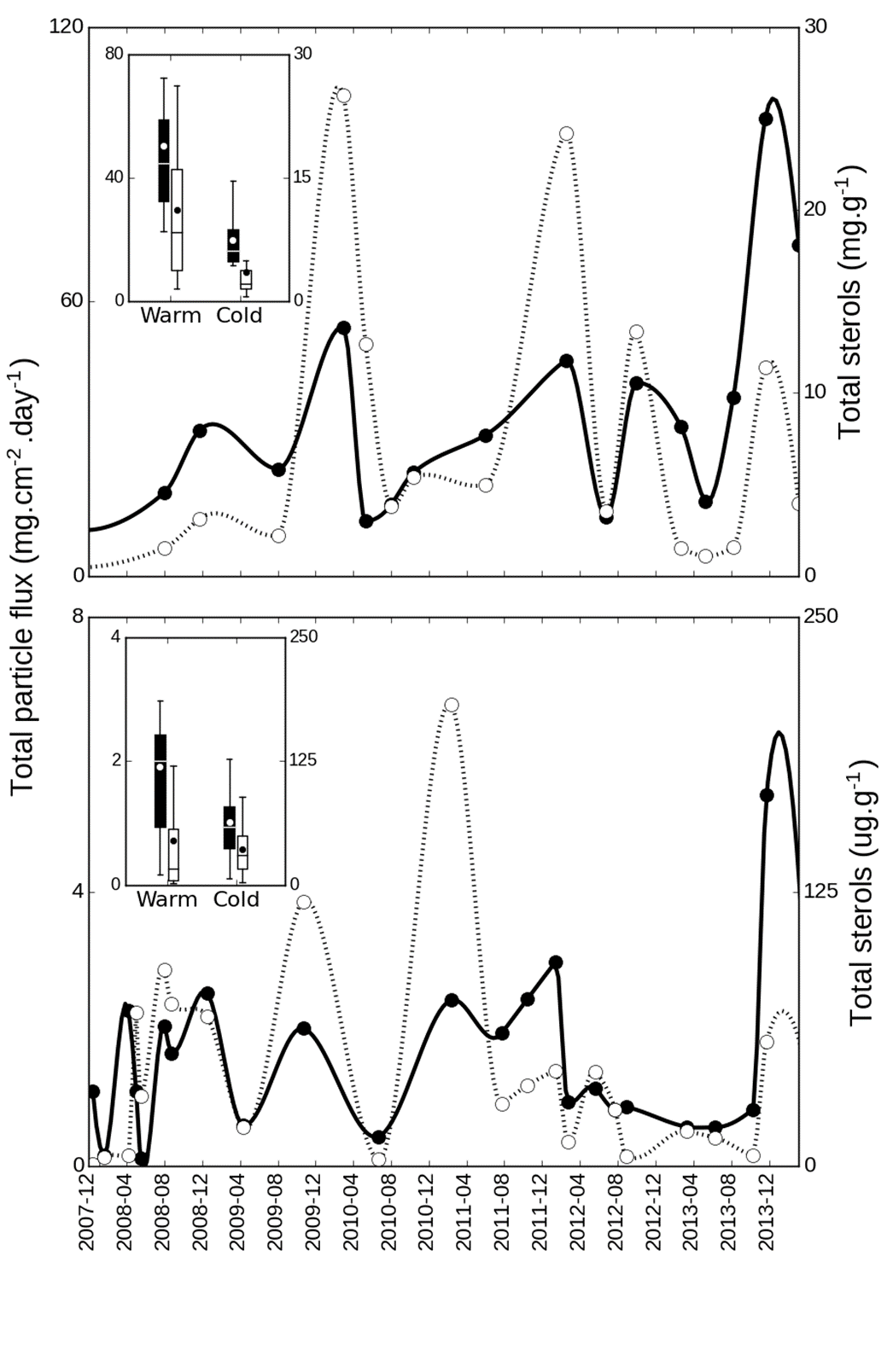
**Fig. 4.** Box plots of different sterol ratios in settling material from North (green) and Buenos Aires (red). Fecal/Phyto: Fecal sterols/phytosterols, Copr/epiCop: Coprostanol/Epicoprostanol, Cop/ethylCop: Coprostanol/24-Ethylcoprostanol, Sito/ethylCop: β-sitosterol/24-Ethylcoprostanol, Chnol/Chrol: Cholestanol/Cholesterol All ratios were significantly different between North and Buenos Aires (p<0.0001).

To simultaneously evaluate the contribution of the different sterol to overall variability in settling material and sediments, multiple regression and multivariate analysis (PCA) was performed (sterols with average concentrations lower than 0.5% were excluded from analysis, Fig.). This model explain 59% of total variability, mainly through principal component 1 (47%), which is loaded in the negative side with coprostanol and epicoprostanol and in the positive side with cholesterol and plant sterols.



**Fig. 5.** Principal component analysis of sterol composition of settling particles (solid squares) and sediments (hollow squares) from Buenos Aires (red) and North (green). Confidence ellipses were set to 90% of normal probability.

In addition to the spatial differences there were clear temporal variations in particle flux and sterol concentrations. Despite the large variability, there is a significant temporal pattern at BA of higher particle fluxes during warm months (September to March, 50±25 mg.cm2.day-1) relative to cold ones (April to August, 20±9.4 mg.cm2.day-1, *p*<0.005; Fig. 6). A similar seasonal variation (1.9±1.2 vs. 1.0±0.60 mg.cm2.day-1 at warm and cold months, *p*<0.05) was observed at N. Sterols at BA were significantly correlated with total particle flux (r = 0.64, *p*<0.05) following its temporal variation, with 11163±9599 μg.g-1 during warm months and 3564±3711 μg.g-1 in cold ones (*p*<0.05, Fig. 6). At N, sterols were also significantly correlated with particle flux (r = 0.60, *p*<0.05), but there was no difference between warm and cold months (45±61 vs. 36±28 μg.g-1 respectively). The sterol composition, on a percentage basis, showed little temporal variation except for coprostanol and epicoprostanol at BA. While coprostanol proportion was higher at warm months than at cold ones (59±9.5 vs 45±8.7; *p*<0.01) and was correlated with total particle flux (*r*: 0.38; *p*<0.05), its epimer showed an opposite trend, increasing at cold months (2.6±2.0 vs 15± 9.2; *p*<0.005) and inversely correlating to total particle flux (*r*: -0.70; *p*<0.005).



**Fig. 6.** Temporal variation of total particles flux (solid line, left axis) and total sterol concentration in settling material (dotted line, right axis) at Buenos Aires (top panel) and North (bottom panel). The boxplot inserts shows the averages for warm months (September to March) and cold months (April to August) for total particle flux (black boxes) and total sterols (white boxes).

**Discussion**

The intense discharge of one of the largest sewer outfall worldwide at BA results in an extraordinarily high sedimentation rate of 4.7±3.3 cm.year-1, in agreement with previous measurements at this area (5.5 ± 2.1 cm.year-1, Colombo et al., 2007). This value is much higher than sedimentation rates reported for nearby areas of this turbid estuary (0.3 -1.3 cm.year-1; Di Gregorio et al., 2007; Bonachea et al., 2010), suggesting than most particles captured by sediment traps at BA are highly organic detritus derived from urban-industrial discharges. This is supported by the high lipid content previously measured in BA settling material (11±7.4 mg.g-1), which was 27-times higher than at the Parana river (0.41±0.28 mg.g-1), which contributes the most to the solid load of the Rio de la Plata estuary (Speranza et al., 2013). The sedimentation rate at N was comparable to values reported by Colombo et al. 2015, which also showed a high variability (1.0±0.88 cm.year-1, range: 0.27-2.7 cm.year-1). In contrast to BA settling material, derived from anthropogenic detritus and Parana River load, the settling material at N came basically from the solid discharge of the Uruguay River, which is much smaller than that the Parana River (Moreira et al. 2013 from Jaime and Menendez 2002). The correlation between total particle flux and river discharge has been previously observed at the Uruguay River and reflects the enhanced transport of eroded material as river flow increases (Colombo et al. 2015).

The tendency of sterols to associate with particulate matter, due to their hydrophobicity, is enhanced by the high organic content of settling particles at BA (total organic carbon: 7.7±5.5%; Colombo et al. 2007), resulting in very high sterol concentration in settling material at this site (~7 mg.g-1). Previous studies dealing with sterols in settling particles were mostly based in ocean waters, relatively deep and clear, which had average concentrations 1-4 orders of magnitude lower compared to this shallow and turbid freshwater environment (Colombo et al. 1996; Parrish et al., 2000; Takada et al., 1994; Burns et al., 2008). In fact, total sterol concentration at BA settling material was comparable to those measured in sewage sludge at wastewater treatment plants (2-9 mg.g-1, Venkatesan and Kaplan 1990; Kelly 1995; Nguyen et al., 1995). The sterol composition of settling material at BA has a clear fecal signature, resembling the composition of human faeces (fecal sterols: 85%, phytosterols: 8.8%, cholesterol: 5.2%, others: 1.2%; Leeming et al. 1996), reflecting the massive discharges of fresh sewage at this site. Moreover, the coprostanol proportion of BA settling material felt in the 50-80% range found in sewage sludges and effluents (Venkatesan and Kaplan 1990), reflecting fresh sewage inputs. Nevertheless, the presence of smaller proportions of epicoprostanol, originated from microbial degradation of coprostanol evidence an incipient degradation of fecal material. Taking into account the length of sewer network (7000 km, main sewer: 35km), implying relatively large residence time of fecal material in this system before reaching the river, thus degradation might occur in sewer pipeline rather than on water column, which is quite shallow (3-4m). The presence of a relatively important proportion of ethylcoprostanol is usually associated with non-human (herbivore) fecal pollution since it is the product of microbial hydrogenation of β–sitosterol, the main sterol in terrestrial vegetation. However, human feces can include significant amounts of ethylcoprostanol (Leeming et al. 1996) so the mere presence of this sterol cannot be unambiguously attributed to herbivore fecal contribution. The significance of coprostanone is difficult to ascertain since it originates in mammalian gut as an intermediary in coprostanol microbial synthesis, but it also can be a product of coprostanol oxidation leading to epicoprostanol formation in oxic sediments (McCalley et al. 1981). The most important phytosterols at BA settling material, i.e. sitosterol, campesterol and stigmastanol, are associated with land plants (Laureillard and Saliot 1993) and are present in modest proportions reflecting the minor contribution of terrestrial inputs at BA. Despite the relative abundance of cholesterol, its utility as biomarker is rather limited since it is present in sewage material but also in multiple organic matter sources, such as algae, zooplankton and benthic fauna (Mudge et al. 1999; Creuzberg and von Elert 2009).

At N, both the sterol concentration and composition were in clear contrast with BA. The ~170-times smaller total sterol concentration is comparable to sterol concentrations reported in particulate matter in tropical and subtropical riverine environments (1-184 μg.g-1; Saliot et al., 2001; Li et al 1995; Jeng and Kao 2002) and reflects the inputs of natural organic matter. Overall, the sterol profile presented a predominant vegetal signature. Despite being found in some algae species, the three major phytosterols at N, β-sitosterol, stigmasterol and campesterol, are strongly associated with land plants (Huang and Meinschein 1979, Volkman 2005), to such an extent that they have used as biomarkers of paper mill pollution (Lahdelma and Oikari 2006). The fecal sterols signal differs both quantitatively and qualitatively from the typical sewage signature observed at BA. The presence of ethylcoprostanol as the main fecal sterol at N could be indicative of small inputs of cattle fecal pollution from the neighboring livestock establishments.

The difference between settling material and sediments on terms of sterol concentrations and composition reflects the tendency of sterol to degrade at the water-sediment interfase, especially under oxic conditions (Sun and Wakeham 1998). Nevertheless, sediments at BA still had remarkably high sterol concentrations, especially of fecal sterols. The coprostanol.concentration was among the highest values reported for The level concentration are remarkable In fact, the level of coprostanol, of sterols, especially coprostanol

tion at the water-sediment interfase had lower sterol concentration observed in sediments reflects COnc in sediment are lower because of well-known degradation (Speranza et al. 2013, Colombo et al.1996). Comparison of coprostanol in sediments with literature data. The sterol concentration in sediments compared to settling material due to degradation at the water/sediment interface mainly aerobic since sterol are prone to degrade under oxic conditions (Sun

Froehner 08:

The results

showed high concentrations of coprostanol, ranging

from 0.25 to 196 μg g−1. (in highly polluted Brazilian river)

Campesterol, β-sitosterol and stigmasterol

are present in large amounts in plants and the

presence of these compounds in aquatic sediments

can be associated with the input of organic matter

whose origin is terrestrial (Volkman et al. 1998).

Venturini 2015 RIO DE LA PLATA!!!:

Total steroids (R Steroids) (mean values of the three sampling

surveys) ranged between 2.28 in station L12 and 34.8 lg g-1 dw

in station B2 (Table 5). The highest concentrations were recorded

in the stations B1, B2, and B5 of Montevideo Bay (Fig. 2). Coprostanol

concentrations ranged between 0.05 lg g-1 in stations L6 and L7 and 21.2 lg g-1 dw in station B2 (Table 5). Epicoprostanol varied

between 0.01 and 1.34 lg g-1, whereas coprostanone ranged from

0.07 to 6.01 lg g-1 dw(Table 5). The highest concentrations of these

compounds were observed in station B2. Cholesterol concentrations

varied from 0.48 in station L6 to 5.07 lg g-1 dw in station B2

(Table 5). Campesterol ranged from 0.13 to 2.13 lg g-1 dw; the lowest

value was obtained in station Zmal and the highest in station B2

(Table 5). Stigmasterol varied from 0.30 to 3.14 lg g-1 dw and the

b-sitosterol ranged between 0.43 and 5.32 lg g-1 dw (Table 5).

Nishimura 1977: This suggests that the degradation of sterols in sediments

could occur efficiently under a relatively oxidative

condition and that the hydrogenation of stenols

predominates over the degradation in a strictly

anaerobic environment. Thus, in the surface sediment (O-l cm in

depth) under a relatively oxidizing environment such

as Lake Suwa, the sterols derived from organisms undergo

preferential degradation of the stenols and the

stanols tend to survive unaltered during the sedimentation

process as compared to stenols. The stenols

which escape the degradation are converted into

stanols by the appearance of anaerobic conditions

after deposition.

The coprostanol concentration is below the thresholds postuled by some authors as indicative of sewage pollution (0.5-0.7; Leeming et al. 1997; Rada et al. 2015). But is over the 0.1 ug.g-1 proposed by Grimalt et al. 1990.

in the oxic water column on both particulate phase and dissolved phase will be assumed to have a rate decay constant of 0.7/day (Writer et al. 1995).

This is in clear opposition with the situation at N.

More recently, it has been reported that coprostanone, contrary to coprostanol, survives biological treatment in urban sewage plants, which renders coprostanone as a potential marker for urban treatment plant effluents (Chaler et al., 2001).

Wakeham 89 Nature: Reduction of stenols to stanols in particulate matter at oxic-anoxic boundary (by bacteria).

Wakeham 89b: In the sterols, the marked

predominance of cholest-5-en-3fl-ol in the trap

material contrasts with the lesser abundance of

cholesl-5-en-3fl-ol and increased abundances of 24-

methylcholesta-5,22E-dien-3fl-ol, 24-methylcholesta-

5,24(28)-dien-3fl-ol and 24-ethylcholesta-5-en-3fl-ol in sediments.

Steroid ketones consistently increase in abundance

relative to other lipids as depth increases (Fig. 2 for

VERTEX IV and Fig. 8 for PARFLUX E). This

increased abundance might be due to preferential

preservation of these compounds compared to other

lipids. However, the increase in vertical flux of the

steroid ketones between 500 and 1500 m suggests that in situ production of steroid ketones may also be involved.

Laureillard 1993: The relative abundance of the higher plant sterols:

sitosterol/stigmasterol/campesterol has been

found for various higher land plants to be (11.5-

31)/(0.5-1.3)/1 (Nishimura, 1977). Furthermore,

in surface sediments of Loch Clair, where

organic matter inputs were attributed to higher

plant origin, the ratios obtained were 6.6/1.6/1

(Cranwell and Volkman, 1981)

This terrestrial signature is in agreement with the previous observation of high PUFA proportion at PAR.

The sterol concentration variability its due to its temporal variation.

La construcción del Emisario Subfluvial Berazategui, se emplazará a una distancia

aproximada de 7500 metros desde la línea de costa, con un área de difusión de 2300

metros de longitud

Canuel et al 1996:

Decreasing rates of sterol degradation with sediment depth.

Venkatesan & Kaplan 1990:

The advective transport of fine particles

as well as better preservation of organic matter in

anoxic deeper waters is reflected in the very high coprostanols

content

Takada dump106:

The presence of coprostanol at the remote sites

limits the utility of coprostanol as an indicator of low

amounts of sewage sludge input.

Flux copr: 30-32ug.m-2.day-1

Ternois 1998:

Early diagenesis of steroids in the

water column has thus been estimated by stanol/ste-

nol ratios (Gagosian et al., 1979; Wakeham, 1987).

Jaffe 1995:

The observation that fluxes of lipids were higher in

the Atabapo (S8) and Orinoco-l (S5) stations during high

waters agrees with the high terrestrial (allochthonous) input

to these rivers during the rainy season, since the respective

catchments of both rivers are dominated by rain forests. In

contrast, the Orinoco-2 (S7) site is strongly impacted by the

Guaviare (S6), which has a discharge twice as high as the

Orinoco above its confluence (at Orinoco-l; S5).

Aerobic bacterial hydrogenation may convert ∆

5

-sterols to 5α(H)-stanols, 5α(H)-

stanones and ster-4-en-3-ones (Gagosian et al., 1982; De Leeuw and Baas, 1986;

Wakeham, 1989).

Galeron 2015:

Hedges and Keil (1995) hinted that sterols associated with waxy higher plant material might not be

as prone to enzymatic degradation as other sterols, which

would explain why sitosterol is only weakly biodegraded in

our samples.

During senescence, unsaturated higher plant lipids (and

notably 15-sterols) may be photodegraded (type II photooxidation),

with chlorophyll acting as a sensitizer (Rontani

et al., 1996). Sitosterol present in higher plant phytodetritus

should thus have been intensely photodegraded on

land. Recently, it has been demonstrated that autoxidation

plays a key role in the degradation of terrestrial (Rontani et

al., 2014b) and marine (Rontani et al., 2014a) vascular plant

debris in seawater. On the contrary, cholesterol is mostly affected by biodegradation (photo- and autooxidation are much less important). All samples are dominated by sitosterol and cholesterol,

with proportions being on average 3 times higher than those

of the other sterols, apart from the 6 March 2012 sample (Table

1).

Leeming 1997:  
In selected environments, 5(3-stanols may be produced in situ by anaerobic bacteria.

However, the ratio of coprostanol to 5a-cholestanol can be used to assess whether

coprostanol in surficial sediments is of fecal origin or, from an in situ microbial source.

A ratio of <0.3 is generally observed for pristine aerobic sediments, with ratios >0.5

for sites impacted by sewage. For Sydney inner-shelf sediments, the ratio was in most

cases >2.

Puerari 12:

This suggests that in the Winter,

which is usually a dry season, the sewage presented an

enhanced level of degradation, as the increased contribution

of cholestanol might be derived from the bacterial reduction

of cholesterol and/or from autochthonous production.

the contamination may also arise

from the washout of manure produced by livestock during

the rainy season, in the Summer, in some sites. The higher

level of sewage contamination in the Winter sampling, a

dry period, suggested a small river ability to disperse and

dilute the effluents in periods of reduced water flow.

The influence of ENSO events on the precipitation and freshwater discharge of the Uruguay River observed in 2006/07, and particularly during the 2009/10 highest peak, is consistent with previous reports which indicate that ENSO timescale variability with characteristic 3.5- and 6-year components associated with Pacific SSTA is most marked in this river (Robertson and Mechoso 1998, Krepper et al. 2003). Superimposed on the ENSO inter-annual variability, the seasonal pattern of low summer-autumn waters and highest spring discharges is very significant, with a single, most significant precipitation and discharge peak in November 2008, coincident with neutral ENSO conditions and the beginning of a moderate

The observed variation of water quality parameters with river discharge basically reflects the enhanced erosion and transport of suspended material (turbidity and particle fluxes increase) and dilution with rainwater during high waters (pH decrease). The significant positive regression of turbidity and river flow (approx. 10–20 to >70 NTU; Fig. 5) reflect enhanced solid transport capacity, as has been observed in other rivers (Kusimi 2008). The flux of material collected by the traps also increases with higher discharges and correlates with turbidity reflecting the transport of eroded material during river floods.

**References**

Gas Chromatography and Lipids by William W. Christie and published in 1989 by P.J. Barnes & Associates (The Oily Press Ltd)

Menéndez, A. N. (2002). “Description and modeling of the hydrosedimentologic mechanisms in the Río de la Plata River.” Instituto Nacional del Agua INA, Buenos Aires, Argentina.

Chalaux, N., Takada, H., Bayona, J.M. 1995. Molecular Markers in Tokyo Bay Sediments: Sources and Distribution. Marine Environmental Research, Vol. 40, No. 1, pp. 11-92,

Nash, D., Leeming, R., Clemow, L., Hannah, M., Halliwell, D., & Allen, D. (2005). Quantitative determination of sterols and other alcohols in overland flow from grazing land and possible source materials. Water, 39, 2964–2978. http://doi.org/10.1016/j.watres.2005.04.063