# Multiple biogeochemical indicators of environmental quality in tropical estuaries reveal contrasting conservation opportunities

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ABSTRACT

Estuaries are under major impacts from pollution even when managed as conservation units. Here we used multiple biogeochemical indexes of contamination and trophic status, including faecal sterols, biopolymers and trace metals, to determine and compare environmental quality of two tropical estuaries with contrasting conservation status. In the metropolitan estuary, eutrophic/hypereutrophic conditions and high concentrations ($> 1.0 \, \mu g \, g^{-1}$) of coprostanol were spatially correlated to sources of raw sewage input. Unexpected eutrophic sediments were also detected at the estuarine reserve, but with low sewage contamination indicating that high organic availability and burial predominated. The natural or polluted eutrophic sediments were determined by comparing multiple contamination indexes, which indicated sediment contamination within the metropolitan estuary. This study indicates that the long-term conservation of estuarine ecosystems on the Atlantic coast of Brazil are threatened by a typically poor sewage treatment and suggests that estuarine sediment quality need to be evaluated by multiple proxies before estuaries can be included in spatial conservation planning.

## 1. Introduction

Estuaries worldwide have been heavily impacted by habitat transformation and pollution with severe risks to their ecological resilience (Lotze et al., 2006). The increasing pollution threaten the ecological services provided by estuaries, especially near urban centers where there is poor management of multiple impacts. In contrast to their low environmental quality, estuaries have become priority areas for conservation and to be managed as marine reserves (Ducrotoy and Elliott, 2006; Gilby et al., 2017). Given that estuarine sediments may concentrate pollutants (Dell'Anno et al., 2002; Baldock et al., 2004; Muniz et al., 2015), estuarine ecosystems need ecological indicators to ensure that protection is targeted to areas with higher environmental quality.

The sedimentary organic matter (OM) and pollutants on estuaries are important drivers of ecological changes observed on benthic assemblages and therefore biopolymers such as carbohydrates (CHO), lipids (LIP) and proteins (PRT), may be used as indicators for estuarine

sediment quality (Nixon, 1995). The biopolymeric carbon (BPC) represent the OM labile fraction that is readily available for benthic organisms through remineralization (Aguiar et al., 2013), and therefore are highly sensitive to spatial and temporal changes in the benthic trophic status associated to both natural and human-induced environmental alterations (Dell'Anno et al., 2002; Pusceddu et al., 2003, 2009; Joseph et al., 2008). High concentrations of CHO with low nutritional value for benthic organisms may represent a degraded organic detritus (Joseph et al., 2008), whereas high PRT content, which is the main source of nitrogen for consumers, may reflect an increased fresh productivity (Danovaro et al., 1999). On the other hand, LIP have been used to indicate available energy to organisms and are indicators of detrital contributions derived from secondary production; furthermore, in polluted areas, LIP have been associated with anthropogenic sources of oil and sewage (Dell'Anno et al., 2002; Venturini et al., 2012). Therefore, the use of molecular markers in estuarine sediments are well established indicators of the benthic trophic status and identifying

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natural or human-induced eutrophication (Dell'Anno et al., 2008; Puscceddu et al., 2011; Venturini et al., 2012; Pita et al., 2017). The sedimentary trophic status is also largely applied to monitor long-term changes in estuarine environmental quality and management (Puscceddu et al., 2009), but their application with sewage and other OM biomarkers are still limited.

Sterols are used as molecular markers to characterize OM sources, either marine or terrigenous, and also to identify anthropogenic faecal contamination (Cordeiro et al., 2008; Martins et al., 2014; Cabral et al., 2018). Sterols are persistent in sediments, easily associate with particulate material and have resistance to anaerobic degradation (Muniz et al., 2015). Faecal sterols, including coprostanol and epicoprostanol, are used as tracers for human waste because they are present in human faecal and sewage effluents (Grimalt et al.,1990; Carreira et al., 2004; Montone et al., 2010; Venturini et al., 2015). Other sterols such as cholesterol and cholestanol are abundant in aquatic systems due to their presence in zooplankton and phytoplankton (Volkman, 2005; Loh et al., 2008). An additional marker of marine systems, trace elements have been increasingly released in estuarine ecosystems from anthropogenic inputs including mining, industry and diffuse sources such as agriculture, combustion, terrestrial and maritime transport, among others (Luiz-Silva et al., 2008; Gomes et al., 2017). Trace metals affect the ecosystem as a whole and human health through the processes of bioaccumulation and biomagnification and their determination in sediments may also support environmental quality assessments (Krull et al., 2014).

This study assessed the trophic status of sediments from two tropical estuarine systems with contrasting conservation and use on the Eastern Brazil Marine Ecoregion, based on determination of multiple organic (biopolymers and sterols) and inorganic (trace metals) indicators. By contrasting a heavily urbanized estuary with another visually well-preserved ecosystem, we aimed to: (i) identify and quantify sources of the sedimentary OM and trace metals, and; (ii) assess the environmental quality of sediment from both estuarine systems in order to aid a basin-wide management of both areas. Low environmental quality within the metropolitan estuary may be expected due to historical record of human activities, whereas the estuary located in a conservation reserve area would be less impacted by pollutants.

## 2. Material and methods

### 2.1. Study area

The study was conducted in two estuaries located in the Eastern Brazil Marine Ecoregion, which has an average monthly rainfall of 145 mm and temperatures from 24 to 26 °C (Bernardino et al., 2015, 2018a). The metropolitan estuary, Vitória bay (VB, 20°18′S, 40°20′W; Fig. 1) receives about 16,000 $m^3 day^{-1}$ of untreated domestic and industrial effluents from several tributaries (Bubu, Itanguá, Marinho, Aribiri rivers and Santa Maria da Vitoria city). VB has an important economic and touristic relevance to the region, but the historical record of degradation has not yet been used towards an improved management of estuarine resources.

The Piraquê-Açú-Mirim estuary (PAM; 17°58′S, 40°00′W) is situated 50 km north of VB and has a well-preserved area with tidal flats and extensive mangrove forests (dominated by *Rhizophora mangle* and *Avicennia schaueriana*), with minor coastal development (Bernardino et al., 2018b; Bissoli and Bernardino, 2018). The PAM estuary is managed as a conservation unit with use of its natural resources by local traditional communities (Servino et al., 2018).

### 2.2. Sampling

Sampling was carried in November 2014, with 19 sites in VB, and 11 sites in the PAM estuary (Fig. 1). Random stations were distributed across the estuaries, with VB02 to VB15 located in the inner VB estuary,

VB17 to VB21 along the Vitória harbor channel and VB24 to VB36 in Espírito Santo Bay. In VB, four stations (PC 02, 04, 06, 07) were sampled along a secondary channel (Passagem Channel, PC) that connects the inner estuary to the Espirito Santo Bay. At the PAM estuary, stations PA01 to PA05 were located in the northern Piraquê-Açu river, stations PM 01 to PM06 in the southern Piraquê-Mirim river, and stations PA07 to PA11 were sampled at the mouth of this estuary. Salinity was measured *in situ* with a calibrated SonTek CastAway CTD. Sediment samples (N = 2 per station) were collected with a Day grab (0.1 $m^2$), and the top 3 cm of undisturbed surface sediment from two independent replicates were mixed into one composite sample for chemical and particle size analyses. For organic compounds, samples were stored in pre-cleaned aluminum container whereas surficial sediments for trace metal analysis were stored in pre-cleaned LDPE containers. Samples remained frozen (− 20 °C) until laboratory procedures.

### 2.3. Laboratory procedures

#### 2.3.1. Bulk sedimentary parameters

In laboratory, sediments were dry weighed and sieved (2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm and 0.63 mm fractions) on a mechanical shaker, and the grain size was determined. Sub-samples of 1.5–2.0 g were analyzed for total organic matter (TOM) by weight loss on ignition at 550 °C for 4 h. Total organic carbon (TOC) and total nitrogen (TN) were analyzed by a LECO Elemental Analyser (Leco, TruSpec CNS). Accuracy of element analysis was checked employing a Soil CRMs (Thermo Soil Reference NCS, Italy and LECO Soil Calibration Sample, USA). Average recovery for TOC (2.36 ± 0.03%) and TN (0.185 ± 0.011%) were 99.8 and 101%, respectively.

#### 2.3.2. Biochemical analysis of organic matter and sediment trophic status categorization

Total protein (PRT) determination followed an extraction with NaOH (0.5 mol $L^{-1}$, 4 h) according to Hartree (1972), modified by Rice (1982). Total carbohydrates (CHO) were analyzed according to Gerchacov and Hatcher (1972) while total lipids (LIP) were extracted by ultrasonication (20 min) in 10 mL of chloroform:methanol (2:1 v/v) and analyzed following Marsh and Weinstein (1966). Blanks for each analysis were performed with pre-combusted sediments at 450 °C for 4 h. PRT, CHO and LIP concentrations were expressed as BSA, glucose and tripalmitine equivalents, respectively. All analyses were carried out in triplicate. Protein, carbohydrate and lipid concentrations were converted to carbon equivalents assuming a conversion factor of 0.49, 0.40 and 0.75 mg, respectively (Fabiano and Danovaro, 1994). The sum of PRT, LIP and CHO carbon equivalents was reported as the biopolymeric carbon (BPC) and used as a reliable estimative of the OM labile fraction (Fabiano et al., 1995). Based on BPC concentrations, estuarine areas were defined as: (i) hypertrophic (BPC ≫ 5 mg C$g^{-1}$); (ii) eutrophic BPC (3–5 mg C$g^{-1}$); (iii) mesotrophic (BPC = 1–3 mg C $g^{-1}$), and; (iv) oligotrophic BPC (< 1 mg C$g^{-1}$) (Puscceddu et al., 2007, 2011). Also, the PRT:CHO and the CHO:LIP ratios were calculated and used as indicators of the status of biochemical degradation processes (Galois et al., 2000).

#### 2.3.3. Sterols

Sterols were extracted from sediments with a Soxhlet apparatus for 8 h with 80 mL of *n*-hexane:dichloromethane (DCM) (1:1) after being spiked with surrogate standard (5α-androstanol) as described by Wisnieski et al. (2016). The extracts were concentrated to 1 mL using a rotary evaporator, purified and fractionated by silica and alumina liquid chromatographic column with an elution of 5 mL of ethanol/DCM (1:9, v/v), followed by 15 mL of ethanol. The purified extract fractions were dried, derivatized (BSTFA/TMCS (99:1) for 90 min at 70 °C), spiked with internal standard (5α -cholestane) before instrumental analyses. Sterols were analysed using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (GC/FID) and an