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THEMATIC ISSUE

Environmental quality assessment by multiple biogeochemical indicators of an intertidal flat under anthropogenic influence from the southwest of Buenos Aires (Argentina)

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Abstract

Chemical measures combined with biological data (biomarkers) are recommended in monitoring programs of marine environments. In the present research, the use of multiples biogeochemical indicators allowed interrelating different environmental measurements to deepen the knowledge of the quality/status of the intertidal flat from Puerto Rosales (Bahía Blanca estuary, Argentina) subjected to anthropogenic pressure. The sediments from this site presented a eutrophic status and high organic matter concentration with high nutritional value for the benthic community. The TPR/TCH ratio (~ 10) would evidence the untreated sewage discharge contribution and increments in protein content due to the complexation of nitrogen during phytodetritus accumulation and degradation. Trace metals levels in the fine sediments were lower than those recommended by international guides for uncontaminated sites. In the sediments, except Cu, all metals analyzed were rarely associated with adverse biological effects. Finally, metallothioneins levels in the burrowing crab *Neohelice granulata* were lower than those found in the literature and significantly higher in females than in males. Even though the different analyses and indices performed indicate that this intertidal flat is in good environmental condition, the wastewater discharge influenced it. Thus, it is advisable to continue with this kind of study by applying multi-combined proxies in this and other impacted tidal flats.

Keywords Trace metals · Intertidal sediments · Trophic status · Biomarkers · Sewage discharge

Introduction

Estuaries are among the most productive natural ecosystems in the world. They are complex systems, and as transitional zones, they especially transfer material from the continent to the oceans, including nutrients, organic matter and contaminants. They are essential nurseries grounds for organisms, recreational areas and are also the place of numerous economic activities (industry, ports, fisheries) being under high anthropogenic pressure. Finally, they play a crucial role as traps for all kinds of suspended and dissolved materials, providing cleaner waters for marine life (Dame 2008; NOAA 2018; Thibault et al. 2019).

Within estuaries, tidal wetlands have been the least studied areas and a deficit of ecological, ecotoxicological and biological knowledge still exists on them, mainly due to their unique characteristics (Rundle et al. 1998; Attrill and Rundle 2002; Sousa et al. 2008). These transitional areas are subjected to several environmental threats, such as eutrophication, chemical pollution, degradation of water

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quality, dredging and other hydrological modifications (Ysebaert et al. 2003). Most of the contaminants available in the water column such as trace metals are adsorbed on suspended particulate matter (SPM) and deposited in sediments by flocculation and sedimentation processes. Then sediments control processes such as adsorption, complexation of metals to organic ligands, clay and mineral surfaces (De Souza Machado et al. 2016). Therefore, they play a significant role as sinks and sources of these contaminants in aquatic systems and hence, are widely recognized and more useful than water as a suitable tool to assess the quality and trophic status of a particular ecosystem (Fernándes et al. 2011; Cheriyan et al. 2015).

The sedimentary organic matter (OM) in estuarine environments comes from different sources such as vascular plants, riverine and marine inputs, benthic primary production or/and exudation of exopolymeric substances of microphytobenthos. In consequence, tidal flat sediments are composed by a mixture of labile and refractory OM. The labile portion of OM mainly consists of simple and/or combined compounds (i.e., biopolymers). It includes carbohydrates, lipids, proteins and nucleic acids that are rapidly mineralized, while the refractory matter, composed by complex substances like humic and fulvic acids, is slowly broken down (Danovaro et al. 2001; Nair and Sujatha 2012). The quantity and quality of the OM, determine the amounts of material potentially available to be consumed by organisms, affect the species biodiversity and the metabolism of the benthic fauna (Fabiano et al. 1995), and define trace metals' dynamics in the sediments. For this reason, it is essential to implement a combined evaluation of the biological and physico-chemical characteristics, such as trace metals content and OM biochemical composition of the tidal flats to perform exhaustive biomonitoring of urbanized ports as the European Union (EU) Water Framework Directive (WFD, CE 2000/60) has already developed it in coastal zones (Renzi et al. 2019) and other developed countries of the world (Venturini et al. 2012; Kumar et al. 2013; Cheriyan et al. 2015; Muniz et al. 2015; Laut et al. 2017; Bigus et al. 2017; Joy et al. 2019).

Thus, to tack this knowledge gap, the present work was conducted in the Bahía Blanca estuary located on the SE coast of Argentina. In this estuary, the rapid development of industrial activities and urban growth in the last decades have caused significant concern due to the potential increase of the contaminants' loads in the system (Marcovecchio et al. 2008; Speake et al. 2020). Moreover, the sewage discharges without adequate treatment in the estuary have been classified as potential sources of contamination (Pierini et al. 2012; Spetter et al. 2015; Berasategui et al. 2018). In this context, the aim of this work was to applied multiple biogeochemical indicators to obtain results of the environmental quality and trophic status of Puerto Rosales intertidal flat, a site influenced by the wastewater discharge from Punta Alta city, and to analyze its potential biological influence. Therefore, the concentration of trace metals in the sediments and suspended particulate matter (SPM); and the content of Chlorophyll *a* (Chl*a*), Phaeopigments (Phaeo), organic matter (%OM), total carbohydrates (TCH) and total proteins (TPR) in the intertidal sediments were analyzed. The concentration of metallothioneins (MTs) as early warning systems of trace metal contamination was determined in the dominant benthic species, *Neohelice granulata* (Brachyura, Varunidae). Finally, different indices were obtained to assess the trace metal contamination of the sediments, its trophic status, and the potential ecological risk to benthic organisms.

Materials and methods

Study area

The Bahía Blanca estuary (BBE) is located on the SW coast of Buenos Aires province, Argentina (38°45′–39°25′ S and 61°45′–62°30′ W) (Fig. 1). This mesotidal system, with semi-diurnal tide prevalence, covers an area of about 2300 km^2 and comprises several tidal channels, extensive tidal flats (1150 km^2) with patches of low salt marshes and islands (410 km^2) (Hayes 1979; Piccolo et al. 2008). On the northern shore of the estuary are established populated cities as Bahía Blanca and Punta Alta and vast industrial complexes (oil, chemical, and plastic factories) whose sewages without an appropriate treatment discharge into the estuarine waters. In addition, it is located the most important deep port system of Argentina composed by Puerto Galván, port of Ingeniero White, Puerto Rosales and Naval Base Puerto Belgrano. This leads into the constant movement of large ships and contributes with the mobilization of tons of sediments, due to the dredging and maintenance activities of the main navigation channel and, therefore, affecting contaminant transport (Marcovecchio 2000).

The present study was carried out in Puerto Rosales (PR) (38°55′ S; 62°04′ W), a tiny coastal breakwater port (Fig. 1), located on the northeast coast in the middle area of the BBE. The mooring sites and the access channel which communicates to the Principal Channel are located on the east side of a 2 km length N–S direction stone shore-connected breakwater. Eastward the breakwater, there is an extensive intertidal zone (2 km approximately), while on the westward is developed a subtidal area.

This area is characterized by a dry temperate climate with a mean annual air temperature of 15.6 °C (mean range from 22.7 °C in January to 8.1 °C in July), low precipitations (mean value of 460.5 mm) and a high evaporation rate (Perillo et al. 2001; Piccolo and Diez 2004). The exchange of water with the estuary is regulated by a semi-diurnal tidal cycle, flooding the extensive tidal

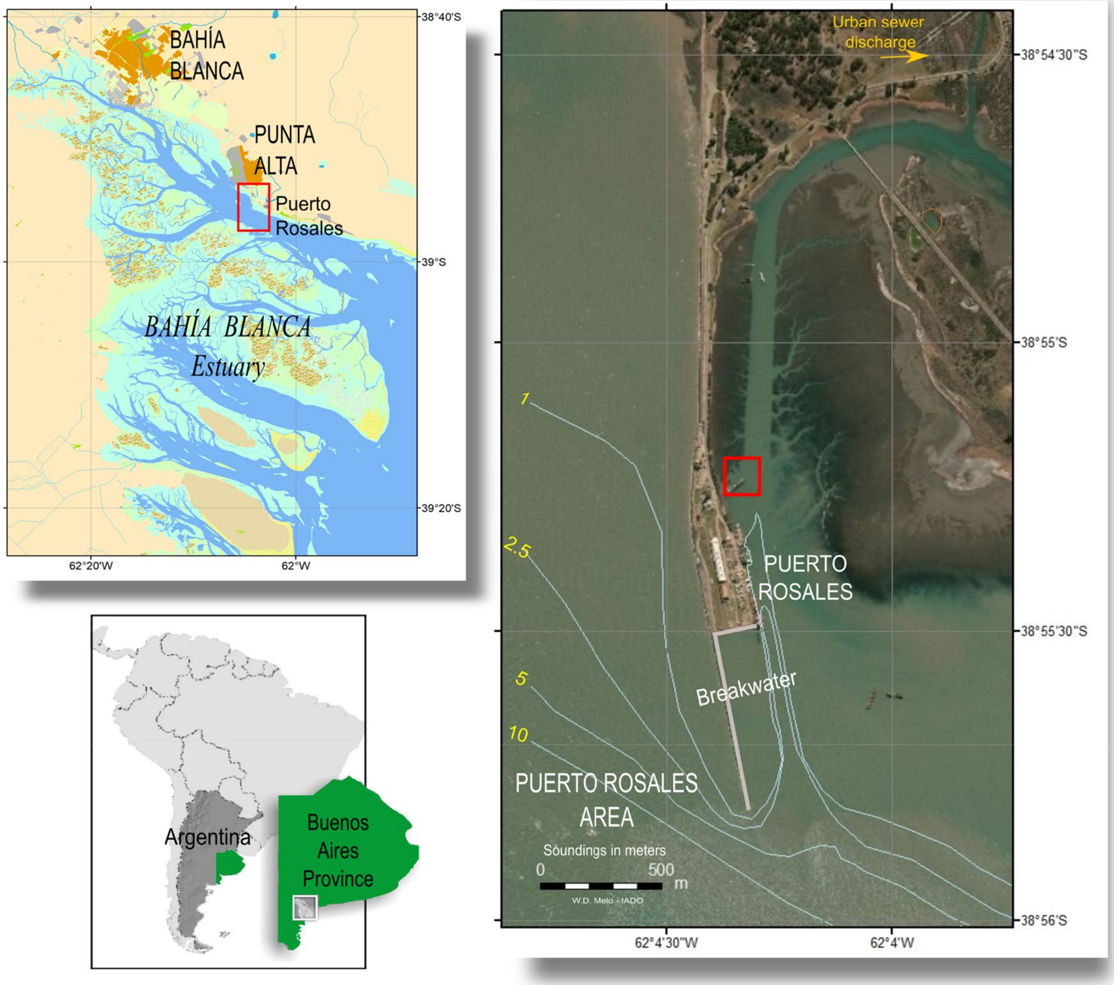

Fig. 1 Map of the Bahía Blanca estuary and location of the sampling site at Puerto Rosales (red square)

flats from PR twice a day. The local wind direction and velocity influences the tide amplitude, being the winds from the NW, NNW and SE the predominant in this area and the wind speed maximum in summer and minimum in autumn and winter (mean monthly values between 15.9 and 32 km h^{-1}) (Piccolo and Diez 2004). The strong winds from the SE cause the reach of the water to the supratidal area at high tide. However, when N and NW winds prevail, the plain is rarely covered with water and the area remains exposed to air. Thus, the intertidal zone at PR is flooded daily by the tide. In contrast, the supratidal zone is reached by water only eventually in syzygy conditions (twice a month) or during storm events. Because of its geographical location, the maritime climate in the area is composed by locally generated wind-waves being short wave-length with periods between 2 and 4 s; and by the swell that enters through the estuary mouth with periods that can reach values of 7–8 s offshore (Cuadrado et al. 2005).

PR tidal flat is composed by fine sediments that range in size from fine sand to mud (Gelós et al. 2004; Fernández et al. 2016). Finally, the macrobenthic community in this area is composed by several crabs like *Cyrtograpsus angulatus*, *C. altinamus*, *Neohelice granulata*, and the southernmost population *Leptuca uruguayensis* (Elías et al. 2007; Truchet et al. 2019). *N. granulata*, the burrowing crab, is the dominant key organism in this ecosystem closely related to the sediments, since it feeds on the associated detritus and burrows its caves (Angeletti et al. 2018a, b; Simonetti et al. 2018).

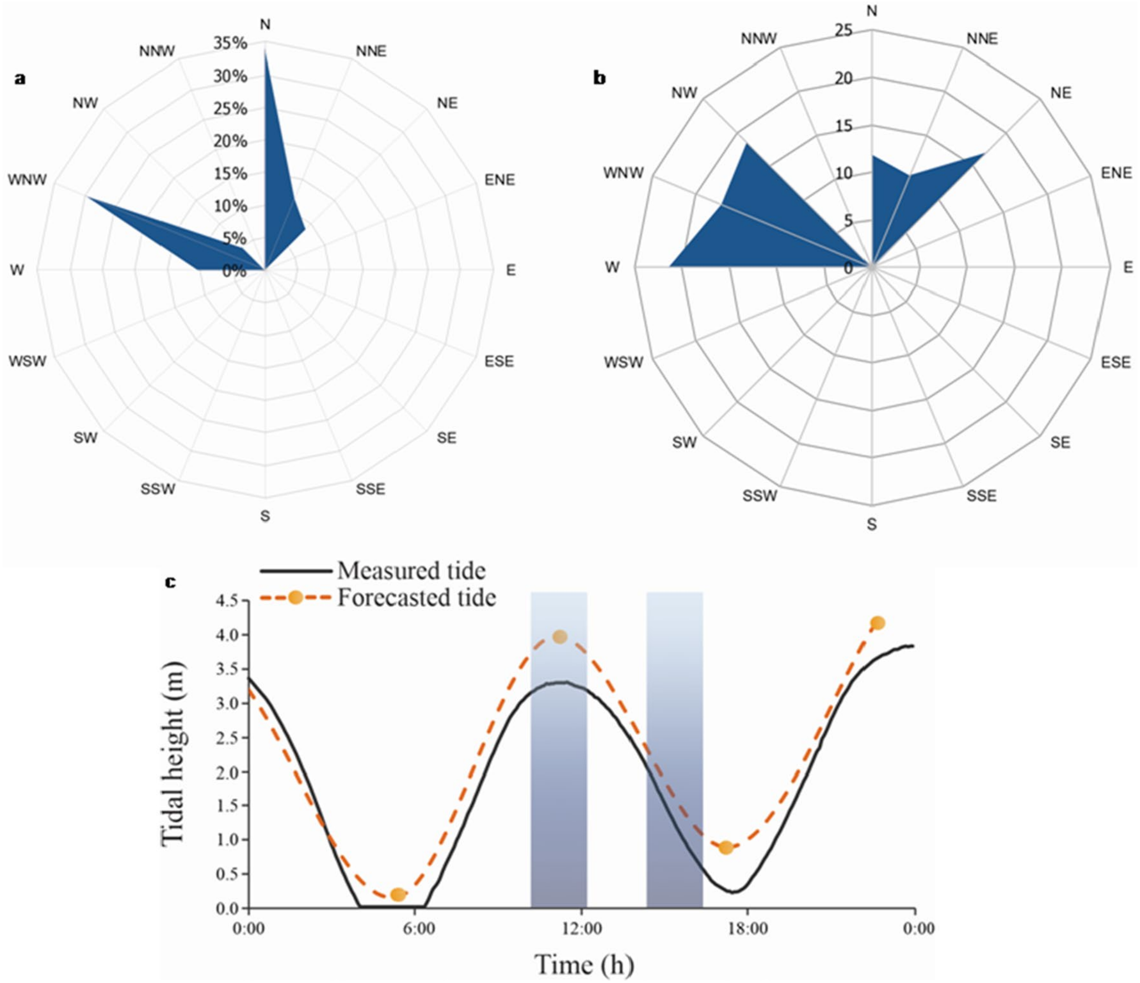

Fig. 2 **a** Wind direction, **b** wind intensity, **c** tidal height during field sampling in Puerto Rosales, Bahía Blanca estuary, Argentina. (The grey columns indicate the sampling moments)

Sampling

The field sampling was carried out on April 22, 2014, on the intertidal flat to the northeast side of the breakwater at PR (Fig. 1). All sediment samples were taken approximately during 2 h at the ebb tide for all the analysis (Fig. 2). For the determination of moisture content (%H), %OM, TCH and TPR, sediment samples were collected by duplicate with small cores of PVC (35 mm i.d.; 120 mm length) (Simpson et al. 2005) and for Chl*a* and Phaeo analysis were employed mini corers (11 mm i.d.; 40 mm length) avoiding exposure to light (Gómez et al. 2009). Sediment samples for trace metals evaluation were taken by duplicate using a plastic spoon and were stored in plastic bags. Simultaneously, temperature, pH and Eh in surface sediment were measured in situ using a Hanna Instruments probe (model HI991003). Surface water samples for the analysis of metals in the SPM were collected manually by duplicate during high tide (precisely one hour after crabs sampling began, Fig. 2), using 1.5 L polyethylene-terephthalate bottles. All the samples were immediately transported to the laboratory in refrigerated boxes.

Neohelice granulata organisms of both sexes were collected with a half-world net from a dock during 2 h, involving 1 h before and 1 h after the high tide (Fig. 2). Deadbeat or damaged crabs were discarded. Twenty adults' male crabs and twenty females in the intermolt stage with a carapace width (maximum distance between the two prominent lateral spines) between 25 and 35 mm and 20 and 30 mm, respectively, were selected. Crabs were transported to the laboratory in thermally isolated boxes with in situ water.

The meteorological parameters were carried out using Coastal Environmental Monitoring Station (EMAC) network (http://emac.iado-conicet.gob.ar/), which was implemented by researchers from the IADO-CONICET. Specifically, at this time, the data were obtained from a tidal gauge at Puerto Belgrano located 4 km toward the inner portion of the estuary from the study area.

Laboratory analysis

Cleaning procedures

Before using all the equipment for the collection, storage, filtration and processing of samples for trace metal determination, they were cleaned and immersed in diluted nitric acid (5%, HNO_3 suprapur, Merk) following internationally recommended protocols (APHA-AWWA-WEF 1998). Millipore HAWP 04700 filters (Mixed Cellulose Esters) (0.45 µm pore size) for metal analysis in the SPM were also soaked in 0.7% HNO_3 for 48 h, rinsed with deionized water, dried to constant weight in individual Petri dishes in an oven at 50 ± 5 °C during 56 h, and then weighed in an analytical balance (OHAUS, Adventurer TM).

For determination of Chl*a* and Phaeo all employed glass material was cleaned with non-ionic detergent and then washed with $MgCO_3$ 1% to remove acid residues. For TCH and TPR analysis, all plastic material was cleaned with HCl 10% (v/v) during 7 days and rinse with ultrapure water, while the glass material was muffled at 450 ± 50 °C during 4 h.

Sediments samples

The sediment samples for %H and %OM were oven-dried at 105 ± 5 °C to constant weight for 12–24 h. Moisture content was calculated from weight differences before and after drying samples (Santisteban et al. 2004) and organic matter content was calculated from weight loss by ignition after drying samples and ashing/combusting at 450 ± 50 °C for 1 h in a muffle furnace (Buhl-Mortensen 1996). The sediment samples for pigment determination were kept in the dark at − 20 °C until analysis. The Chl*a* and Phaeo content was analyzed following the method suggested by Gómez et al. (2009). Pigments were extracted with acetone 90% during 20–24 h, immersed in an ultrasonic bath at a controlled temperature and then refrigerated in the dark at − 4 °C for 24 h. The pigment concentrations were determined by spectrophotometry (Jenway 6715 UV–Vis) using the equations of Lorenzen (1967) and the results were expressed in µg g^{-1} sediment dry weight (dw). The TCH and TPR content in sediments were determined colorimetrically following the methods described by Venturini et al. (2012). The concentrations were expressed as glucose and bovine serum albumin (BSA) equivalents, respectively. Blanks for each analysis consist of sediments that had been pre-combusted at 450 °C for 4 h.

The sediment samples for trace metals analysis were oven-dried at 50 ± 5 °C until constant weight. Before grounding, debris and biota fragments were removed from the dried sediments to be finally sieved to obtain the smallest fraction (FF < 63 µm). Two subsamples (about 0.5 g) of FF sediments were taken to determine total metal concentrations (Cd, Cu, Pb, Ni, Zn, Cr, Mn, and Fe) using the method described by Marcovecchio and Ferrer (2005). It includes acid digestion with a mixture $HNO_3/HClO_4$ (5:1) at 110 ± 10 °C in a glycerin bath on a hotplate to obtain an extract of about 1 mL. Finally, each extract was filled with 0.7% HNO_3 up to 10.00 mL. The same digestion procedure without sample was performed to act as blanks.

For particle size determination, sediments were treated with hydrogen peroxide 35% (130 Vol.) during 48 h at room temperature to remove the organic matter content (wet oxidation). Then, the samples were placed on a hotplate (50 ± 5 °C) to accelerate the elimination process, and the grain size was determined using a Malvern Masterziser 2000 based on the principle of laser dispersion.

Water samples

Water samples were vacuum filtered (750 mm de Hg) and the filters with the retained material (SPM) were dried at 50 ± 5 °C to a constant weight and then were weighed. Subsequently, trace metal determination was done according to the methodology of Marcovecchio and Ferrer (2005) described above. The same digestion procedure was performed for filters without particles to act as blanks.

Trace metal quantification

All the fractions (sediments FF and SPM) were analyzed by duplicate to ensure the reproducibility of the method using ICP-OES Optima 2100 DV Perkin Elmer with a Cross-Flow nebulizer. For the analytical quality control, reagent blanks, certified reference materials and analytical grade reagents were used. The recovery percentages for all the trace metals in CRM (Certified Reference Material No. 2. NIES, Japan, Pond Sediments) were higher than 90%. The method detection limit (MDL) for each metal was calculated by performing the complete analytical procedure on blank replicates (20). The MDL was calculated by multiplying the standard deviation of these replicate (n) measures by the Student's t value at the 99% confidence level (at $n - 1$ degrees of freedom) (Federal Register 1984). The MDL for Cd, Cu, Pb, Zn, Ni and Cr, Mn and Fe in sediments was 0.003, 0.062, 0.094, 0.106, 0.011, 0.010, 0.045 and 0.614 mg L^{-1}, respectively. The MDL for Cd, Cu, Pb, Zn, Ni and Cr, Mn and Fe in SPM was 0.003, 0.090, 0.040, 0.110, 0.050, 0.050, 0.230 and 3.00 µg g^{-1} dw, respectively.

Biological samples

Once in the laboratory, for MTs analysis, males and females' crabs were anesthetized by freezing, weighed and their carapace width was measured with an electronic caliper (0.01 mm accuracy). Then, the organisms were carefully dissected to obtain the hepatopancreas, which was weighed and stored at − 80 °C. The condition index (CI) [ratio between total body wet weight (g) and cephalothorax width (cm)] (Pereira et al. 2006) and the hepatosomatic index (HSI) (percentage of hepatopancreas wet weight to the total body wet weight) (Ferreira et al. 2006; Comoglio et al. 2008) of *N. granulata* were evaluated. Hepatopancreas tissues from three crabs were pooled according to sex and ice-cold homogenized (1:4) (0.5 M sucrose, 20 mM Tris–HCl (pH 8.6), 0.5 mM phenylmethanesulfonyl fluoride (PMSF) and 1 mM dithiothreitol (DTT)) in a Potter–Elvehjem glass/Teflon homogenizer. Homogenates were centrifuged at 30,000*g* for 30 min at 4 °C. Then, MTs content was evaluated following the spectrophotometric method described by Viarengo et al. (1997). The absorbance was read at 412 nm in a Jenway 6715 UV–Vis spectrophotometer and MTs concentration was quantified using reduced glutathione (GSH) as a reference standard. The amount of MTs was calculated based on cysteine content in the decapod *Eriocheir sinensis* (18 cysteine mol^{-1}, GenBank accession no. ADV31337.1; 59 aa) assuming a similar SH group content in *N. granulata* MTs, since the first one is a grapsid crab, phylogenetically related to the varunid ones. MTs assay was carried out by triplicate, blanks were performed without sample and the MTs concentration was reported as μg MT g^{-1} wet tissue (wt).

Indicators of sediment quality and trophic status

The contamination degree by trace metals and sediment quality was evaluated through the application of four indices, namely Enrichment Factor (EF), Geo-accumulation Index (I*geo*), Pollution Load Index (PLI) and the mean Probable Effect Level quotients (m-PEL-Q).

I*geo* and EF are calculated for individual elements and supply valuable data about the degree and extent of trace metals burden in the sediments compared with background values. The I*geo* is expressed as follows (Müller 1979):

$$I_{\mathrm{geo}} = \log_2\left(\frac{C_n}{1.5 \times B_n}\right), \tag{1}$$

where C_n is the measured concentration of the analyzed trace metal (n) in the sediment samples, B_n is the geochemical background concentration of the metal (n) and 1.5 is the background matrix correction factor due to lithogenic effects. The degree of sediment contamination was classified according to the scale of Muller (1981). The following equation was used to estimate the EF of metals in the study site (Ergin et al. 1991):

$$\mathrm{EF} = \frac{(\mathrm{M/Fe})_{\mathrm{sample}}}{(\mathrm{M/Fe})_{\mathrm{background}}}, \tag{2}$$

where $(\mathrm{M/Fe})_{\mathrm{sample}}$ is the ratio of metal concentration (μg g^{-1}dw) to Fe concentration (% dw) in the sediments sample and $(\mathrm{M/Fe})_{\mathrm{background}}$ is the corresponding ratio of the background. In this equation, Fe was employed as a conservative element to normalize the trace metals data. The EF is widely used to estimate the metal source (anthropogenic or natural) and the pollution degree (Selvaraj et al. 2004). Elements which are naturally derived have an EF value of nearly a unit, while metals of anthropogenic origin have EF values of several orders of magnitude. In this study, the categories given by Sutherland (2000) were considered.

On the other hand, PLI provides global information on sediment pollution. The PLI is calculated as follows (Tomlinson et al. 1980):

$$\mathrm{PLI} = \left(\mathrm{CF}_1 \times \mathrm{CF}_2 \times \mathrm{CF}_3 \ldots \mathrm{CF}_n\right)^{1/n}, \tag{3}$$

where CF is the concentration factor of the metal (n) concerning the background value in the sediment ($\mathrm{CF} = C_{\mathrm{metal}}/C_{\mathrm{background}}$) (Angulo 1996). The PLI indicates how many times the metal content in the sediments exceeds the background concentration. The PLI value of > 1 means polluted, whereas < 1 indicates no pollution (Barakat et al. 2012). Due to the absence of trace metals background concentration at the study area, average shale standards of trace metals described by Turekian and Wedepohl (1961) were taken as geochemical reference values in the present work.

Finally, to analyze possible biological effects of metals mixtures, *m*-PEL-Q (mean PEL quotient) was calculated following Long et al. (1998). All measured trace metals except Fe and Mn (no PEL values) were considered during the m-PEL-Q calculation.

$$\text{m-PEL-Q} = \sum_{i=1}^{n} \frac{(C_i/\mathrm{PEL}_i)}{n}, \tag{4}$$

where C_i is the sediment concentration of measured metal (i), PEL_i is the probable effects level values of metal (i) and n is the number of metals. PEL Sediment Quality Guideline indicates the metal concentrations in sediments above which adverse biological effects frequently occur (MacDonald et al. 2000). The value obtained from the application of the mean PEL quotient is associated with a certain probability of there being a toxic stress to the biota, being m-PEL-Q < 0.1: 8% probability of toxicity; 0.11–1.5: 21% probability of toxicity; 1.51–2.3: 49% probability of toxicity; and > 2.3: 73% probability of toxicity (Long et al. 2000). Consequently, four relative levels of priority (non toxic, slightly toxic, medium toxic and highly toxic) have been proposed.

The trophic status and organic matter quality were evaluated following Dell'Anno et al. (2002) classification according to protein and carbohydrate thresholds: hypertrophic (TPR > 4.0 mg g^{-1}; TCH > 7.0 mg g^{-1}; TPR/TCH > 1), eutrophic (TPR = 1.5–4.0 mg g^{-1}; TCH = 5.0–7.0 mg g^{-1}; TPR/TCH > 1) and meso-oligotrophic (TPR < 1.5 mg g^{-1}; TCH < 5.0 mg^{-1}; TPR/TCH < 1). To evaluate sediment quality, the Chl*a*/MO (mg g^{-1}) ratio was used and low values correspond to low photo-autotrophic capacity (Niell 1980; Moreno and Niell 2004). Besides, the Chl*a*/Phaeo ratio was used as an indicator of the physiological state of the microphytobenthos or as an indirect grazing index, being this ratio lower than unity when the community is declining.

Statistical evaluation

The statistical analysis was carried out using STATISTICA7.0 (StatSoft, Inc.). The average result for MTs, CI and HSI came from the different pools of female or male crabs. Statistical significance of compared results was analyzed by the one-way analysis of variance (one-way ANOVA; level of significance at $p < 0.05$). When necessary, data were log-transformed to satisfy the parametric assumptions. Data in the figures were not transformed. Finally, in the figures, tables or text, the error values represent the standard errors (SEs).

Results and discussion

Estuaries are dynamic environments due to the mixing of salt and fresh waters and the strong chemical and physical gradient which characterized them. In these areas, sediment quality plays a crucial role in marine life and biodiversity and two actions influence it in these environments: (1) the interaction between sediments and water dissolved components and (2) the sedimentation of the SPM.

Table 1 Intertidal sediment characteristics and environmental conditions of the sampling day in Puerto Rosales

Parameter	Intertidal sediment	Environmental conditions
pH	6.3	–
Eh	175	–
Temp (°C)	21.2	12.3 ± 0.2
Atmospheric pressure (Bar)	–	956.0 ± 38.3
%H	27.6 ± 2.0	76.0 ± 0.6
%OM	2.6 ± 0.3	–
Grain size	Clay: 17.65% Silt: 78.50% Sand: 3.85%	–

The physical and chemical characteristics of the PR intertidal sediments and the environmental temperature, pressure and humidity during the field sampling are summarized in Table 1. The temperature, pH and Eh values detected in the sediments were within the ranges reported for the area (Spetter et al. 2015). Moreover, pH value remained in the typical range values for estuarine sediments (5–7) (Caçador et al. 2004; Reddy and DeLaune 2008). Considering the Eh value registered (175 mV), the intertidal sediments would be reduced (Cronk and Fennessy 2001), in concordance with Reddy and DeLaune 2008), who argue that the Eh values between 0 and 200 mV represent anaerobic conditions of the sediment and a facultative microbial metabolism typical of wetlands in a transition zone. The air temperature was between the range reported for the area during the autumn season (in Southern Hemisphere) (Piccolo and Diez 2004).

The wind direction and speed; and tidal level recorded on the sampling day are shown in Fig. 2. N and WNW winds dominated most of the sampling (34.03 and 29.86%, respectively) (Fig. 2a). Mean wind speed was low and fluctuated between 10 and 21 km h^{-1} being the highest mean speed recorded from the W (10.42% occurrence) (Fig. 2b). These data agree with previous observation by Piccolo and Diez (2004) and Cuadrado et al. (2005), who reported that predominant wind direction in the area is from the NW and NNW. In the same way, the wind speed during the sampling day was within the range of the reported monthly mean speed for PR (Piccolo and Diez 2004). The comparison between the forecasted tide and the actual tide records showed substantial differences in tidal height, being the sea-level measurement lower in both cases, during high and low tide (Fig. 2c). Although the mean wind speed recorded was not high, this decrease in tidal height prediction was expected due to the N–WNW wind direction during the sampling day. Strong SE winds produced an increase of the tidal height prediction. In contrast, NW–NNW winds caused the opposite effect (Menéndez et al. 2012).

Estuaries are active biogeochemical regions, where organic matter inputs from a variety of sources undergo numerous transformations during the transfer from land to the ocean. The mean total OM content in the dry intertidal sediments of PR was 2.60 ± 0.30% (Table 1). This value is similar to the reported by Quintas et al. (2019) in the intertidal sediments (2.63 ± 0.30%) and by Spetter et al. (2015) in the supratidal sediments (2.82 ± 0.20%) at PR where, as in this sampling site, inhabits the burrowing crab *N. granulata,* but lower than the reported by Negrin et al. (2019) in the same port but in a salt marsh dominated by *Spartina alterniflora*. In addition , the %OM detected is comparable to the values determined in Cochin (India) estuarine sediments (0.38–4.80%) by Joseph et al. (2008). Several factors may be influencing the amount of OM in this intertidal flat; on one side, as was suggested by Middelburg and Herman (2007) the OM composition in estuaries is continuously changing, according to the modification of environmental variables, such as light penetration, salinity and tidal range and, in addition, it is also affected by the residence time of the water. In this sense, the periodic flooding of the PR tidal flat is directly related to the OM´s dissolution or mineralization. On the other, the proximity to the discharge channel of the untreated urban wastewater of the Punta Alta city (~60,000 inhabitants) is probably a significant source of organic matter. Moreover, organic matter in sediments is a crucial source of energy for benthic organisms and it has been observed that OM, together with trace metals in the sediment, can undergo modifications due to the activity of these organisms. In the present study, the bioturbation of sediments and the construction of burrows by *N. granulata* in the tidal flats of PR are factors that influence the amount of OM and the remobilization, speciation and distribution of trace metals (Botto et al. 2006; Fanjul et al. 2015; Duan et al. 2019; Andrade et al. 2019; He et al. 2019). At the same time, this species modifies the sediment dynamics, affecting its porosity and permeability throughout the destabilization of cohesive sediments (Escapa et al. 2008).

To know the biochemical composition of the OM in the sediments is a useful tool to understand the biogeochemical processes that influence the distribution and biodiversity of the benthic fauna in marine and estuarine environments (Incera et al. 2003). In this context, the TPR and TCH content evaluation is suitable to determine the trophic status, the origin and the age of sedimentary organic matter and the factors influencing their diagenesis (Dell'Anno et al. 2002; Cresson et al. 2012). High concentrations of TCH with low nutritional value for benthic organisms may represent degraded organic detritus (Joseph et al. 2008), whereas high TPR content, which is the primary source of nitrogen for consumers, may reflect an increased fresh productivity (Danovaro et al. 1999). In PR, the TPR concentrations ranged from 1.11 to 1.66 mg g^{-1} dw and TCH from 0.10 to 0.18 mg g^{-1} dw (Fig. 3) and accounted for 0.5 and 6.1% of the total organic carbon (TOC) of sediments (Fernández unpublished data). Therefore, comparing both components, TPR was the most abundant component of the OM. Moreover, in this case, organic nitrogen content (i.e., proteins) is not a limiting factor to deposit feeder organisms, as is mention by Fabiano et al. (1995) and Fanjul et al. (2015). The sediments from PR intertidal flat showed a high nutritional quality in terms of *N* contribution. In this sense, Fanjul et al. (2015) observed that the mounds generated by *N. granulata*, contains low net amounts of OM but are important in nutritional terms due to their relatively high content of organic nitrogen. The results of TPR and TCH in the intertidal sediments from PR are between the range of those reported by Cotano and Villate (2006) in the Mundaka estuary, Bay of Biscay. Considering American estuarine and coastal systems, the values of TPR and TCH detected were lower than the ones reported by García-Rodriguez et al. (2011) and Venturini et al. (2012) in sediments from Montevideo coast (Río de la Plata estuary); and by Laut et al. (2016, 2017) in two lagoons from Brazil. According to the protein and carbohydrate concentrations, the intertidal sediments from PR should be considered in the meso-oligotrophic category given by the classification proposed by Dell'Anno et al. (2002) (Fig. 3).

The study of pigment concentrations in the sediments is essential for evaluating productivity in an estuarial system. The concentration of Chl*a* and Phaeo in intertidal sediments of PR ranged from 5.41 to 8.57 μg g^{-1}dw and 5.87 to

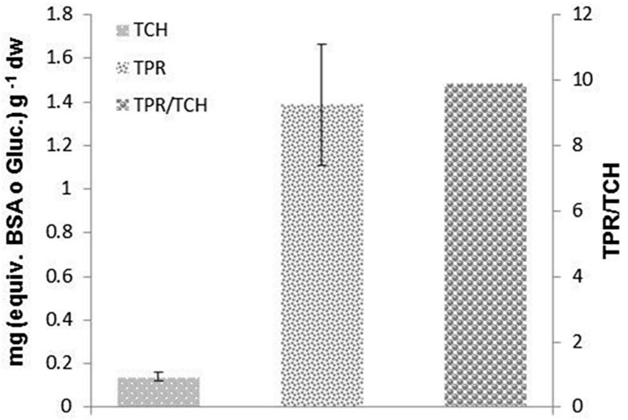

Fig. 3 Total proteins content (TRP; mg equiv. BSA g^{-1} dw), total carbohydrates content (TCH; mg equiv. Gluc. g^{-1} dw) and TPR/TCH ratio in the sediments from Puerto Rosales intertidal flat. Values are mean ± SE

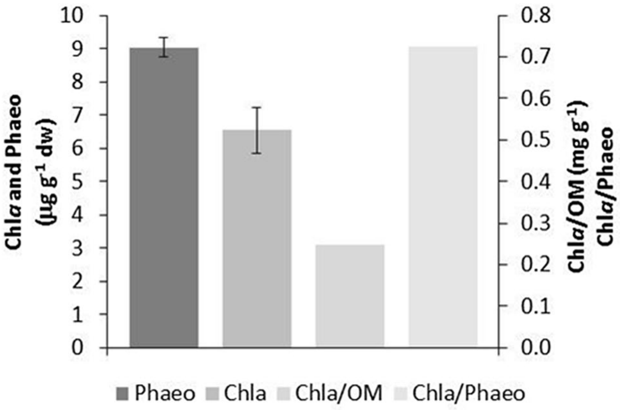

Fig. 4 Pigments content (μg g^{-1}dw): chlorophyll (Chl*a*) and phaeopigments (Phaeo) and Chl*a*/OM and Chl*a*/Phaeo ratios in the sediments from Puerto Rosales intertidal flat. Values are mean ± SE. *OM* organic matter

13.75 μg g^{-1} dw, respectively (Fig. 4). These results were similar to that found by Spetter et al. (2015) in the same tidal flat from PR and to the detected in others estuarine and coastal zones worldwide (de Brouwer et al. 2003; Moreno and Niell, 2004; Lessen 2006; Albano et al. 2013; Bergamino et al. 2018).

Coastal and estuarine ecosystems are hot spots of environmental variability, biogeochemical transformations, and biological interactions, where dynamic exchanges of energy, mass, or nutrients occur between the benthic and pelagic compartment via diverse pathways. The structure and function of these systems are strongly affected by anthropogenic pressures and seasonal variability; however, the exchange of inorganic nutrients and organic matter is not yet fully known between the sediments and the water column (Griffiths et al. 2017; Goméz-Ramírez Eddy et al. 2019). Thus, the distribution of contaminants between dissolved and SPM fractions and sediments is continuously modified in these coastal areas (Paucot and Wollast 1997; De Souza Machado et al. 2016). Changes in the environmental conditions influence the release and suspension of contaminants from the sediments, causing their redistribution between phases (Wang et al. 2016). Among all the factors, the salinity is strongly involved in the subdivision of contaminants among sediments, overlying, and interstitial water (McComb et al. 2014). In this work, the trace metals in the intertidal sediments' FF and in the SPM from PR were analyzed. All the trace metals evaluated, except Cd in the SPM, were above the detection limit of the method (Table 2). The average concentrations of trace metals in both components revealed the following order: Fe > Mn > Zn > Cu > Cr > Ni > Pb > Cd. Of the eight metals studied, Fe was found to be the most abundant metal present in the sediments and in the SPM. Although Cu and Fe concentrations tended to be higher in FF, the only metals that showed significant differences were Zn and Mn, with higher concentrations in the SPM. Similarly, high Zn concentrations in the SPM were found by Fernández Severini et al. (2017) in different sites of the BBE influenced by the sewage discharges of Bahía Blanca city and by the industrial discharges of the petrochemical refineries and fertilizer plants. Overall, the trace metals' concentrations in the SPM are variable over time and space. This variation is related to the proportion of sediments and seston (detritus and living material, predominantly phytoplankton) and the composition of the suspended particles, which are influenced by physical, chemical and biological processes in this highly dynamic environment (Fernández Severini et al. 2018). At the intertidal flat evaluated, a value of 3062 mgC m^{-3} of particulate organic matter was detected (Spetter unpublished data) due to the contribution of OM by the wastewater discharge. For this reason, the concentration of trace metals in the SPM would be expected to be higher than in the sediments. In this study, no major differences were found in the metal content between both compartments, probably due to physical factors, rather than biological ones. Moreover, this assumption is supported by the low abundance of phytoplankton (Chl*a* in the water column was not detected, Spetter unpublished data). Perillo and Piccolo (1991) and Piccolo et al. (2008) reported that in the BBE, tides and winds are the main sources of energy involved in the resuspension of fine sediments (silt and clay) from the tidal flats causing high system turbidity. However, by acting PR as an enclosed dock due to the presence of the breakwater, sediment deposition is promoted within this area, because it is protected from the wave's action and small tidal currents occur (Cuadrado et al. 2006). In consequence, in this study, it is assumed that the similarity in trace metals concentrations between fine sediments and SPM is due mainly to the excessive siltation rate. In addition, Arena et al. (2019) observed that due to the progressive decrease of the tidal range from the head to the widened mouth of the Principal Channel, the intensity of the tidal currents declines to cause a decrease in the SPM concentration from the head to the mouth of the estuary. In this sense, the mean SPM

Table 2 Summary of trace metal concentration (μg g^{-1}dw; Fe in mg g^{-1}dw) in the suspended particulate material (SPM) and fine sediments fraction (FF) from Puerto Rosales (PR)—Bahía Blanca estuary

	Cd	Cu	Zn	Ni	Cr	Pb	Mn	Fe
SPM-PR	< MDL	12.61 ± 1.53	49.1 ± 0.37	7.24 ± 0.02	10.77 ± 0.32	6.98 ± 0.79	398.15 ± 18.05	20.53 ± 795
FF-PR	0.090 ± 0.027	20.60 ± 3.93	33.10 ± 6.33	5.33 ± 0.56	7.29 ± 1.30	6.30 ± 0.73	261.88 ± 12.53	26.64 ± 3.30
TEL-SQGs[a]	0.68	18.70	124.00	15.90	52.30	30.24	nd	nd
PEL-SQGs[a]	4.21	108.20	271.00	42.80	160.40	112.18	nd	nd
RV	0.11[b,c]	33.00[b]	95.00[d]	56.10[c]	77.20[c]	19.00[d]	770[d]	41[b,d]

TEL, PEL-SQGs and RV comparison. (MDL: method detection limit; RV: recommended values of unpolluted sediments)

[a] Buchman (1999)

[b] GESAMP (1982)

[c] IAEA (1990)

[d] Salomons and Förstner (1984)

concentration detected on the sampling day was 93.60 mg L^{-1} at port of Ingeniero White, an inner port of the BBE, and 37.70 mg L^{-1} at PR (Fernández Severini unpublished data). Thus, under lower energy conditions, the cohesive material transported in suspension are subjected to deposition (Cuadrado et al. 2004).

It is well known that several forcings, like the tidal currents, waves, and associated winds and drainage, determine the hydrodynamic of intertidal flats (Le Hir et al. 2000). In this context, Cuadrado et al. (2006) reported that in the presence of local N–NW winds, the concentration of the suspended sediments in the intertidal flat from PR increases but to a lesser extent than when the wind direction is from the SE. This is attributed to the erosive action of locally generated waves with winds blowing from the SE that resuspend cohesive materials (silt and clay) from the extensive tidal flats neighboring PR. In the present study, during the field sampling, the N-WNW winds prevailed (Fig. 2a, b), therefore, probably another factor involved, but less important, in the similar trace metals content in both compartments' analyzed, could be the resuspension of fine sediments by tidal currents during the flood, as was observed by Cuadrado et al. (2006). They reported that with N–NW winds, the increase of the suspended sediments concentration is due to the sediment resuspension of the same tidal flat caused by the increased speeds of the tidal currents during the flood, because this area is not affected by the mentioned winds. Moreover, Cuadrado et al. (2005) recorded maximum currents intensities for the area between 0.30 and 0.40 m s^{-1} during the flood tide.

Naturally, waves generated by local winds will likely resuspend sediments. Cuadrado et al. (2013) observed that when NNW wind speed was lower than 40 km h^{-1}, as in this study, the wave heights were lower than 0.5 m. In a previous work carried out in the intertidal mudflat at Villa del Mar, a small town located near PR, Pratolongo et al. (2010) reported that short wind-waves (periods from 1 to 3 s) with heights never exceeding 0.2 m, were the major hydrodynamic forcing factor above the mudflats producing bottom shear stresses. Similarly, Perillo and Sequeira (1989) observed that small waves of about 0.05–0.1 m in height and periods of 1–3 s characterize the BBE tidal flats during high tide. Then, these authors mentioned that the continuous impinging of these very steep waves might be a critical factor in the sediment resuspension in the tidal flats during high tide. Thus, the local wind-waves action in the sediments resuspension above the intertidal flat evaluated should not be ruled out as another factor involved in the similar metal concentrations between FF sediments and SPM found in this study.

In addition, the burrowing crab *N. granulata*, during the construction of its burrows produces an intense sediment destabilization within the intertidal flat and exposes the removed material to the action of tidal currents and waves, increasing the mobilization of soft sediments toward the water column (Murray et al. 2002; Zapperi et al., 2016; Angeletti et al. 2018a, b). On the other hand, the burrows act as traps of sediment and organic matter that arrives with the tide, since the deposition of particles into the burrows is favored by the disturbance (turbulence) that generates velocities in the vertical direction (Hetsroni 1989; Iribarne et al. 2000). However, more studies are necessary about the geochemical partition of trace metals between the benthic–pelagic compartments in PR as well as in the BBE. Regarding the SPM, trace metals values detected in this study are within the lower concentrations detected by other studies in the same estuary (Fernández Severini et al. 2009, 2013, 2018). Considering the sediments, the metals detected in the FF are into the range reported in previous works in the sediments from the Principal Channel, near PR (Marcovecchio and Ferrer 2005), from the access channel (Pizani 2008) and from a supratidal flat colonized by microbial mats (Spetter et al. 2015). However, it should be noted that in these works the analysis was performed in the total sediments and not in the FF. Buzzi et al. (personal communication) reported lower concentrations of trace metals in fine sediments but higher in SPM from Puerto Cuatreros, a small artisanal/recreational fishery port in the inner area of BBE. It is probable that the high %OM together with the higher clays sediment content in PR compared with the inner zone favors the complexation to trace metals (Burdige 2011; Negrin et al. 2013), retaining these contaminants and preventing their release to the water column. Additionally, OM influences the speciation, bioavailability and transport of trace metals in sediments (Bianchi 2007; Blankson and Klerks 2017); and its interaction through the formation of simple or mixed complexes affects their environmental behaviors in sediments (Wallschlager et al. 1998; Szkokan-Emilson et al. 2014). In addition, trace metals concentrations in the intertidal sediments did not exceed the recommended values by GESAMP (1982), IAEA (1990) and Salomons and Förstner (1984) for unpolluted sediments (Table 2). Moreover, the present results are below the trace metals concentrations thresholds given by Villaescusa-Celaya et al. (2000), Buggy and Tobin (2008) and Yang et al. (2012) for contaminated environments. Finally, considering the Sediment Quality Guidelines (SQGs) (MacDonald et al. 1996), only Cu concentration in the FF of sediments from PR is occasionally associated with adverse biological effects, since its value is above the marine threshold effect level (TEL) but below the probable effect level (PEL) (Table 2).

Metallothioneins are biomarkers of low molecular weight that belong to the class of cytoplasmic stress-defense proteins and particularly respond to environmental trace metal exposure. Its induction is regarded as an early warning signal of environmental contamination by trace metals before longer-term or higher-level changes are detected, and in this way, they provide crucial information on the potential impact of metals on the organisms' health (Lam and Gray 2003). Even though several abiotic and physiological variables may induce MTs concentration in aquatic invertebrate tissues, the level of induction is usually lower than that caused by metals (Kägi 1993). Pavičić et al. (2006) reported that the hepatopancreas of estuarine invertebrates is the more appropriate tissue for biomonitoring purposes. Moreover, this organ plays an important role in the uptake, detoxification and excretion of metals in crustaceans (Barrento et al. 2009; Rainbow and Luoma 2011). In this study the analysis of MTs induction was performed in the hepatopancreas of *N. granulata*, but unfortunately, there are not many works about MTs in crustaceans and even less those evaluating the differences between females and males. The concentrations of MTs in both sexes were lower than the reported by Buzzi and Marcovecchio (2016) for the same crabs in the inner area from the BBE. Comparing with studies carried out in crabs from other coastal areas of the world, the levels of MTs in males were similar to those reported by Wedderburn et al. (1998) in *Carcinus maenas* from different sites (estuaries and coastal zones) around the southwest United Kingdom subjected to different kind of human pressures. On the other hand, the adaptation to chronic pollution has also been considered a point of disagreement between organisms from a contaminated area that barely can synthesize MTs and organisms from a clean site that synthesize high levels of these biomarkers (Ballan-Dufrançais et al. 2001). Sex-related differences in MTs concentrations in *N. granulata* from PR were observed, being in adult females significantly higher than in male crabs ($p < 0.005$) (399.71 ± 24.83 µg MT g^{-1} wt vs 228.07 ± 19.96 µg MT g^{-1} wt, respectively), specifically 1.8 times higher in females than in males. This behavior was observed in *N. granulata* from the inner area of the BBE (Buzzi and Marcovecchio 2016), in *Pachygrapsus marmoratus* and *C. maenas* from Gironde estuary, France (Legras et al. 2000; Mouneyrac et al. 2001) and in *Callinectes* sp. from southeastern Brazil (Lavradas et al. 2014). Additionally, the levels of MT in both genders of *P. marmoratus* and *C. maenas* were higher than those detected in this research. The reason of the differences between sexes is still unknown, but it might be related to biological factors, such as the development, molting, gonadal status, protein metabolism and/or trace metal bioaccumulation (Overnell et al. 1987; Hamza-Chaffai et al. 1999; Legras et al. 2000; Mouneyrac et al. 2001; Lavradas et al. 2014). Though according to the SQGs, Cu is the only metal that is occasionally associated with adverse effects on the biota, more studies under controlled conditions are necessary, since the eight metals evaluated were considered jointly and not each separately, in the MTs induction. Considering that MTs are early warning signals of metal contamination, it would be recommended to pay attention to the Cu input in the study area. However, it has been reported that MTs' concentrations vary among different species and in response to each metal exposure and might also vary considerably for the same species depending on seasonality and or the environmental conditions (Amiard et al. 2006).

Finally, biological variables concerning the organisms and organ health status were estimated. In this sense, the CI and the HSI of *N. granulata* showed highly significant differences between females and males crabs ($p < 0.005$). The CI in males was higher (0.48 ± 0.02) than in females (0.30 ± 0.01), while the HSI was higher in females crabs (3.45 ± 0.24%) than in males (2.07 ± 0.14%). The CI is an organism-level response influenced by the food quality, pathogens and toxic contaminants' exposure, while the HSI gives information about the hepatopancreas status. In this sense, its change in size due to environmental factors occurs faster than changes in weight or length at the organism level (Ferreira et al. 2006). Thus, the highest HSI in female crabs could be linked to the levels of MTs detected, which would cause an enlarged organ due to the increased detoxification activity, as reported in other organisms (Adams et al. 1989). On the contrary, Sabatini et al. (2009) observed a decrease in the HSI of *N. granulata* exposed to Cu under low salinities. Anyway, López Greco and Rodríguez (1999) reported the HSI increase in the same crab species by March, just after the reproductive season, probably related to the accumulation of reserves before molting during April and May.

Sediment quality and trophic status

Several indices have been developed in the last decade to assess trace metal contamination of sediments and/or its ecological risk (Spencer and Macleod 2002; Caeiro et al. 2005). The I*geo* (Eq. 1) for each of the eight trace metals analyzed in the sediments FF from PR was < 0, thus according to Muller (1981) classification, the intertidal sediments from this flat are unpolluted by these metals (class 0). In the same way the EF (Eq. 2) was < 1 for each trace metal evaluated, specifically for each element was: Cu (0.81) > Zn (0.61) > Pb (0.56) ≥ Mn (0.55) > Cd (0.53) > Cr and Ni (0.14). According to Sutherland (2000), these EF values indicate background concentration; thus the trace metals detected at PR intertidal flat are naturally derived. Considering the global metal contamination, the PLI (Eq. 3) was 0.25; indicating as well as the I*geo* and the EF that the intertidal sediments from PR are unpolluted by trace metals. Finally, to integrate metal-specific SQG values and the possible effect of trace metals mixtures on biota, the m-PEL-Q (Eq. 4) was calculated, resulting in 0.09 for PR surface sediments, indicating a 8% probability of being toxic to biota. Thus, according to this result, the intertidal sediments from PR are categorized as non toxic.

The evaluation of phaeopigments content is especially important in sediments, because they are degradation products from different sources. They are mostly produced in situ by the cells death of the microphytobenthos, the grazing and the great contribution of detritic material present in the column water, and, therefore, of detritic chlorophyll. The fact that Chl*a*/Phaeo < 1 (Fig. 4) indicated a trend to the predominance of more degraded OM, a low contribution of microphytobenthos and a high sedimentation of partially degraded organic detritus associated with different sources of natural and anthropogenic disturbances (García-Rodriguez et al. 2011; Cheriyan et al. 2015). In addition, this trend has been reported in eutrophic aquatic ecosystems under anthropogenic pressures (Venturini et al. 2012; Albano et al. 2013; Cheriyan et al. 2015).

The TPR/TCH ratio is used as an index to determine the origin and age of the materials present in sediment and to distinguish the presence of materials recently formed (Cauwet 1978; Danovaro et al. 1993; Cividanes et al. 2002). Values TPR/TCH < 1 has been ascribed to old detritic material, i.e., OM in more advances degraded state and with lower nutritional value for benthic organisms, whereas TPR/TCH > 1 has been associated to fresh detritic material or newly generated (Dell'Anno et al. 2002; Cividanes et al. 2002; Haldlich et al. 2018). Since proteins are more readily utilized than carbohydrates and are rapidly bound into refractory compounds, low TPR/TCH ratios suggest the presence of age OM (Cividanes et al 2002). In the studied site, the TPR/TCH ratio showed a value of 9.87 (Fig. 3). The high protein content indicates OM newly generated, but also evidencing the contribution of the sewage discharge and the accumulation of phytodetritus (partially degraded material derived from primary production in the water column) that may contribute to the prevalence of TPR associated with refractory material, as evidenced by the Chl*a*/MO < 1. This added to the estuary´s hypoxic conditions; allow the material of the water column to reach the sediments with a lower degradation level (Cotano and Villate 2006). In addition, the ratio of chlorophyll *a* to the organic matter in surface sediments was 0.25 mg g^{-1} indicating low sediment quality and OM highly degraded (Fig. 4). According to Dell'Anno et al. (2002), a TPR/TCH > 1 ratio indicates that the PR intertidal flat is in a eutrophic state. Since we did not get the same trophic classification using the threshold values for TPR, TCH and TPR/TCH ratio, we decided to use the "precaution principle" whose classification is the least favorable i.e., the surface sediment from PR would be classified as eutrophic. Moreover, in the water column of the study site, Spetter (personal communication) has evidenced for one studied year a moderated eutrophic state associated to moderated Chl*a*, dissolved inorganic nitrogen and phosphorous concentrations.

Conclusion

This study aimed to provide a snapshot of the current situation regarding inorganic contamination and trophic status of an intertidal flat from Puerto Rosales. The sediment characteristics were thoroughly evaluated for the enrichment of trace metal, the ecological risks regarding SQG and the trophic status with respect to sedimentary organic matter and its biochemical composition. Trace metal concentrations in fine sediments of the intertidal and SPM, except Zn and Mn, were similar probably due to the owner system´s characteristics. Regarding fine sediments, metals values were lower than those recommended for uncontaminated ones and according to the analysis of different indices, the sediments of the intertidal evaluated at Puerto Rosales showed not to be contaminated by these elements. Considering the possible effects of trace metals on organisms and comparing with the values given by TEL/PEL SQG, the concentrations of the trace metals analyzed, except Cu are rarely associated with adverse biological effects. In addition, the use of metallothioneins as biomarkers of metallic pollution in *Neohelice granulata* can be considered a relatively simple tool to complement current and classic monitoring methodologies. However, the inclusion of the metallothioneins analysis in *N. granulata* as a potential biomarker in biomonitoring programs requires some precaution, since it might vary according to the sex of the organisms. Even though there is no metallic contamination, the TPR/TCH ~ 10 ratio would be evidencing the wastewater discharge influence from Punta Alta city in this tidal flat. Moreover, this relationship would indicate that this intertidal area is in a eutrophic state.

Finally, although the tidal flat is affected by the sewage discharge, the multi-combined proxies and the different indices applied, allow concluding that the intertidal site evaluated at Puerto Rosales is not contaminated by trace metals and its trophic status is according to its features (organically enriched sediments). Moreover, the measured variables are within the international environmental values for uncontaminated sediments.

This information is a baseline analysis for future integrated studies related to quality and trophic status of tidal flats and a useful tool to generate strategies for the management and monitoring programs in this and other similar aquatic systems.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

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