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Biochemical and behavioral responses in the estuarine polychaete *Perinereis gualpensis* (Nereididae) after *in situ* exposure to polluted sediments

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ABSTRACT

In situ translocation experiments are advantageous relative to traditional laboratory experiments, particularly for understanding the bioavailability of trace metals like mercury (Hg). Individuals of the polychaete *Perinereis gualpensis* were translocated from a reference site (Raqui estuary, Chile) to an estuarine site with significant sediment Hg concentrations (Lenga estuary: 1.78–9.89 mg/kg). Individuals were exposed in polluted and non-polluted sediments for 21 days and sampled every 7 days with cages deployed at three different depths. Tissue Hg concentrations were measured in conjunction with oxidative stress responses. Translocated polychaetes rapidly accumulated Hg. Glutathione S-transferase (GST) activities measured from posterior body regions were 2-fold higher than control activities after 21 days of exposure. Other antioxidant measures were idiosyncratic. Distinct burrowing behavior differences were observed; control polychaetes exhibited more homogenous vertical distributions, whereas in Lenga, worms tended to remain in upper layers. These studies demonstrate that under natural conditions, Hg is highly bioavailable to polychaetes affecting both biochemical and behavioral responses after relatively short-term exposure.

1. Introduction

Estuaries are among the most ecologically productive and economically important ecosystems. However human activities often result in considerable contamination in estuaries, and sediments are often the ultimate sink for these contaminants. Thus, the assessment of sediment contamination is of crucial importance for the management of estuarine ecosystems (Moreira et al., 2005; Amiard-Triquet and Rainbow, 2009).

In dynamic systems such as estuaries, the fluctuations of abiotic factors (e.g., salinity and temperature) and the particular biogeochemistry in their sediments can strongly affect the bioavailability of some pollutants like metals (Moreira et al., 2006). The extrapolation of results obtained using laboratory assays to estimate contaminant risks in estuarine environments generally lack the integration of the natural fluctuating environmental conditions, creating unrealistic conditions (Burton et al., 2005). As environmental assessment tools, *in situ* approaches offer key advantages over traditional laboratory tests and field sampling, since they allow organisms or assemblages to be exposed in the field, which could be particularly important for highly dynamic ecosystems such as estuaries (Moreira et al., 2006; Crane et al., 2007). In this respect, *in situ* tests with caged organisms in sediments can reduce artifacts related to the collection, storage, and manipulation of sediment samples, which can ultimately affect bioavailability and toxicity of contaminated sediments (Moreira et al., 2005; Ramos-Gómez et al., 2011; Rosen and Miller, 2011). The use of benthic species is ideal for *in situ* studies due to the abundance, low mobility and key roles within the local food web and in sediment processes (Baird et al., 2007).

The nereid polychaetes including *Perinereis gualpensis* (Jeldes, 1963) posses several advantageous features that may make it ideal for use in environmental studies: (1) high densities in estuaries (Jaramillo et al., 2001), (2) sedentary life-styles, (3) important roles in food webs and biogenic sediment processes (Scaps, 2002), (4) effective biomonitors for heavy metal bioaccumulation and (5) measurable toxicological responses in estuaries under different anthropogenic pressure (Bertrán et al., 2001; Díaz-Jaramillo et al., 2010;

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Díaz-Jaramillo et al., 2011a). Thus, *P. gualpensis* represents a good choice for *in situ* caging experiment in estuarine polluted sediments.

Estuaries in south central Chile range from near pristine to highly impacted by industrial and urban activities. The Lenga estuary (36°46′15′S; 73°10′06″W) is impacted by historic mercury (Hg) pollution from chloro-alkali industries, which utilized Hg cells in their processes from the 1970s until 1990s (Díaz et al., 2001). Due to the high persistence of Hg (Tchounwou et al., 2003), it is still common to find high Hg levels in sediments (0.4–12.6 mg/kg d.w.) and biota (0.73–2.41 mg/kg d.w.), that in some sites are above threshold effect levels (TELs) and probable effect levels (PELs) for some marine sediments quality guidelines (CCME, 2001; Díaz-Jaramillo et al., 2011a, 2013). Therefore, Hg pollution in Lenga sediments represents an ideal scenario to evaluate the suitability of *P. gualpensis* as an effective assay tool to examine Hg biovailability and effects from sediment to biota.

Effects of Hg in biota range from subtle biochemical and behavioral effects to lethality. The biochemical/antioxidant responses such as reduced glutathione (GSH), the enzyme glutathione-S-transferase (GST), total antioxidant capacity against peroxyl radical (ACAP), and lipid oxidative damage (TBARS), between others, have been applied as biomarkers of contaminant exposure in polychaetes (Durou et al., 2007; Monserrat et al., 2007; Díaz-Jaramillo et al., 2010; Díaz-Jaramillo et al., 2011a,b). These endpoints are valuable for assessing potential risks posed by metal contaminated sediments and can be applied to organisms exposed *in situ* (Moreira et al., 2006).

In addition, behavioral responses to contaminants can also profoundly affect an organism's ability to feed and evade predation. Burrowing is an important behavior in sediment dwelling organisms, both in terms of life history attributes and in creating physicochemical changes (e.g. redox conditions) in sediments that not only can affect the mobility/bioavailability of contaminants but also habitat suitability for other species (Moreira et al., 2005; Buffet et al., 2011). As Hg exposure is known to alter neurologic and neuromuscular functions (Vieira et al., 2009), the burial capacity in *P. gualpensis*, (described as the ability to build deeper tubes in the sediment), is considered to be an ecologically relevant measure of Hg effects.

Thus, the objectives of this study were to use *P. gualpensis* to determine: (1) Hg bioavailability from Lenga sediments; (2) the temporal dynamics of Hg bioaccumulation over a three week exposure duration (3) biochemical responses to Hg bioaccumulation (GST activity, total antioxidant capacity, levels of GSH, TBARS); and (4) compare behavioral responses such as burial capacity in resident *P. gualpensis vs.* individuals transplanted from a reference site. Together, these studies evaluate the suitability of this species as test organism for *in situ* sediment evaluations.

2. Material and methods

2.1. Study sites

Lenga is a small estuary (3.2 km^2) located in San Vicente Bay, Biobío, Chile (36°45′ S; 73°10′W). It is within an important chemical industrial complex, including an oil refinery, steel and chemical industries. Raqui estuary (37°14′S; 73°26′W) is a small coastal-type basin estuary with an important salt marsh area. It receives minimal anthropogenic pressure and it is relatively similar and close to the Lenga estuary, making it an appropriate reference site for comparison (Díaz-Jaramillo et al., 2011a). Raqui estuary has been previously used as reference site in studies of biomarker expression in polychaetes and their sediments have been characterized with low levels of Hg and polycyclic aromatics hydrocarbons (Díaz-Jaramillo et al., 2011a,b, in press). Two sampling sites, Lenga (36°46′11.48″S; 73°10′06.29″W) and Raqui (37°14′32.59″S; 73°26′21.86″W), were established in

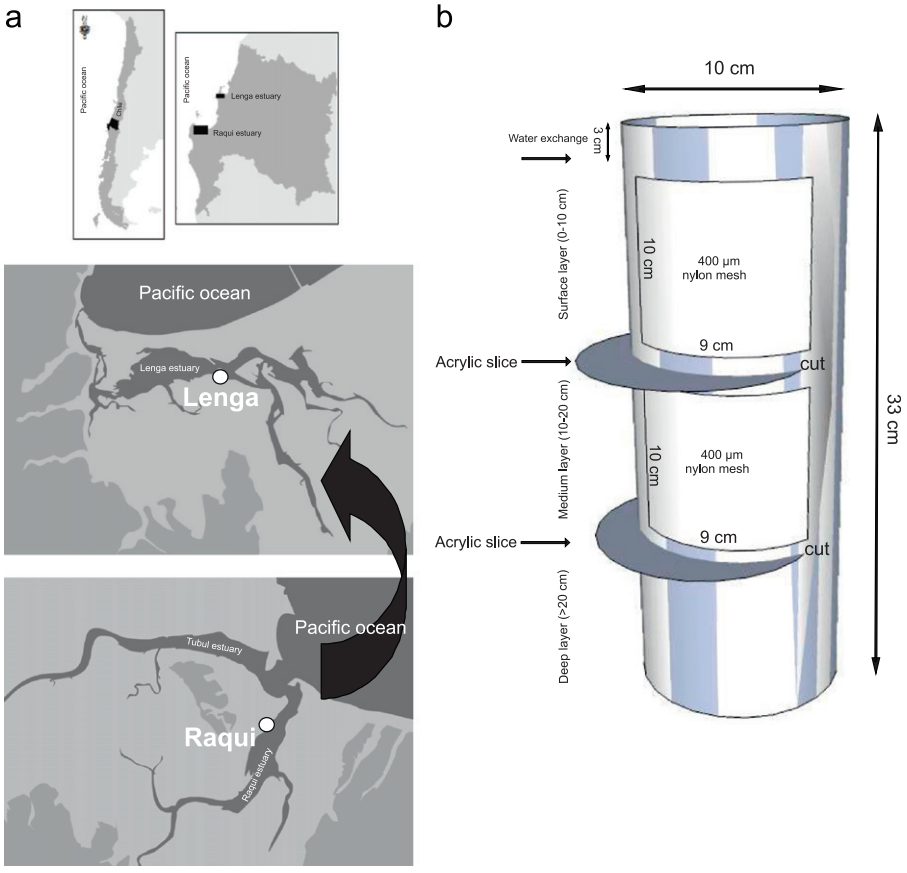

Fig. 1. (a) Experiment locations for *Perinereis gualpensis in situ* sediment exposures from Lenga and Raqui estuaries, 2009 and (b) design of modified assay chamber for *in situ* sediment test assay (modified from Moreira et al., 2005).

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estuarine areas according with previous characterization and determination of similar physicochemical water and sediment characteristics (Fig. 1a). *In situ* exposures were conducted at the end of the summer season (March–April, 2009) because water physical–chemical variables are similar between sites and more stable than during the rainy season when flow and salinity conditions are more highly variable.

2.2. Sediment characterization

Sediment samples were collected in sampling sites at low tide using a plastic PVC corer (33 cm × 10 cm) sliced in three 10 cm depth layers (surface, medium and deep) sub-sampled for different sediment attributes (see below). Sediment cores were kept at −20 °C until analysis ($n=2$). Additionally *in situ* measurements of redox sediment condition were performed in the same PVC corer with holes in each depth layer (0–10, 10–20, and 20–30 cm) using an ORP probe (WTW, Germany).

2.2.1. Organic matter and sediment size

Total organic matter content (OM) was determined by the ash free dry weight (AFDW) method by ashing the samples in a furnace for 4 h at 550 °C. Sediment size was assessed by using standard grids of 4.0 and 1.0 phi in order to separate the principal textural fractions: mud, sand and gravel. The coarse fraction (sand and gravel) was analyzed in a digital decantation tube (Emery type) and the fine sediment fraction (mud and clay) by microparticle analyzer (ELZONE®282 PC, Particle data Inc., USA). The data were represented on a phi (ϕ) logarithmic scale. For mean size classification and selection, data obtained were grouped according to Gray (1981).

2.2.2. Mercury in sediments

Total Hg in sediment, was determined by cold vapor atomic absorbance spectrometry (CVAAS; Perkin–Elmer FIMS-400, Perkin–Elmer Corp., USA). The analytical quality control of Hg determinations was performed by using certified reference materials PACS-2 from National Research Council Canada (NCR, Canada), performed in triplicate. Total sediment Hg was determined in each deep layer from freeze-dried sediments ($n=2$, per layer) following the modified EPA method 245.5 (USEPA, 1991), with recoveries averaging 113%.

2.3. Assay chamber

Assay chambers were modifications of the chambers described by Moreira et al. (2005) for *in situ* assays with *Hediste diversicolor*, a related nereid specie close to *P. gualpensis*. Each chamber consisted of a 33 cm long and 10 cm inner diameter PVC tube (0.3 cm thickness) with open ends (slimmed bottom edge), and two 400 μm nylon mesh rectangular windows (9 cm × 10 cm) to prevent the escape of the test organism and allow the proper exchange of interstitial waters. Nylon mesh (400 μm) squares were secured to the top of each chamber to allow the overlying water exchange while preventing the escape and predation by non target organisms.

Additionally, two thin slits (0.5 cm wide; 9 cm long) allowed us to further divide the chambers by depth layers (0–10; 10–20; >20) (Fig. 1b). These slits were taped during the experiment to avoid the escape of test organism. When the chambers were removed/harvested from the sediments at different exposure times, tape was removed and chambers were divided by introducing acrylic slices into the slits (Fig. 1b).

2.4. In situ exposure

Adult polychaetes (1.8–2.0 mm L3 length; L3=prostomium+peristomium+first segment) were collected from a reference site (Raqui) at low tide using a shovel and manually extracted from the sediment. Initial storage of the polychaetes in cold site water (4°–5°) prevented their entanglement (and subsequent damage) and allowed us to select only undamaged and apparently healthy individuals. Prior to field deployment, water temperature was increased gradually to the field temperature (15 °C), to minimize thermal stress. The collection and translocation of worms from the reference site were carried out on the same day.

Multiple chambers ($n=9$ per site) were deployed approximately 1 m apart in parallel in the sediment from both estuarine sites at low tide. Chambers were pushed into the sediment to a depth of about 30 cm, with 3 cm exposed to the overlying water. Twenty five individuals were introduced onto the surface of each chamber. This density was chosen match the observed density of individuals from the reference site. The assay chambers were then covered with mesh. On days 7, 14, and 21, 3 chambers were retrieved from the sediment during low tide, and the polychaetes were carefully extracted from the sediment for later analysis. During the experiment, *in situ* water measures of salinity, dissolved oxygen concentration, temperature and pH were taken every 7 days with a multi-parameter probe (YSI professional plus, YSI Inc., USA).

2.5. Perinereis gualpensis Hg levels and biochemical responses

Hg tissue levels and biochemical responses were performed in individuals of *P. gualpensis* at each exposure period (7, 14, and 21 days) in addition to reference site individuals collected prior to the initiation of the experiment (T0). For tissue Hg analysis, individuals were transported to the laboratory and kept in filtered seawater (adjusted to field salinity) for 24 h in order to eliminate sediment particles and gut content. Due to the relatively small size of *P. gualpensis*, individuals were pooled ($n=5$ per replicate), kept frozen (−20 °C) and then freeze dried for subsequent Hg analysis. Individuals for biochemical responses were immediately frozen in liquid N_2 and subsequently kept at −80 °C. Prior to biochemical analyses, each individual was weighed and subdivided into anterior (first 40 segments) and posterior regions (remaining body segments) according to previous studies that found differences in some antioxidant responses associated with different body regions (Ferreira-Cravo et al., 2009).

2.5.1. Hg analysis

Total mercury in biological tissues was determined using approximately 60 mg of freeze-dried pooled tissues ($n=3$, per treatment). Total Hg in *P. gualpensis*, was determined by flow-injection cold-vapor atomic fluorescence spectrophotometry (CVAFS; Leeman laboratories Hydro AF Gold plus analyzer, Leeman Labs Inc., USA). Quality control of Hg determinations was performed by using certified reference materials (NIST mussel 2976). Total Hg was determined following the USEPA method 1631 version E (USEPA, 2002), with recoveries averaging 110%.

2.5.2. Biochemical analysis

For the biochemical measurements of protein content, GST, GSH and total antioxidant capacity, organisms ($n=8$–10 per site) were homogenized (1: 3 w/v) in ice-cold buffer (20 mMTris-base, 1 mM EDTA, 1 mM DL-dithiothreitol, 500 mM sucrose and 150 mM KCl) with pH adjusted to 7.60 (Geracitano et al., 2002). Homogenates were centrifuged at 9000*g* for 45 min (4 °C) and the supernatants were collected and stored at −80 °C for later use. For TBARS measurements, polychaetes ($n=5$–7 per site) were homogenized in 1.15% KCl (1:5 w/v) containing 35 μM butylatedhydroxytoluene (BHT), and stored at −80 °C until analysis (Oakes and Van Der Kraak, 2003). The activity of glutathione-S-transferase (GST) and levels of reduced glutathione (GSH) were evaluated according to Díaz-Jaramillo et al. (2010). Total antioxidant competence against peroxyl radicals (ACAP) was measured in a 96-well microplate format according to Amado et al. (2009). Briefly, 10 μL of the supernatant of each homogenate were pipetted into each of six replicate wells per sample. The reaction buffer (127.5 μl), containing 30 mM HEPES (pH 7.2), 200 mM KCl and 1 mM $MgCl_2$, was added to the wells with samples. In three of the six wells of each sample, 7.5 μL of the pro oxidant 2,2′-azobis 2 methylpropionamidine dihydrochloride (ABAP; 4 mM; Aldrich) were added. In the other three wells, the same volume of ultrapure water was pipetted. The microplate was then incubated in a fluorescence microplate reader (Victor 2, Perkin–Elmer), at 35 °C. At this temperature, peroxyl radicals are produced by thermal decomposition of ABAP. Immediately prior to reading the plate, 10 μL of the fluorescent probe 2′,7′-dichlorofluorescein diacetate (H_2DCF-DA) in a final concentration of 40 μM was added to all wells. H_2DCF-DA is cleaved by esterases that are present in the samples' supernatants. Thereafter, the non-fluorescent compound H_2DCF is oxidized by ROS to the fluorescent compound DCF, which is detected at wavelengths of 488 and 525 nm, for excitation and emission, respectively. The thermal decomposition of ABAP and ROS formation was monitored for 30 min, with readings every 5 min. Total fluorescence production was calculated by integrating the fluorescence units (FU) along the time of measurement, after adjusting FU data to a second order polynomial function. The relative difference between ROS area with and without pro oxidant (ABAP) was considered a measure of antioxidant capacity, with greater differences reflecting lower antioxidant capacity to neutralize peroxyl radicals (Amado et al., 2009). Lipid peroxidation was evaluated using fluorometric assays for the determination of thiobarbituric acid reactive substances (TBARS), expressed as nmoles TBARS/mg of wet tissue, using tetramethoxypropane (TMP) as standard (Oakes and Van Der Kraak, 2003).

2.6. Perinereis gualpensis behavioral responses

Behavioral responses were evaluated *in situ* based on burrowing depth profiles, (the presence of individuals in three different layers of sediment (surface, middle and deep) at each exposure period). The presence of individuals at different depth layers was evaluated by rapidly subdividing the sediment of each assay chamber with acrylic blades introduced into pre-cut slits (see above) (Fig. 1b). Then each polychaete was gently removed, and its depth was recorded. Data is presented as frequency (%) of occurrence at different depths, based on total survivors.

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2.7. Data analysis

Changes in biochemical responses were evaluated by analysis of variance (ANOVA) using Neuman–Keuls test for post-hoc comparisons ($\alpha = 0.05$). Data were checked to meet the assumptions of normality and homogeneity of variances prior to analysis. The significance in behavioral responses in the different depth layers at different exposure times was evaluated by 3×2 contingency tables using chi-square test ($p < 0.05$) of categorical frequency data (Rao and Scot, 1984).

3. Results

Water physical–chemical variables were generally similar between the estuaries throughout the experiment, though small differences in oxygen and temperature were observed (Table 1). Sediment granulometric values were comparable in the predominance of the sandy fraction (> 92%) in both sampling sites (Fig. 2a). Sorting values showed similar profiles in both estuarine sediments sites where well to moderately well sorted sediments were found in surface and deep sediment layers (Fig. 2b), while in the middle sediment depth layers were poorly sorted in both estuarine sites sampled (Fig. 2b). Organic matter content was similar at all depths in both estuarine sites, and did not exceed the 2% (Fig. 2c). Reduced sediments were more pronounced in surface and middle sediment profiles from Lenga relative to Raqui, while deeper sediments were more similar (Fig. 2d). Total Hg concentrations in the Lenga sediments ranged from 1.78 ± 0.09 to 9.84 ± 0.95 mg Hg/kg d.w. and total Hg concentrations in the Raqui sediments ranged from 0.005 ± 0.001 to 0.08 ± 0.005 mg Hg/kg d.w. (Fig. 2e). In each site, the higher Hg levels were found in the middle depth layers (Fig. 2e).

Total Hg level in *P. gualpensis* tissues reflected their native sediments. Polychaetes from Lenga (0.21 ± 0.03–0.41 ± 0.08 mg Hg/kg d.w.) were significantly more contaminated than polychaetes from Raqui (0.01 ± 0.00–0.04 ± 0.01 mg Hg/kg d.w., Fig. 3) ($p < 0.05$). Polychaetes translocated to Lenga, rapidly increased in Hg concentrations over the first 14 days, but tended to stabilize by 21 days of exposure (Fig. 3).

A significant increase in GST activity was found principally in individuals translocated to the Lenga estuary in comparison with worms caged in Raqui ($p < 0.05$; Fig. 4a). Indeed, GST activity in Lenga was 1-fold higher in the anterior body region after 14 days exposure relative to the control chambers (Raqui) and 2-fold higher after 21 days when compared with the control chambers ($p < 0.05$; Fig. 4a). Also, GST activity in posterior body region from Lenga worms showed the same pattern after 14 and 21 days of exposure relative to control worms ($p < 0.05$; Fig. 4a). Concentrations of GSH, total antioxidant capacity and TBARS in polychaete body regions did not consistently reflect Hg bioaccumulation differences between sites (Fig. 4b–d). An unexplained elevation in TBARS levels was observed in both body regions from both sites at day 7, but not in any other samples ($p < 0.05$; Fig. 4d).

Burial depth profiles in polychaetes from Lenga and Raqui showed significant differences at 7 and 21 days of exposure ($p < 0.05$; Fig. 5). Polychaetes in Raqui sediments appeared mainly in middle and deep sediment layers whereas polychaetes in Lenga sediments appeared principally in surface and middle sediment layers ($p < 0.05$; Fig. 5).

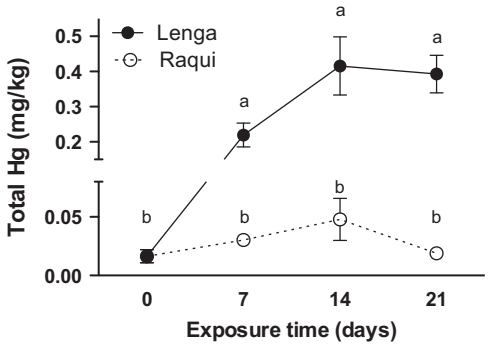

Fig. 3. Total Hg uptake of *Perinereis gualpensis* tissues from *in situ* exposures to Lenga and Raqui estuary sediments. Different letters indicate significant differences among sampling sites and times of exposure ($p < 0.05$).

Table 1
Water physicochemical variables (mean ± 1 S.D) for the *Perinereis gualpensis in situ* sediment assay sites in Lenga and Raqui estuaries during 21 days.

Variable	Day 0		Day 7		Day 14		Day 21	
	Lenga	Raqui	Lenga	Raqui	Lenga	Raqui	Lenga	Raqui
Temperature (°C)	17.1 ± 0.1	15.5 ± 0.5	17.6 ± 0.1	20.1 ± 0.3	16.1 ± 0.1	14.4 ± 0.2	19.4 ± 0.0	16.8 ± 0.1
Oxygen (mg/L)	11.4 ± 0.2	7.4 ± 0.3	7.5 ± 0.1	9.0 ± 0.1	9.7 ± 0.1	6.9 ± 0.1	8.7 ± 0.2	9.2 ± 0.0
Salinity (PSU)	25.3 ± 0.0	24.8 ± 0.4	25.2 ± 0.6	25.0 ± 0.2	25.3 ± 0.1	25.5 ± 0.4	25.8 ± 0.0	24.7 ± 0.4
pH	8.4 ± 0.1	7.8 ± 0.0	8.3 ± 0.1	8.4 ± 0.1	8.1 ± 0.0	7.9 ± 0.0	8.2 ± 0.0	8.2 ± 0.1

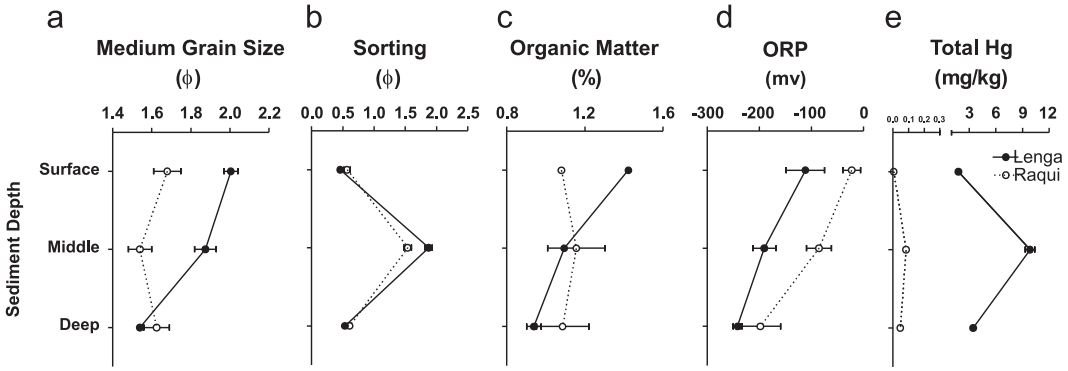

Fig. 2. Sediment physical-chemical variables; (a) medium grain size, (b) sorting, (c) organic matter, (d) redox and (e) total Hg for *in situ* sediment exposures in Lenga and Raqui estuaries at different depth layers (surface, middle and deep).

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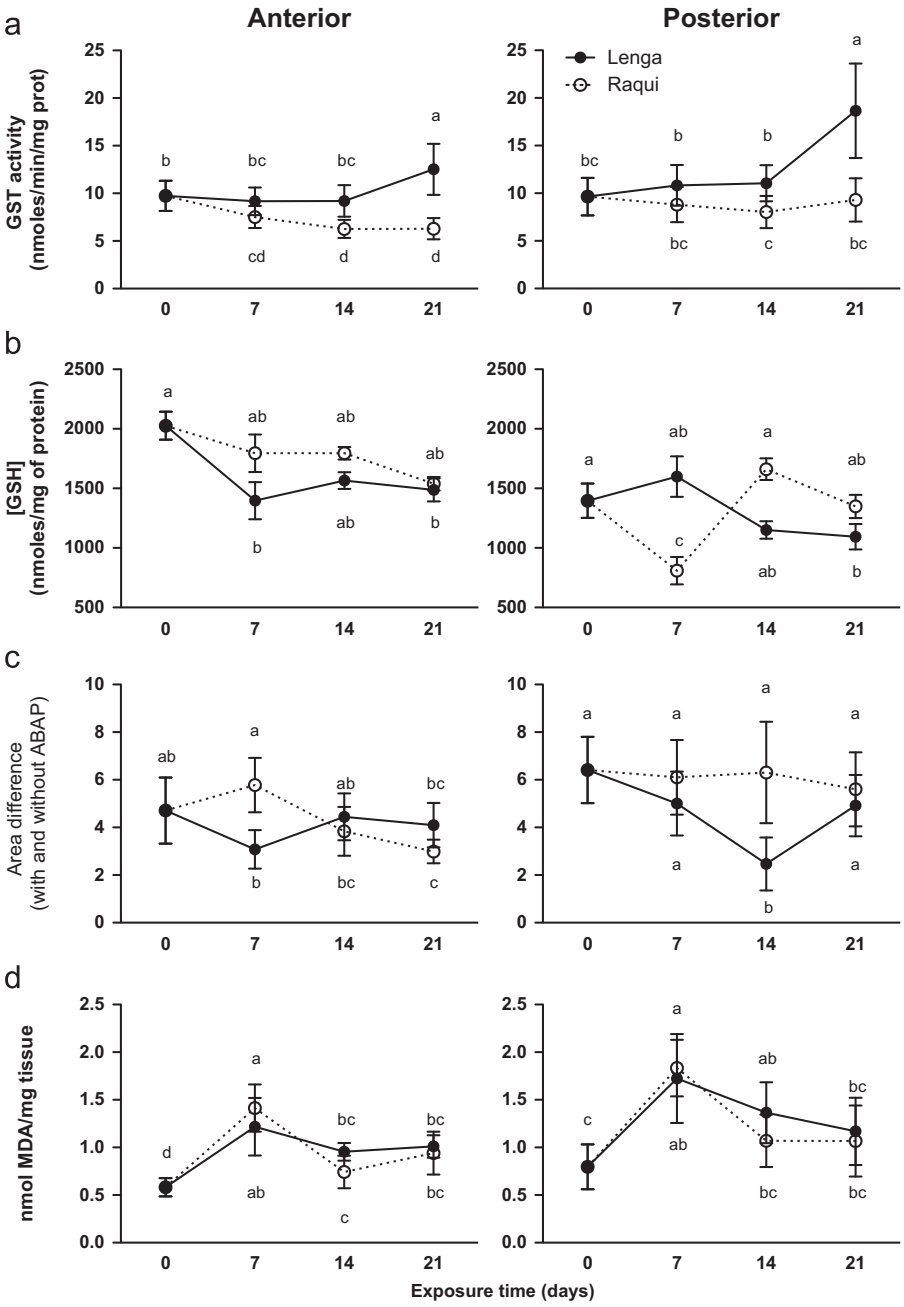

Fig. 4. Values for (a) glutathione-S-transferase (GST) activity, (b) reduced glutathione (GSH) levels, (c) total antioxidant capacity (ACAP) and (d) thiobarbituric acid reactive substances (TBARS) levels in the anterior and posterior body regions of *Perinereis gualpensis* from Lenga and Raqui estuaries. Lower values of relative area in ACAP data indicate higher antioxidant capacity. Different letters indicate significant differences among sampling sites and times ($p < 0.05$).

4. Discussion

The Lenga and Raqui sites are generally well paired for translocation experiments based on physical and chemical characteristics (Constabel, 1993). Granulometric characteristics and OM content characteristics that profoundly affect borrowing behavior were particularly similar in these two sites. However, reduced sediments were more evident at deeper layers of Lenga sediments, indicating the potentiality of oxygen depletion by aerobic bacteria (Lapota et al., 2000) and also suggest the low infauna/bioturbation activity to homogenize the sediment vertically (Gray et al., 2002). The primary difference between these sites was in Hg content, with core data suggesting dramatic Hg spill events in recent decades resulting in contamination levels being similar to or even higher than some of South America's more polluted estuaries (De Marco et al., 2006).

After 21 days of exposure, polychaetes translocated to Lenga sediments accumulated approximately 50% of the tissue concentrations found in resident adults. Resident Lenga polychaetes collected in cold and warm seasons averaged 1.11 ± 0.21 mg Hg/kg Hg d.w., (Díaz-Jaramillo et al., 2013). This finding is interesting, because we did not observe changes in tissue Hg concentratios between the 14 and 21 day sampling periods. Yet other studies suggest 120–180 days were needed to reach Hg steady state conditions in *Hediste diversicolor* (Cardoso et al., 2009). However, differences in steady states could be found in populations chronically exposed to polluted sediments in comparison to worms exposed to high Hg levels during short/medium term. The

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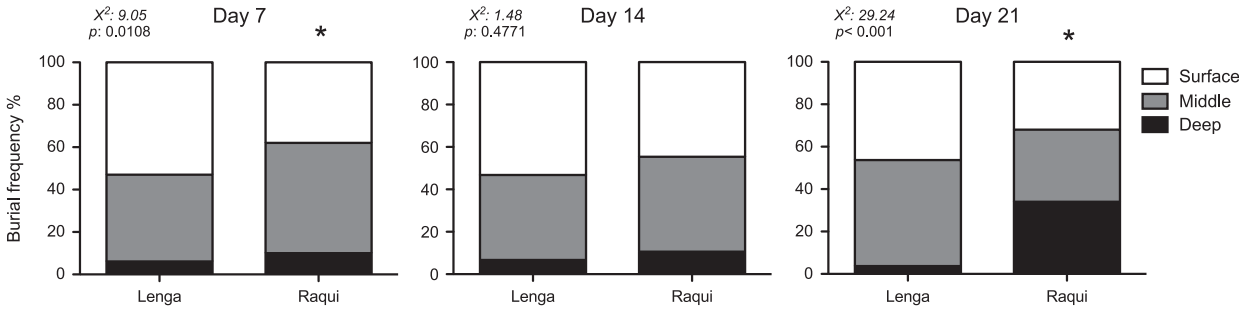

Fig. 5. Burrowing depth profiles (%) at three depths in *Perinereis gualpensis* in Lenga and Raqui estuary sediment. Chi-square (X^2) and *p*-values from burial frequency percentage in three different depth layers at different exposure time (days) are given.

bioaccumulation rates of Hg in *P. gualpensis*, estimated to be, 0.1326 μg g^{-1}/day, is higher than other related species (Cardoso et al., 2009), corroborating its heavy metal biomonitoring capability (Díaz-Jaramillo et al., 2013), even in reduced sediment conditions, where bioavailability of Hg decreases (Stoichev et al., 2004). Also due to the capacity of many nereid species to change their feeding behavior according to food availability (Olivier et al., 1997), the capacity to ingest contaminated sediment by *P. gualpensis* inside the assay chamber (with low food availability) could increase the uptake of Hg contaminated sediments. In addition, the stable Hg levels between 14 and 21 days of exposure, suggested a "point of saturation", where elimination rates begin to balance uptake rates (Rainbow, 2007).

The observed differences in ROS production between different body regions reiterate previous studies of oxidative responses in worms from Lenga sediments and other nereid species (Díaz-Jaramillo et al., 2011a; Ferreira-Cravo et al., 2009).

The significant increase in GST activity in both body regions after 21 days of exposure could be regarded as an activation of detoxification processes or antioxidant action against Hg, because GST helps in eliminating reactive compounds by forming metal conjugates with glutathione like Hg-GSH conjugates (Zalups, 2000, Maria et al., 2009). GST activity may represent an important detoxification/induction pathway of Hg in *P. gualpensis* as suggested in other marine and estuarine species (Elumalai et al., 2007). Furthermore, it can be inferred that GST induction found in worms from Lenga sediments have a medium term response despite short term GST responses were observed in other estuarine species under *in situ* exposure to contaminated sediments (Moreira et al., 2006; Ramos-Gómez et al., 2011). However, it is difficult to establish cause-effects relationships of these endpoints with only Hg exposure, as other contaminants are present in Lenga estuary sediments (Pozo et al., 2011; Díaz-Jaramillo et al., 2011a). But, relative to Sediment Quality Guidelines (SQG) values of other contaminants present, Hg appears to be the most problematic contaminant in this system.

The differences of TBARS levels between field worm levels after 7 days of exposure reflect the lack of a clear relationship between MDA, Hg body burdens and sediments, suggesting other environmental parameters acting as confounding factors (Nesto et al., 2010). However, returned TBARS levels close to T0 and control values at latter time intervals indicates efficient mechanisms for metabolizing the aldehydic product of peroxidation by this species (Hermes-Lima et al., 1998).

The inability of polychaetes to reach the lower layers of Lenga sediment cannot be ascribed to granulmetric characteristics of differences in organic carbon resources. We cannot discount the possibility that the more reduced conditions found in deeper Lenga sediments (relative to Raqui) may have impeded borrowing by transplanted polycheates (see Cardoso et al., 2010). However, it is equally plausible that the polychaetes were behaviorally affected by their exposure to the heavily contaminated middle depth Lenga sediments. We suggest that the burrowing behavior of Raqui polychaetes resulted in the more oxygenated deeper sediments in that less contaminated scenario. Given the importance of burrowing in biogeochemical and ecological processes, we suggest that burrowing behavior is an underappreciated factor to consider in contaminated environments.

4.1. Conclusions

P. gualpensis is an excellent choice for biomonitoring because it rapidly bioaccumulates metals such as Hg, often to very high concentrations. Some biochemical responses such as the antioxidant GST are tractable. The ability to use both behavioral and biochemical responses in this species over medium-term exposures (weeks) is also useful. Finally, the ability to quantify burrowing activity appears to be a promising endpoint to evaluate ecologically important functions performed by this specie.

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