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Seasonal mercury concentrations and $\delta^{15}N$ and $\delta^{13}C$ values of benthic macroinvertebrates and sediments from a historically polluted estuary in south central Chile

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HIGHLIGHTS

- Hg in sediments and biota from Lenga Estuary were elevated compared to nearby estuary.
- Invertebrates showed interspecific and seasonal differences in terms of organic Hg %.
- Total Hg levels in the ragworm best reflect Hg sediment gradient in Lenga Estuary.
- Interspecific variation in $\delta^{13}C$ signatures indicated different feeding modes.
- Organic forms of Hg in invertebrates were mainly related to the carbon sources.

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ABSTRACT

The Lenga Estuary is one of the most industrialized sites in south central Chile where the historic operation of chlor-alkali plants resulted in large quantities of mercury (Hg) being deposited into the estuary. This historical contamination may still represent a risk to the biota in the estuary. To investigate this four macroinvertebrates, *Neotrypaea uncinata* (ghostshrimp), *Elminius kingii* (barnacle), *Hemigrapsus crenulatus* (shore crab) and *Perinereis gualpensis* (ragworm) were collected seasonally from three different sites in the Lenga Estuary and one in a reference estuary (Tubul Estuary), and analyzed for Hg and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$). Mercury concentrations in Lenga sediments ranged from 0.4 ± 0.1 to 13 ± 3 mg/kg, while those in Tubul sediments ranged from 0.02 ± 0.01 to 0.07 ± 0.09 mg/kg. Total Hg concentrations of invertebrates were significantly different between estuaries ($p < 0.05$), but not by species or season for each estuary ($p > 0.05$). In contrast, organic Hg concentrations were different by species and season with shore crab muscle tissues exhibiting the greatest percent difference. Site-specific relationships demonstrated that total Hg concentrations in ragworm best reflected the total Hg sediment mercury concentrations. Signatures of $\delta^{13}C$ were correlated to the organic Hg % rather than total Hg. This suggests that organic Hg concentrations in these species were related to the carbon sources.

1. Introduction

Estuaries are among the most productive and economically important ecosystems, and generally support diverse human activities making them potential sites for pollutant concern (Amiard-Triquet and Rainbow, 2009). Recently, contamination by mercury (Hg) has become a world-wide concern (Hylander and Goodsite, 2006). This metal is recognized as a priority pollutant due to the significant inputs into the environment, its mobility, persistence and toxicity to living organisms. In addition, organic forms of mercury biomagnify in estuarine food chains becoming concentrated in species consumed by humans (Tchounwou et al., 2003). A legal framework for controlling global mercury pollution is being developed by the United Nations Environment Programme (UNEP) and will likely be adopted in the near future as a convention (UNEP, 2011).

Sediments represent the major compartment for trace metal storage in aquatic environments (Ramalhosa et al., 2006). Benthic macroinvertebrates are key components of estuarine food chains and have been used to evaluate trace metal bioavailability, including mercury (Coelho et al., 2007; Nunes et al., 2008; Chen et al., 2009). Furthermore, relatively high abundance, ease of collection, and small home ranges facilitate the use of macroinvertebrates for studies of site-specific pollution (Nunes et al., 2008; Díaz-Jaramillo et al., 2010). These organisms also integrate contaminant responses over time making them valuable assessment tools (Dauvin et al., 2007).

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Macroinvertebrates are a possible route of exposure of metals, and as such Hg, to higher trophic levels including fish. Since it is well known that there are differences in bioconcentration factors of potentially toxic compounds among benthic species (Rainbow, 2002), it is desirable to compare multiple species that include different feeding strategies, detoxification capacity and habitat utilization in environmental assessments and biomonitoring programs. Species such as bivalve molluscs are frequently used for biomonitoring but are often not available in the areas or habitats of interest (Coelho et al., 2007). In addition, seasonal variability in habitat and biological factors may influence the bioavailability of pollutants in the estuarine food webs (George and Batzer, 2008).

Food is considered a major source of uptake for contaminants such as methyl-Hg (George and Batzer, 2008). Assessing the different feeding strategies of benthic biota is therefore a crucial factor to account for bioaccumulation within food webs. Stable isotope signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have become a commonly used tool to investigate trophic interactions, track the origin of carbon sources within complex food webs and predict the transfer of contaminants such as Hg to biota (Post, 2002; Chen et al., 2009).

South central Chilean estuaries can experience a range of anthropogenic pressures due to current and historical human and industrial activities. The Lenga Estuary (36° 46′ 15″ S; 73° 10′ 06″ W) has experienced anthropogenic organic matter enrichment over the last few decades (Moscoso et al., 2006) and is also impacted by Hg pollution from chlor-alkali industries which utilized mercury cells in their processes from the 1970s until the 1990s (Hoffmann, 1978; Díaz et al., 2001). Within the estuarine benthic fauna of south central Chile, the ragworm (*Perinereis gualpensis*; sediment living organism), epibenthic shore crab (*Hemigrapsus crenulatus*; intertidal/subtidal living organism), ghostshrimp (*Neotrypaea uncinata*: sediment living organism) and barnacle (*Elminius kingii*: hard-substratum living organism) are common species with a wide geographical distribution and a relatively well described biology (Riquelme-Bugueño, 2006; Castillo-Blasco et al., 2009). Recently, these species have been proposed as organisms to be used in biomonitoring of the environmental quality of Chilean estuarine ecosystems (Díaz-Jaramillo et al., 2010, 2011). Thus, there is a need for better understanding of the trophic relationships and bioaccumulation potential (Hg) of these species in estuarine environments. The objective of the work presented here was to assess the seasonal and spatial variation in bioavailability of Hg (total and organic) in sediments and macroinvertebrates in an impacted (Lenga) and reference (Tubul) estuary in south central Chile.

2. Materials and methods

2.1. Study area

Lenga is a small estuary (3.2 km^2) located in San Vicente Bay, of the Biobio Region of Chile (36° 45′ S; 73° 10′ W). It is within an industrial area containing an oil refinery, steel and chemical industries. Tubul is a small coastal estuary with an ecologically important salt marsh area (37° 14′ S; 73° 26′ W). It receives minimal anthropogenic inputs of pollution and the relative similarity and proximity to the Lenga Estuary make it an appropriate reference site for comparison (Díaz-Jaramillo et al., 2011). Three sites in the Lenga Estuary (L1, L2, and L3) and one site in the Tubul Estuary (T) were sampled (Fig. 1a,b). Considering freshwater inputs in these estuaries two seasons can be defined the cold/rainy and warm/dry seasons (Díaz-Jaramillo et al., 2011). Sampling was performed during representative months; June (winter 2008) and January (summer 2009). Sites were selected within the estuaries with similar physico-chemical parameters including salinity, oxygen concentration, temperature and pH.

2.2. Sediment sampling

Sediment samples were collected at all sampling sites during low tide using an acrylic corer (10 cm × 6 cm) and were kept at −20 °C until analysis. Additionally in situ measurements of sediment redox (reduction–oxidation) condition as Eh were performed at several depths (2, 4, 6, 8 and 10 cm) using a plastic corer with holes at each 2 cm of depth and a multi-parameter probe (YSI Professional Plus, YSI Inc., USA).

2.3. Biological sampling

Four invertebrate species were collected from both estuaries during the two seasons (Fig. 1). Ragworm, *P. gualpensis* (Polychaeta: Nereididae), epibenthic shore crab, *H. crenulatus* (Crustacea: Varunidae), ghostshrimp, *N. uncinata* (Crustacea: Callianasidae) and barnacle, *E. kingii* (Crustacea: Chthamalidae) were collected manually using a blade, sediment pump or by hand, from each site where available; ghostshrimp and barnacles were not available/collected at all sites (Fig. 1). Individuals were transported to the laboratory and kept in filtered-seawater (adjusted to field salinity) for 24 h in order to eliminate sediment particles. Biometric measurements of total wet weight (*P. gualpensis*), cephalothorax width (*H. crenulatus*), cephalothorax length (*N. uncinata*) and operculum length (*E. kingii*) were measured (Fig. 1; Table 1). Soft tissues, hepatopancreas and muscle were obtained from the various species. For each sample of *P. gualpensis* or *E. kingii* five composites were made of soft tissues (10 individuals per composite) (Table 1). For each *N. uncinata*, and *H. crenulatus* sample, five composites of 5 individuals each were made with male hepatopancreas (Table 1). The water content (%) of 4 samples of each tissue was determined by freeze drying the tissues (Table, 1). Additionally, given the high abundances and wide distribution in the Lenga Estuary, *P. gualpensis* and *H. crenulatus* were chosen to study site-specific relationships between total Hg concentration in sediments and Hg levels in tissues (Fig. 1). All composites were kept frozen (−20 °C) and then freeze dried/powdered for subsequent mercury analysis. A subsample of each freeze dried composite sample was also taken for stable isotope analysis.

2.4. Sediment physico-chemical analysis

Sediment cores from each site (n = 2 per sampling site) were sub-sampled in five 2 cm slices, freeze dried and subsamples were taken for the different sediment analysis procedures. Sediment mean grain size, sorting and organic matter (OM) content were evaluated by the momentum method (McManus, 1988) according to Díaz-Jaramillo et al. (2011). Total organic matter content was determined by the ash free dry weight (AFDW) method by ashing the samples in a furnace for 4 h at 550 °C.

2.5. Mercury analysis

Total Hg (inorganic and organic Hg forms) and organic Hg (MeHg and other organic Hg forms) were determined by cold vapor atomic absorbance spectrometry (CVAAS; Perkin-Elmer FIMS-400, Perkin-Elmer Corp., USA). The detection limit for Hg in the digested sample solutions was 0.1 μg/L. Calibration of Hg determinations was done using certified aqueous standard of 1000 mg/L (Merck 170226, Darmstadt, Germany) traceable to Standard Reference Materials (SRM) of the National Institute of Standards and Technology (NIST). Analytical quality control of the total and organic mercury determinations included Certified Reference Materials (CRMs) from the National Research Council Canada (NCR, Canada), PACS-2 (for sediments) and DORM-2 (for biota) and was performed in triplicate (for each analytical run). Analytical procedural precision under the operating conditions was 1.6% (corresponding to the relative percent of

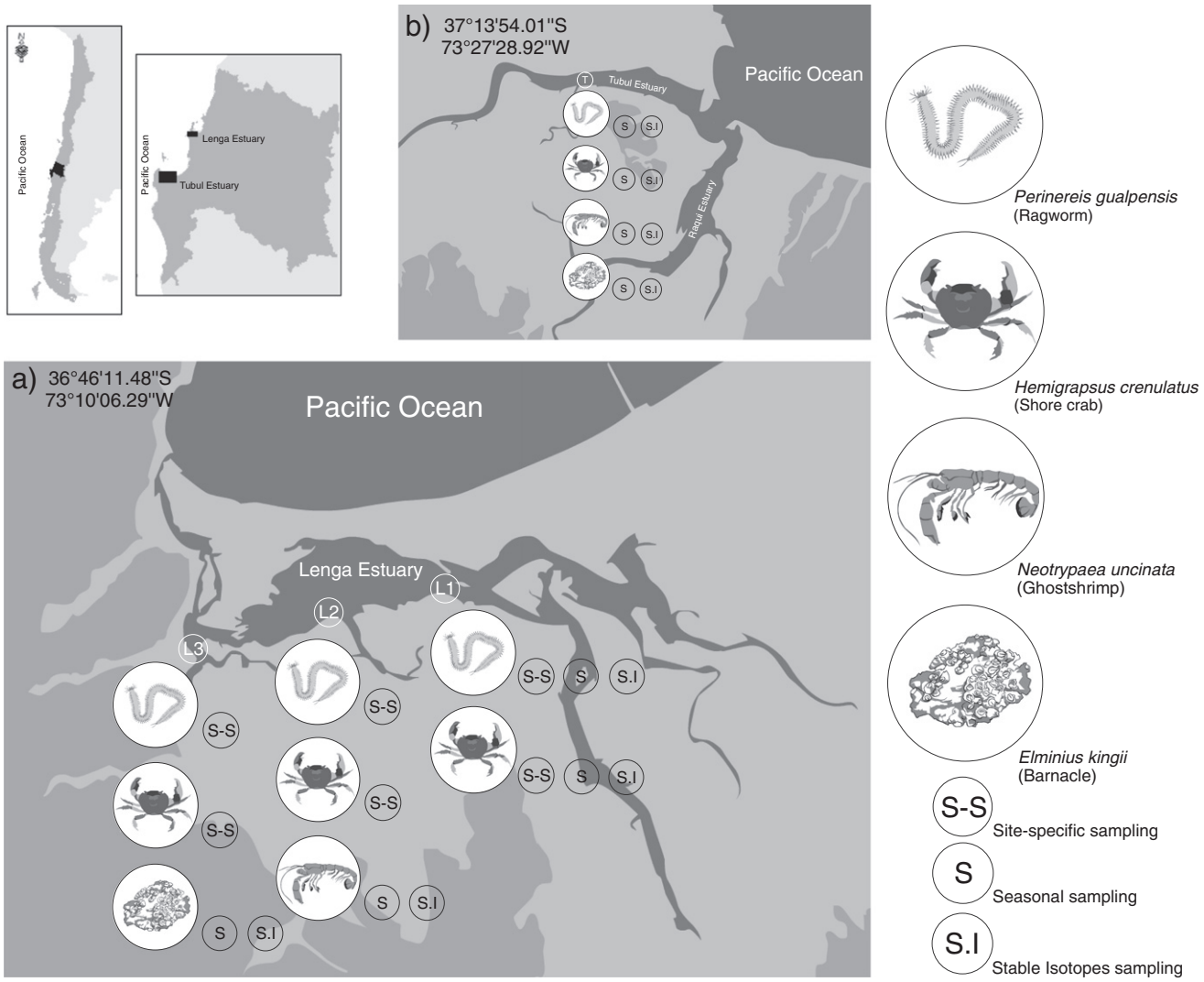


Fig. 1. Sediment and benthic macroinvertebrate sampling sites from a) Lenga and b) Tubul Estuaries, 2008–2009.

difference). The values of extraction efficiency obtained for the CRM analysis ranged from 70.2% to 117.0% with an average of 98.0% ± 10.8%. Relative percent difference (RPD) between two duplicated measurements ranged between 1.8 and 8.5%.

Total Hg in freeze-dried sediments was analyzed (n = 2 per layer) following modified EPA method 245.5 (USEPA, 1991). Sediment CRM recoveries ranged between 86% and 116% with a mean of 101% ± 9% (method detection limit; 0.01 mg/kg).

Total Hg concentrations in biological tissues (n = 5 composites per species/tissue at each sampling site) were determined using approximately 30 mg of freeze-dried tissues, digested with a mixture of 1 mL H_2O_2 and 5 mL H_2SO_4:HNO_3 (1:1). Samples were placed in a microwave (Millestone, ETHOS) until the complete digestion of the material (200 °C, 30 min). Then, 8 mL of $KMnO_4$ (5%) was added and maintained overnight before being neutralized with 12% hydroxylamine chlorhydrate ($HONH_3CL$ + NaCl) (Bastos et al., 1998). CRM

Table 1
Number of individuals per composite, number of composite per sample, tissue type (water percentage) and seasonal biometric measurements (±S.D.) in four macroinvertebrate species sampled from Lenga and Tubul Estuaries.

Species	Common name	No. of ind./ composite	No. of composite/ sample	Tissue (% water)	Type of measurement	Lenga	Tubul	Lenga	Tubul
						Winter		Summer	
Perinereis gualpensis	Ragworm	10	3–5	Whole individual (79 ± 3)	Wet weight (g)	0.20 ± 0.04	0.19 ± 0.05	0.33 ± 0.04	0.29 ± 0.04
Hemigrapsus crenulatus	Shore crab	5	3–5	Muscle (76 ± 3) hepatopancreas (79 ± 2)	Cephalothorax width (cm)	3.47 ± 0.30	2.74 ± 0.11	3.66 ± 0.27	3.31 ± 0.15
Neotrypaea uncinata	Ghostshrimp	5	3–5	Hepatopancreas (62 ± 6)	Cephalothorax length (cm)	1.40 ± 0.16	1.88 ± 0.08	1.79 ± 0.17	1.81 ± 0.13
Elminius kingii	Barnacle	10	3–5	Whole individual (81 ± 10)	Operculum length (cm)	1.18 ± 0.11	1.10 ± 0.09	1.41 ± 0.29	1.15 ± 0.04

recoveries were between 70% and 117% with a mean of 101 ± 10% (method detection limit; 0.08 mg/kg).

The concentrations of MeHg and other forms of organic mercury in biological tissues (n = 3 to 5 composites per species/tissue) were determined according to Wagemann et al. (2000) who followed the procedure of Uthe et al. (1972). Approximately 200 mg of freeze-dried tissue was homogenized with an aqueous solution of acidic KBr (5 mL of 30% in 4 N H_2SO_4) and $CuSO_4$ (7.5 mL of 2.5% in 4 N H_2SO_4). Organic mercury was then extracted by vortexing the tissue homogenate with a 3:2 v/v mixture of dichloromethane (DCM)–hexane (5 mL), followed by centrifugation to separate the organic and aqueous layers from the solid material pellet. An aliquot of 1 mL of the DCM–hexane supernatant (organic phase) was withdrawn, added to a test tube containing 5 mL of a HNO_3–H_2SO_4 mixture (1:4 v/v) and heated for 30 min at 60 °C. CRM recoveries were between 70% and 90% with an average of 84.8% ± 6.0% (method detection limit; 0.06 mg/kg).

2.6. Stable isotopes analysis

Dried sediment (2 cm and 10 cm depth layer; n = 3 subsamples per section) and tissue samples (n = 5 composites per sampling site for each species/tissues) were ground and aliquots (0.25–0.30 mg) were weighed into tin cups for submission to the University of Waterloo Environmental Isotope Laboratory for determination of stable isotope of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) (Drimmie and Heemskerk, 2005). A Delta Plus continuous flow stable isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) coupled with a Carlo Erba Elemental Analyzer (CHNS-O EA1108, Milan, Italy) was used to calculate % elemental composition.

2.7. Statistical analyses

To characterize the sediment Hg concentrations and their relationship to other sediment physico-chemical characteristics, principal component analysis (PCA) was performed after data standard normalization, using the software PRIMER V.6 developed in Plymouth Marine Laboratory, UK (Clarke and Gorley, 2005). Total Hg concentrations in invertebrate tissue samples were compared with those measured in organisms from the reference estuary and evaluated by season using a multi-factorial analysis of variance (ANOVA) followed by a Neuman–Keuls test for post-hoc comparisons ($p<0.05$). Hg and stable isotope relationships were evaluated by Spearman correlation analysis using the software INFOSTAT 2010 developed in Universidad Nacional de Córdoba, Argentina (Di Rienzo et al., 2010).

3. Results

3.1. Sediments

The concentration of total Hg in sediments cores was different between the two estuaries and between the three Lenga Estuary sites (L1, L2, and L3; Fig. 2). Total Hg distribution through the sediment profile did not show marked seasonal variation in the estuaries. Conversely, differences were evident among sites (Fig. 2). The highest Hg concentrations were found at site L1 which also showed the most variable Hg concentrations through the sediment profile (Fig. 2; note difference in scale for figures). Total Hg gradually increased from the surface (1.61 ± 0.35 mg/kg) to the deeper layers (12.63 ± 2.56 mg/kg) (Fig. 2). Site L2 sediment concentrations were lower than those measured at L1 concentrations with little variation between the surface and deeper sediment layers with values (0.77 ± 0.47 versus 1.55 ± 0.10 mg/kg (Fig. 2)). L3 sediments exhibited the lowest Hg concentrations among all studied sites with average surface concentrations (0.39 ± 0.01 mg/kg) being less than that measured in deeper (1.23 ± 0.05 mg/kg) layers (Fig. 2). Average surface sediment Hg concentrations were seasonally different for the Tubul Estuary (T) (Fig. 2) with mean values of 0.07 ± 0.04 and 0.01 ± 0.00 mg/kg for the surface and deeper layers respectively (Fig. 2).

Principal components analysis (PCA) was performed using the seasonal average of sediment physico-chemical parameters (redox, OM%, sorting and mean grain size) and total Hg concentration for all the samples (Fig. 2b). PCA indicated that OM% (positive) and sorting (positive), and Hg concentration (negative) explained 79% of the variation (Fig. 3). This analysis showed a clustering of samples from L3 relative to other sites. Samples from this core were characterized by poorly sorted fine and very fine sand (2.59 to 3.43 Φ), high organic matter (2.8 to 4.3% OM), and low Eh in sediments (−125 to −330 mV) in lower layers (Fig. 3). L1 had high total Hg concentration and reduced sediments in deeper layers (Fig. 3). Sites L2 and T were characterized by fine sand (2.05 to 2.75 Φ), low OM content (1.5 to 2.0% OM) and more oxygenated sediments (−32 to −230 mV; Fig. 3).

3.2. Total Hg in biota

Total Hg concentrations of macroinvertebrates during both seasons, from the contaminated estuary (Lenga), exhibited significantly higher Hg values than those from the reference estuary in most of the analyzed tissues ($p<0.05$; Table 2). Hg concentrations in macroinvertebrates from the Lenga Estuary exhibited significant differences ($p<0.05$) for only barnacles *E. kingii* for both seasons and in the ragworm *P. gualpensis* in summer relative to the other target species (Table 2). Seasonal differences were only observed for

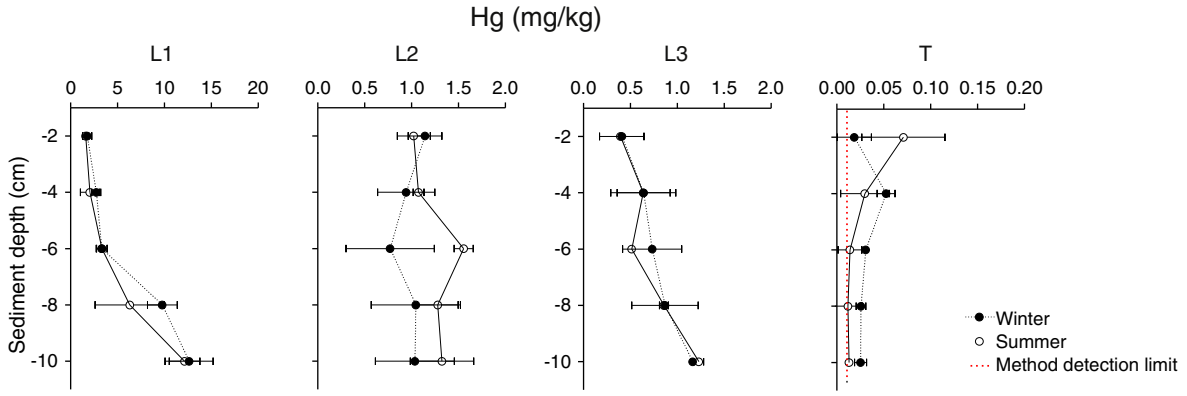
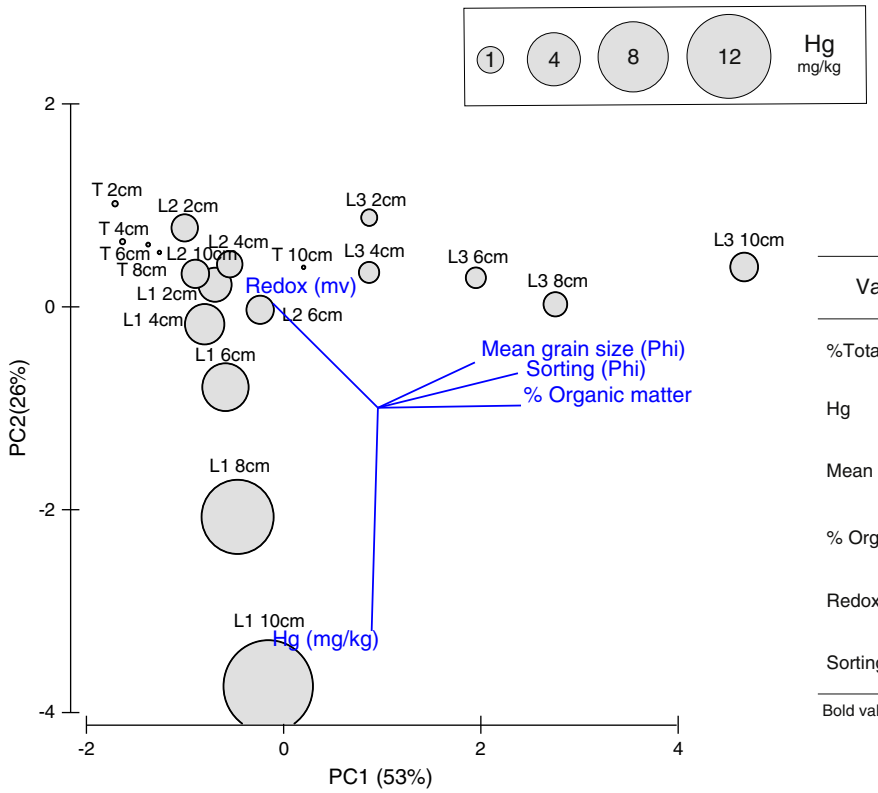


Fig. 2. Total Hg in sediments (±S.D.) versus depth in a) seasonal vertical Hg profiles (mg/kg D.W.) in three sites from Lenga (L1, L2, and L3) and Tubul (T) Estuaries.



Variable	PC1	PC2
%Total Variance	53	26
Hg	-0.025	-0.882
Mean grain size	0.394	0.180
% Organic matter	0.581	0.009
Redox	-0.427	0.413
Sorting	0.569	0.137

Bold values indicate significant correlations.

Fig. 3. Principal component analysis (PCA) for the seasonal average of Hg sediment concentrations (bubbles) and physico-chemical parameters in sediment layers of different depths (2–10 cm) from Lenga and Tubul Estuaries. The variability percentage explained by each principal axis is provided. The direction of blue lines indicates the steepest increase in the variable, and the length indicates the strength relative to other variables. Table of variable correlations and principal component percentages (PC1, PC2) of Hg concentrations and physico-chemical sediment parameters is provided.

barnacles ($p<0.05$; Table 2). In the Tubul Estuary, significantly higher concentrations of total Hg were found in the summer relative to winter for 3 of the 4 studied taxa (*P. gualpensis*, *N. uncinata* and *E. kingii*, respectively) ($p<0.05$; Table 2).

3.3. Organic Hg in biota

In terms of organic Hg, significant differences were observed between species for both seasons ($p<0.05$; Fig. 4). The muscle of the shore crab *H. crenulatus* showed the highest percentage of organic Hg ranging from 80% to 98% of total Hg (Fig. 4). The ragworm and the ghostshrimp *N. uncinata* did not show seasonal differences in terms of organic Hg proportions ($p<0.05$; Fig. 4). Significant seasonal differences in the percentage of organic Hg were observed in the shore crab hepatopancreas (digestive gland), and the barnacle with the highest percentages of organic mercury in the winter season ($p<0.05$; Fig. 4). However organic Hg concentrations in the barnacle were similar during both seasons (Fig. 4). The concentrations of organic Hg in invertebrates from the Tubul Estuary were below the detection limits.

3.4. Site-specific responses

There was a significant decrease in total Hg in the ragworm from the inner estuarine sites (L1) to the mouth (L3) ($p<0.05$; Fig. 5a). In contrast, both tissues of the shore crab did not show a consistent gradient of total Hg concentration across the same sites (Fig. 5a). The $\log_{10}$ transformed bioaccumulation factor [BAF = $\log_{10}$ (mean Hg biota tissue/average Hg sediment depth layers)] where lower $\log_{10}$ BAF values indicate less difference in Hg concentrations between biota and sediment, showed a significant sequential increase in both shore crab hepatopancreas and muscle from the inner estuary (L1)

Table 2
Seasonal total Hg (mg/kg dry weight) in macroinvertebrate biota (±S.E.): *P. gualpensis*, *H. crenulatus* (hepatopancreas and muscle), *N. uncinata* and *E. kingii* tissues from Lenga and Tubul Estuaries. The same letter indicates the absence of significant differences between sampling sites and species ($p>0.05$). Asterisk indicates seasonal significant differences ($p<0.05$). Italic values indicate means below method detection limits.

Species	Common name	Total Hg (mg/kg)			
		Lenga	Tubul	Lenga	Tubul
		Winter		Summer	
Perinereis gualpensis	Ragworm	0.89 ± 0.08^{a}	$\mathit{0.05 \pm 0.01}^{cd}$	1.11 ± 0.21^{bc}	$0.22 \pm 0.02^{d*}$
Hemigrapsus crenulatus (hepatopancreas)	Shore crab	1.50 ± 0.06^{a}	0.14 ± 0.05^{c}	1.59 ± 0.15^{ab}	0.21 ± 0.03^{d}
Hemigrapsus crenulatus (muscle)		1.51 ± 0.15^{a}	0.10 ± 0.03^{cd}	2.05 ± 0.28^{a}	$\mathit{0.07 \pm 0.09}^{e}$
Neotrypaea uncinata	Ghostshrimp	1.91 ± 0.41^{a}	0.12 ± 0.02^{c}	2.41 ± 0.35^{a}	$0.39 \pm 0.07^{d*}$
Elminius kingii	Barnacle	0.32 ± 0.05^{b}	$\mathit{0.04 \pm 0.01}^{d}$	$0.73 \pm 0.09^{c*}$	$0.32 \pm 0.04^{d*}$

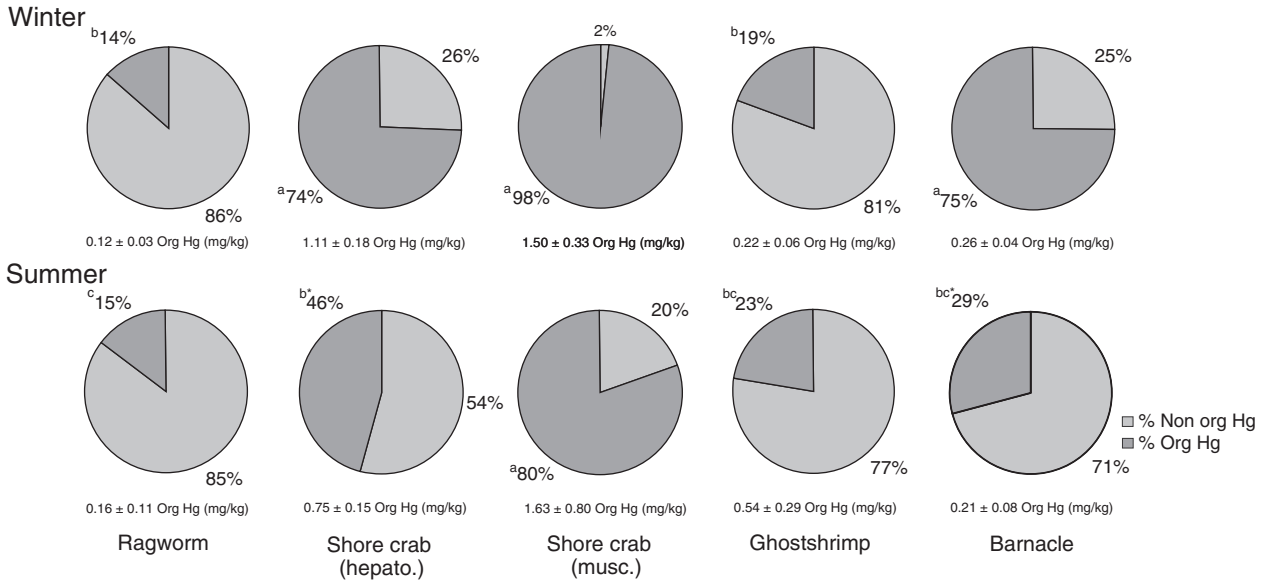


Fig. 4. Seasonal organic Hg concentrations (±S.E.) and percentages (percent of Total Hg as organic Hg) in macroinvertebrate biota: *P. gualpensis* (ragworm), *H. crenulatus* (shore crab hepatopancreas and muscle), *N. uncinata* (ghostshrimp) and *E. kingii* (barnacle) tissues from Lenga Estuary. The same letter indicates the absence of significant differences between species % ($p>0.05$). Asterisk indicates seasonal significant differences in % ($p<0.05$).

to the mouth sites (L2, L3; Fig. 5b). Conversely the ragworm exhibited significantly lower $\log_{10}$ BAF values in all sites compared to the shore crab ($p<0.05$; Fig. 5b). Site-specific organic Hg percentages did not show differences in the shore crab tissues between sites, while the ragworm showed a significantly greater percentage of organic Hg in the L2 site ($p<0.05$; Fig. 6).

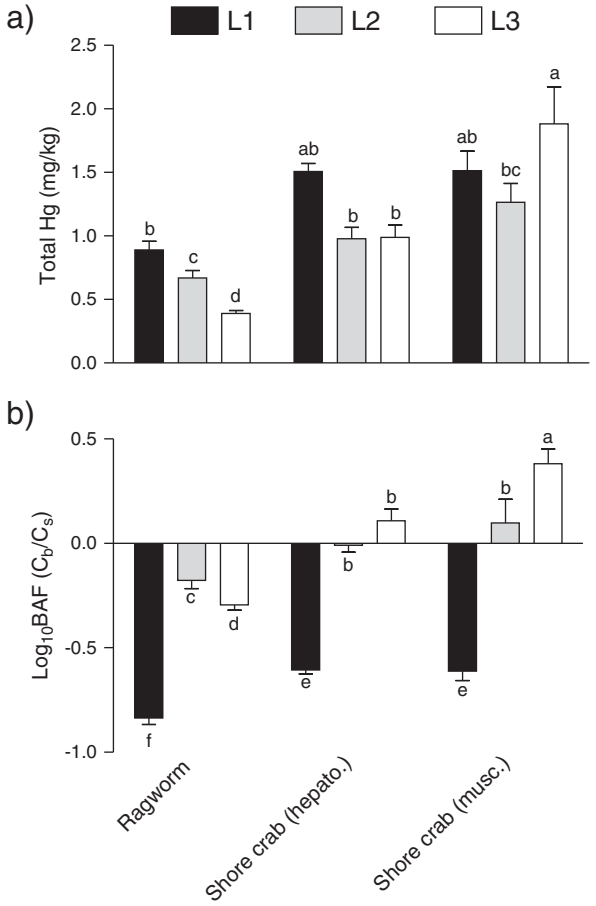


Fig. 5. Site-specific relationships in *P. gualpensis* (ragworm) and *H. crenulatus* (shore crab) from Lenga Estuary sampling sites in winter. a) Total Hg (±S.E.; mg/kg dry weight) in *P. gualpensis* and *H. crenulatus*. b) BAF levels $\log_{10}$ (±S.E.; mean Hg biota tissue/aver. Hg sediment depth layers) in *P. gualpensis* and *H. crenulatus*.

3.5. Stable isotope analysis

Sediment stable isotope ratios of carbon and nitrogen (δ^{15}N and δ^{13}C) in the Lenga Estuary sites did not show significant differences ($p<0.01$) between superficial and deeper sediments in the majority of the sampling sites for both seasons, while the Tubul Estuary sediments showed consistent significant differences in δ^{13}C between layers in both seasons ($p<0.01$; Fig. 7). Isotope values for macroinvertebrates showed differences between species with ghostshrimp values most similar to sediment δ^{13}C signatures (Fig. 7). In the Lenga Estuary shore crab tissues showed a slightly higher trophic position than the other species in the cold season in terms of δ^{15}N values ($p<0.01$; Fig. 7a). On summer, δ^{15}N signatures in shore crab, ragworm and barnacle showed no significant differences ($p>0.01$; Fig. 7a). Also in the Lenga Estuary the ghostshrimp showed a significant depletion in terms of δ^{15}N values in both seasons ($p<0.01$; Fig. 7a). High variability in δ^{13}C values was found among species, in both estuaries ($p<0.01$; Fig. 7a,b). In the Lenga and Tubul estuaries, ghostshrimp showed the most significant depletion in terms of δ^{13}C signatures, whereas shore crab muscle exhibited significant enriched δ^{13}C signatures ($p<0.01$; Fig. 7a,b). δ^{15}N and δ^{13}C ratios in macroinvertebrates from the Tubul Estuary showed a similar pattern to the Lenga Estuary, however the ragworm ratios in the Tubul Estuary showed a significant seasonal difference that was not evident in the same species in the Lenga Estuary ($p<0.01$; Fig. 7a,b). In general, the isotope differences between the four species exhibited more variability for δ^{13}C (ranging from −11 to −20 per mil) than δ^{15}N values. For all estuaries ghostshrimp had the lowest δ^{15}N values (Fig. 7a, b).

3.6. Hg and stable isotopes relationships

Total Hg concentrations in the four invertebrate species from the Lenga Estuary were not correlated with δ^{13}C ($r_s=-0.07$; $p=0.84$) values but were inversely correlated with δ^{15}N values ($r_s=-0.67$; $p<0.05$). In contrast, the percentage of organic Hg showed a strong positive correlation with δ^{13}C signatures ($r_s=0.82$; $p<0.05$) while δ^{15}N signatures were poorly correlated ($r_s=0.36$; $p=0.24$).

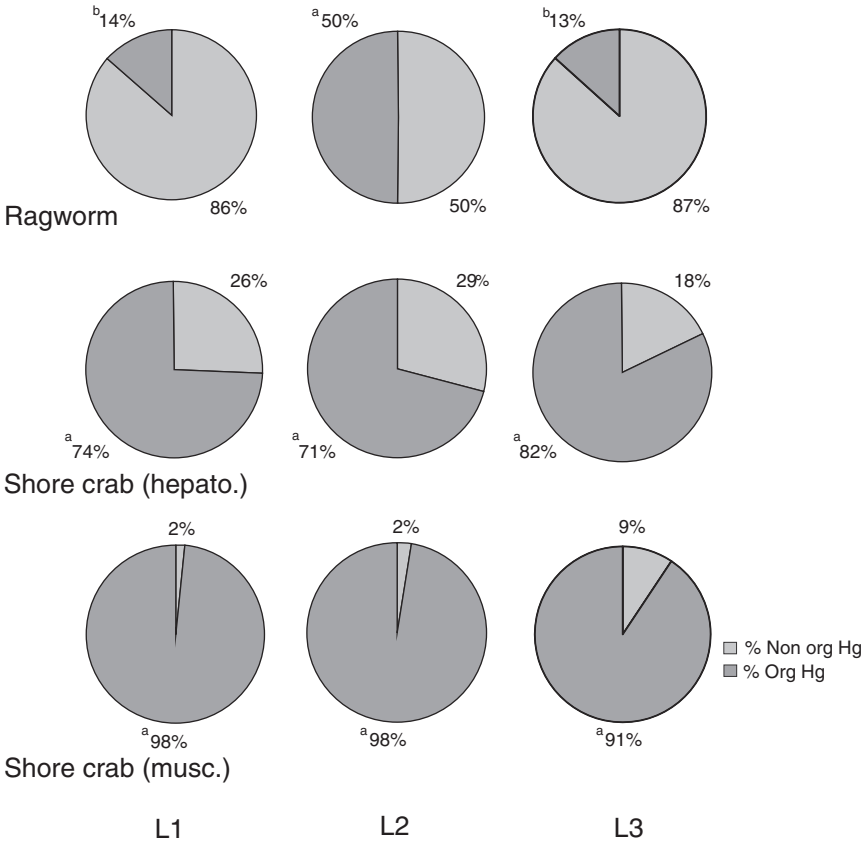


Fig. 6. Site-specific relationships in *P. gualpensis* (ragworm) and *H. crenulatus* (shore crab) from Lenga Estuary sampling sites in winter. Organic Hg percentage in *P. gualpensis* and *H. crenulatus*. The same letter indicates the absence of significant differences between sampling sites and species ($p > 0.05$).

4. Discussion

Mercury concentrations in Lenga Estuary sediments were one-to-two orders of magnitude higher than those measured in the reference estuary (Tubul). Hg concentrations observed in Lenga Estuary sediments were higher than those reported for European and other South American estuaries (Bryan and Langston, 1992; Ouddane et al., 2008; Mirlean et al., 2009) and were above threshold effect levels (TELs; 0.13 mg/kg) and probable effect levels (PELs; 0.70 mg/kg) for some marine sediment quality guidelines (Long et al., 1995; CCME, 2002). High sediment Hg concentrations in L1 in contrast to L3 suggest input from anthropogenic sources at the head of Lenga Estuary, corresponding with the vicinity of historic chlor-alkali chemical industries. Vertical differences at L1 may represent an archive of Hg inputs and suggest a recent decline of Hg inputs in Lenga Estuary. Moreover the relative homogeneity in Hg depth profiles in the majority of sampling sites suggest bioturbation effects by benthic fauna (Cardoso et al., 2008), which can redistribute particles and associated elements to depths of at least 1 m and on time scales of days to months (Hirschberg et al., 1996). The stronger vertical profile at L1 suggests the anoxic sediments had little infauna/bioturbation to homogenize the sediment vertically (Gray et al., 2002). The lack of relationship between smaller particle size and OM content with the total Hg concentrations in L1 is not consistent with other studies that have shown positive relationships between these variables (Stoichev et al., 2004; Ouddane et al., 2008; Chen et al., 2009). These results suggest that the Hg concentrations in Lenga Estuary sediments are dependent on the distance from the source rather than on physico-chemical characteristics, which diminished from the vicinity of the Hg source (chlor-alkali plant) to the mouth of Lenga Estuary.

Total Hg concentrations in macroinvertebrates from the Lenga Estuary were generally equal to or higher than those reported for similar species from Hg polluted estuaries (0.1–0.9 mg/kg: Muhaya et al., 1997; 0.01–0.2 mg/kg: Coelho et al., 2007; 0.2–2.0 mg/kg: Morillo and Usero, 2008). Total Hg concentrations increased slightly during summer for all macroinvertebrates and were higher for barnacles. This suggests a relatively constant pattern across seasons. The significantly higher value Hg in the barnacle may be related to the larger size of the individuals collected during summer in the Lenga Estuary, however a summer increase was also seen in individuals from the reference estuary for organisms of similar size in both seasons. Allometric differences in the seasonal collections (different cohorts of individuals) may also have caused observed differences. The more significant change in the barnacle Hg concentrations could also be due to the fact that these organisms are suspension/filter feeders and are exposed to other Hg sources like suspended particulate matter (SPM) or microalgae which are known to be important seasonal sources of Hg (Amiard-Triquet and Rainbow, 2009). The increase of total Hg concentration during the summer in individuals from the reference estuary suggests an alternative source of Hg during low flow conditions, which may be related to a change in Hg uptake/bioavailability in different life stages of test organisms or changes in the sediment biogeochemistry in the warm season.

Organic Hg percentages in shore crab muscle were equal to or greater than those reported for other estuarine shore crabs (70–95%: Pereira et al., 2009, 90%: Coelho et al., 2007). This has ecological implications since crabs are an important food source for predatory birds. Observed organic Hg percentages in the crustaceans *H. crenulatus* and *E. kingii* were greater during the winter compared to the summer which is in agreement with the findings of Rothenberg et al. (2008) and Muhaya et al. (1997).

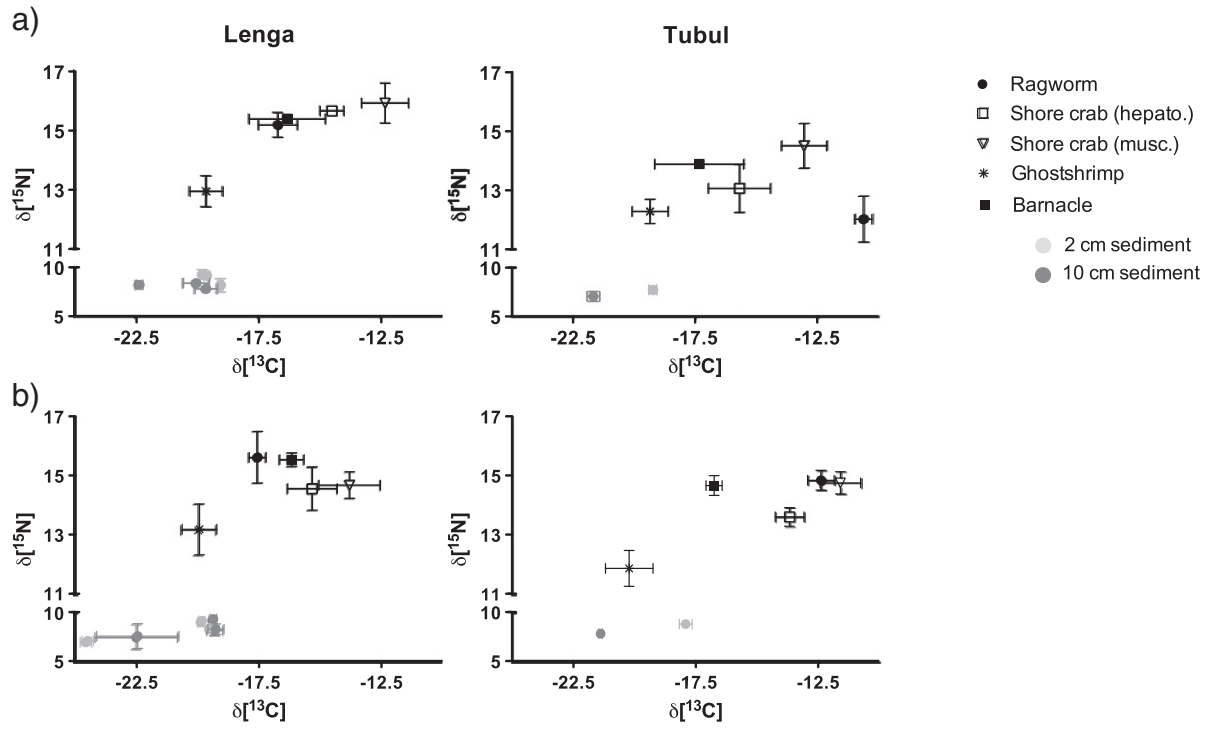


Fig. 7. Seasonal stable isotopes analysis ($\delta^{15}N$, $\delta^{13}C$) signatures in invertebrate biota: *P. gualpensis* (ragworm), *H. crenulatus* (shore crab hepatopancreas and muscle), *N. uncinata* (ghostshrimp) and *E. kingii* (barnacle) tissues and superficial and deeper sediments from the Lenga and Tubul Estuaries. a) Winter and b) summer seasons.

In terms of site-specific relationships, the ragworm Hg concentrations best reflected the gradient in sediments from the head to the mouth of the estuary, reaffirming the utility of this species for estuarine biomonitoring (Díaz-Jaramillo et al., 2010, 2011). This was not the case for the shore crab that exhibited an inconsistent pattern of Hg concentrations in their tissues. This could be due to different pathways of for Hg exposure due to feeding modes and the mobility of this species within the estuary.

The biogeochemical behavior of Hg in sediment is dominated by the redox conditions and the S-cycle due to sulfate reducing bacteria (Muhaya et al., 1997; Stoichev et al., 2004). High organic Hg ratios in the ragworm tissues from L2 might be due to the particular sediment physico-chemical characteristics at this sampling site. Sediments with high OM contents and under anoxic conditions are frequently associated with a high rate of MeHg production (Ouddane et al., 2008). However, in this work, similar to results from Muhaya et al. (1997), sandy oxygenated sediments with low OM percentage were found to be associated with high concentrations of organic Hg in ragworm species.

Sediment isotopes signatures ($\delta^{15}N$, $\delta^{13}C$) showed vertical differences only in $\delta^{13}C$ samples from the Tubul Estuary, with surface sediment being more enriched. Values of $\delta^{13}C$ in these sediments ranged from about −22 to −18‰ suggesting the importance of littoral production from attached algae and detritus in the estuarine carbon flow (Post, 2002). Furthermore enriched surficial sediment values of $\delta^{13}C$ might indicate carbon sources related to allochthonous inputs (i.e., atmospheric CO_2 and weathered carbonates, −20 to −14‰) rather than heterotrophic respiration (−28 to −20‰ ;Post, 2002). Our results suggest that differences in the $\delta^{13}C$ values between the two estuaries and deeper/surface sediments are driven by differences in primary production.

Ghostshrimp, stable isotopic ratios were most similar to the sediment values reflecting the feeding behavior of this benthic crustacean (Schlacher and Wooldridge, 1996). The higher trophic position of the shore crab and the seasonal changes of $\delta^{15}N$, and $\delta^{13}C$ signatures measured for this organism in both estuaries reflected the opportunistic feeding behavior of this epibenthic species possibly due to the seasonal changes of food availability (Reichmuth et al., 2010). The high variability of $\delta^{13}C$ values in the barnacle in the winter could be explained by seasonal changes of food supplies in the water column. During winter, the absence of plankton blooms that mainly occur during the warm season, and the increased runoff from the land result in these organisms being exposed to suspended particles with different $\delta^{13}C$ sources. The differences among sites in $\delta^{15}N$ and $\delta^{13}C$ signatures in the ragworm reflected the opportunistic characteristics of this species.

Taking into consideration that $\delta^{15}N$ typically increases by 3 to 4‰ per trophic level (Post, 2002), the results presented here imply that most of the studied species are at a similar trophic level. Ghostshrimp $\delta^{15}N$ was consistently 3 to 4‰ below that of the other species except in winter for Tubul, indicating a lower trophic level. Despite studies across broader trophic ranges reporting a strong relationship between organic Hg bioaccumulation and $\delta^{15}N$ (Senn et al., 2010) in lower trophic levels of benthic food webs, the assessment of % organic versus organic Hg concentrations and the relationship with carbon sources, may better link to Hg biotransfer to the food web. This might be due to the low variability of MeHg concentrations in benthic secondary producers (Chen et al., 2009).

The differences in the $\delta^{13}C$ signatures between macroinvertebrate species indicated different carbon sources in their diets (Carmichael et al., 2004; Michener and Kaufman, 2006; Chen et al., 2009; Filgueira and Castro, 2011). In the Lenga Estuary the $\delta^{13}C$ signatures of ghostshrimp, barnacle and ragworm reflected a diet of benthic microalgae, *Spartina* detritus and plankton (−15 to −20‰), while for epibenthic shore crab the enriched $\delta^{13}C$ signatures mainly reflected a diet of benthic invertebrates (−12 to −15‰). This study suggests using $\delta^{13}C$ signatures and organic Hg percentages due to the importance of the detritus cycle and benthic diatoms as a potential pathway of Hg exposure to macroinvertebrates.

4.1. Conclusions

This study documents elevated Hg concentrations in invertebrates and sediment from a historically polluted estuary in south central Chile, compared with a nearby reference estuarine site. Hg concentrations in sediment and macroinvertebrates associated with a historically contaminated site (chlor-alkali plant) have remained high relative to other estuaries despite closure of the chlor-alkali facility. The polychaete, *P. gualpensis*, showed a strong sediment site-specific response and may provide a valuable biomonitoring tool for Chilean decision makers and environmental authorities. Sediment physicochemical characteristics are important in the transfer of organic forms of Hg from the sediment to the biota. Stable isotope signatures provide a means of understanding the feeding habits of macroinvertebrates and the relationships with organic Hg percentages suggest that the organic forms of Hg in the studied species are mainly related to the carbon sources. Future studies should focus on determining the influence of age/size and feeding habits on Hg uptake, the speciation of specific organic forms of Hg such as methyl-Hg and factors influencing the bioaccumulation of Hg from sediment contaminated estuaries.

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