



Spatial Variation in Mercury Bioaccumulation and Magnification in a Temperate Estuarine Food Web

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Estuaries are renowned sinks or repositories of contaminants and reflect historical pollution of persistent compounds. In particular, mercury (Hg) contamination is widespread in coastal environments and occurs in both inorganic (THg) and highly toxic organic forms (OHg) with high bioaccumulation potential. Trophic magnification factors have been increasingly used to quantify biomagnification and represent the average rate of change in contaminant concentration throughout a food web. Here, we assessed small-scale spatial variation in THg and OHg concentrations, as well as variations in local trophic magnification factors in three segregated areas of the Tejo estuary. Selected sites covered a gradient of contamination from industrial Hg hotspots to a natural reserve area, and are key nursery areas for multiple fishes. We analyzed concentrations in sediment and biota, representing the entire local food webs. Samples included sediments, primary producers (salt marsh plants), primary consumers (macrobenthic invertebrates) and top consumers (fish muscle and liver), and the trophic web structure was characterized via SIAR mixed-modeling of nitrogen and carbon isotopic ratios. Spatial variation in Hg concentrations was observed in sediment and biota (but not for all species), with highest concentrations in the area near historical mercury input. Hg concentrations increased with trophic level, and so did the OHg fraction (% of OHg relative to THg), with mean maximum values up to 48.7 and 94.9% in invertebrates and fish, respectively. Trophic magnification factors were positive for all sites ($p < 0.05$ for all regressions), ranging between 1.56 to 1.76 and 1.78 to 2.47 for THg and OHg, respectively. Overall, rates of mercury bioaccumulation were similar across sites with variations in biota Hg concentrations reflecting baseline differences in site environmental levels. Understanding mercury bioaccumulation and magnification in estuarine biota is critical to safeguard the multiple ecologic functions and economic benefits estuaries provide.

Keywords: mercury, trophic transfer, isotopic signature, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, nursery area, Tejo estuary

INTRODUCTION

Mercury (Hg) pollution is a prevailing issue worldwide due to the long-distance transport and persistence in the environment of Hg, coupled to the high biomagnification and toxicity potential of this element in animals and humans (Minamata Convention on Mercury, UN treaty 2013). In marine and coastal environments, Hg occurs in many forms (Gworek et al., 2016). Inorganic Hg (Hg^{2+}) is the most common, however, it can be biologically (and chemically) converted to the highly toxic organic form of methylmercury (OHg), that biomagnifies through trophic transfer in marine foods webs (e.g., Baeyens et al., 2003; Chen et al., 2009; Coelho et al., 2013).

Trophic magnification factors (TMF) have been increasingly used to quantify the biomagnification of chemicals in the environment, from legacy-type contaminants (e.g., organochlorines) to emerging chemicals of concern, such as pharmaceutical compounds (e.g., Houde et al., 2008; Liu et al., 2017; Matulik et al., 2017). TMF refers to the average rate of change in contaminant concentrations from diet-to consumer along the trophic web, which differs from classic bioaccumulation assessments based on single predator-prey relationships (Borga et al., 2012). The TMF approach relies on combining bioaccumulation data with a characterization of trophic structure, usually via nitrogen and carbon stable isotope analysis and fractionation. Overall, nitrogen isotopic ratios are indicators of species trophic level given the predictable stepwise $\delta^{15}\text{N}$ enrichment across trophic levels of consumers (typically 3.4‰) (Minagawa and Wada, 1984; Post, 2002). On the other hand, carbon isotopic ratios are associated with sources of primary producers in the trophic web (e.g., salt marsh plants, seagrass) and have reduced trophic fractionation throughout trophic levels [ca. 0.4‰ following Post (2002)]. Hence, TMF is determined from the slope of a regression between the chemical concentration and trophic level of organisms in the food web. In TMF, slope values above zero are indicative of biomagnification (i.e., $\text{TMF} > 1$), whilst the intercept of the regression reflects the concentration of contaminants at the base of the food web (Borga et al., 2012).

Trophic magnification factors assume that diet is the main exposure route to contaminants, hence trophic level is the main driver for bioaccumulation. However, various confounding factors and uncertainties potentially influence TMF, including differences in species biology and/or ecology (e.g., metabolism, reproductive stage, life-cycle), seasonal and spatial variation in isotopic ratios, as well as other local or ecosystem characteristics such as productivity and physical or chemical parameters (Borga et al., 2012). In fact, Borga et al. (2012) argued that different chemical input among sites within a system, or local hotspots, would be reflected in local food webs and in the variability of TMF at small spatial scales.

Estuaries have been described as sinks or repositories of contamination, reflecting historical contamination of Hg and OHg, particularly in bottom sediments and plants (e.g., Ridgway and Shimmield, 2002; Canário et al., 2010). These are highly productive ecosystems, with conspicuous environmental variability and habitat complexity, thus providing an appealing

natural setting to test small-scale spatial dynamics in Hg and OHg bioaccumulation and biomagnification along food webs. Moreover, estuaries have long been preferred areas of human settlement, and are globally threatened by multiple human activities that jeopardize their environmental quality and consequently their many ecological functions (Beck et al., 2001; Kennish, 2002). The Tejo estuary is no exception. Whilst renowned for its importance as a nursery area for multiple commercially important fish species (Vasconcelos et al., 2010; Reis-Santos et al., 2013), it also supports a wide array of economic activities (e.g., industrial and urban development, large shipping and port activities, fishing) (Vasconcelos et al., 2007). This estuarine system is one of the largest estuaries in Europe and is the most impacted along the Portuguese coast, with high levels of Hg contamination in sediments and water from historical anthropogenic sources (Figuéres et al., 1985; Canário et al., 2005; Cesário et al., 2017). However, there are no published data on Hg concentration in biota from the Tejo, and we still lack an understanding on small scale variability in Hg contamination, and how this may underpin local bioaccumulation processes.

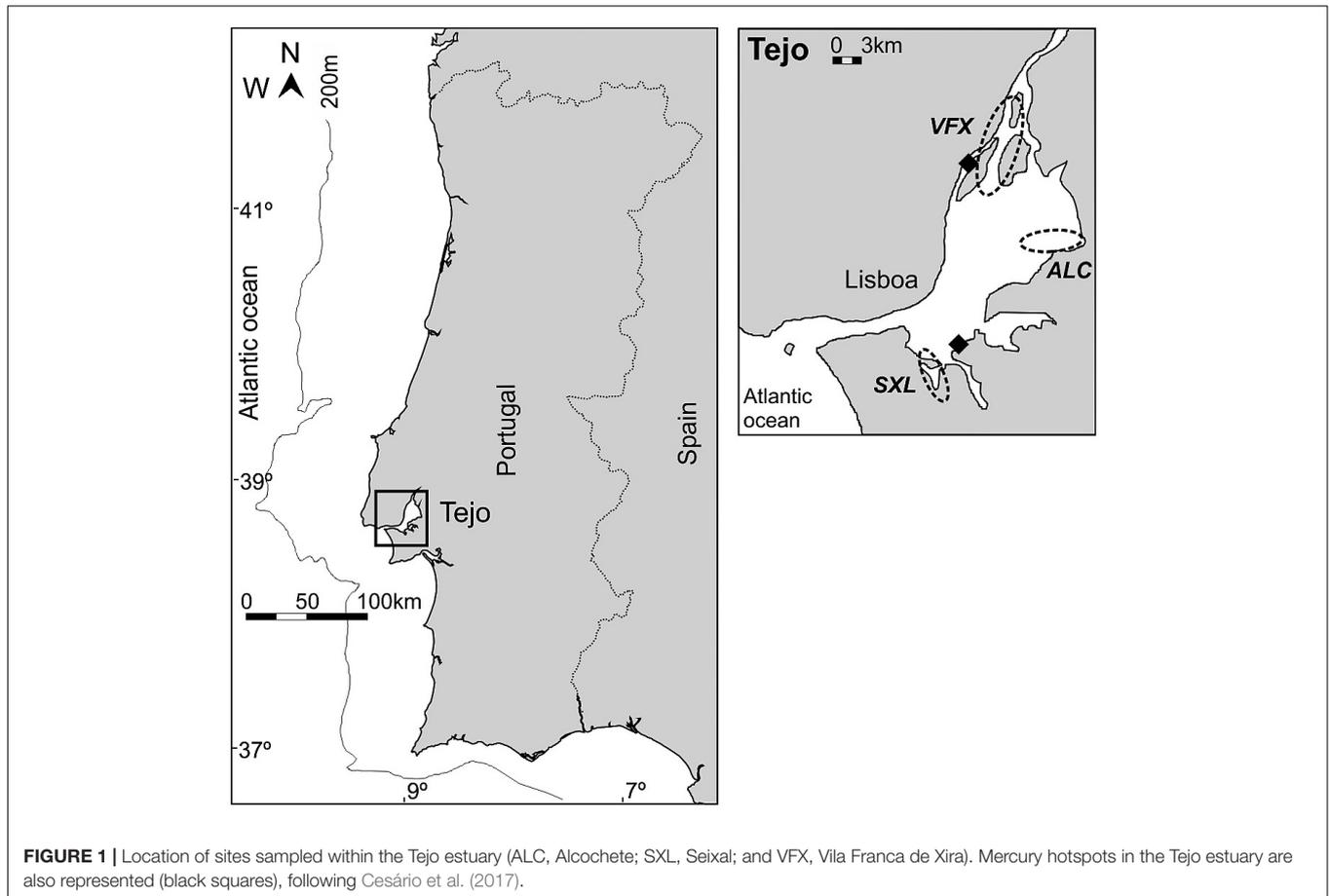
In this context, we hypothesize that mercury trophic magnification processes in the Tejo estuary are driven by spatial variability in mercury concentration. Hence, the main goal of this work is to assess small-scale spatial variability in mercury concentrations (THg and OHg) in sediments, biota, and in trophic magnification processes among segregated nursery areas with different abiotic conditions within a large temperate estuary.

MATERIALS AND METHODS

Study Area

The Tejo estuary, a permanently open temperate estuarine system with a surface area of 320 km², has extensive intertidal mudflats fringed by salt marshes that make up to 40% of the estuarine area, and play a vital ecological role for numerous invertebrate and fish species (e.g., Fonseca et al., 2010; Duarte et al., 2018). Previous work has identified key segregated nursery areas or important habitats for highly commercial fish species, such as Senegalese sole (*Solea senegalensis*), European sea bass (*Dicentrarchus labrax*) or Lusitanian toadfish (*Halobatrachus didactylus*) (Vasconcelos et al., 2010; Cotter et al., 2013; Reis-Santos et al., 2013); with muscle stable isotope ratios and otolith elemental signatures, among others, demonstrating high site fidelity and restricted intra-estuarine fish movements among segregated nursery areas (Cotter et al., 2013; Tanner et al., 2013; Reis-Santos et al., 2015, 2018).

In terms of mercury pollution, historic contamination has been described in water, sediments and plants of the Tejo estuary, with two areas identified as Hg hotspots from industrial sources (Cesário et al., 2017) (Figure 1). Consequently, three estuarine areas were sampled based on prior information on species occurrence and abundance, trophic web structure and mercury level contamination (França et al., 2011; Fonseca et al., 2013, 2014; Cesário et al., 2017). Sampled



areas were selected to reflect an expected gradient of Hg contamination: with lower Hg concentrations at a site located in the Nature Reserve of the Tejo estuary (ALC) and distant from Hg hotspots; an intermediate contaminated site in the bay of Seixal (SXL), south of an Hg hotspot, and with hydrodynamics and particle exchange dominated by the outer estuary; and a third site with higher Hg levels, in the vicinity of a renowned Hg contamination hotspot (VFX) (**Figure 1**). All sites are nursery areas for juveniles of commercially important fish species.

Sampling Survey

Sampling occurred over three consecutive days in late September 2016 and included three to five replicate samples per site of surface sediments, salt marsh plants (*Halimione portulacoides*, *Sarcocornia fruticosa*, *Spartina maritima*), endobenthic and epibenthic macroinvertebrates (*Hediste diversicolor*, *Scrobicularia plana*, *Carcinus maenas*) and fishes (*Pomatoschistus microps*, *H. didactylus*, *D. labrax*, and *S. senegalensis*). Epibenthic macroinvertebrates and fish were collected with a beam trawl during ebb-tide at each site. Hauls were performed at a constant speed for 5–10 min, and GPS coordinates registered. Collected *D. labrax*, *S. senegalensis*, and *H. didactylus* were age 0⁺/1 juveniles from the same yearly cohort; and all *P. microps* (a small sized goby) were c. age 1⁺/2 (Cabral and Costa, 2001;

Leitão et al., 2006; Cotter et al., 2013; Tanner et al., 2013; Reis-Santos et al., 2015). Surface sediments (top 5–8 cm), endobenthic invertebrates (*H. diversicolor*, *S. plana*) and primary producers (salt marsh plant species) were collected by hand at low tide in intertidal areas in each site, on the same day and locations of fish sampling. This sediment layer was selected to best represent both the main vertical habitat use of sampled endobenthic macroinvertebrates (Cardoso et al., 2010) and the depth of increased root biomass and root metal retention of salt marsh plants (Duarte et al., 2010). Salinity and water temperature were measured with a multi-parameter probe to characterize and compare the environmental conditions of sampling locations.

Upon collection samples were transported on ice to the laboratory, where individuals were measured and weighed, and appropriate tissues collected (three to five replicates per species and tissue). Tissues with highest accumulation potential were selected, whenever possible, specifically: roots in plant species, digestive gland in *C. maenas* and *S. plana*, and muscle and liver in all fish species except for *P. microps*. The latter were processed as whole individuals as were polychaetes *H. diversicolor* due to their small size. Sediment samples were weighted, freeze-dried, ground and sieved through a 1-mm sieve and stored prior to Hg determination. All biological samples were washed with ultra-pure water, prior to being freeze-dried,

and subsequently ground to a fine powder for dry-weight determination and Hg analysis.

Mercury Quantification

Total Hg in sediments (SedHg) was determined by atomic absorption spectrometry (AAS) with thermal decomposition and gold amalgamation, using a LECO 254 advanced mercury analyser (AMA) (Costley et al., 2000). The accuracy and precision of the analytical methodology for SedHg determinations was assessed by replicate analysis of certified reference materials (CRM), namely Mess-3 (marine sediments). Precision of the method was better than 9% ($n = 17$), with recovery efficiency of 119.8% ($n = 4$). Blanks evaluated between each sample were always <1% of the SedHg.

Total mercury (THg) determination in biological samples was carried out in three to five replicate samples per species and per site, by AAS, as described for SedHg determination. The accuracy and precision of the analytical methodology for THg determinations were assessed by daily replicate analysis of certified reference materials, namely ERM-CD200 (seaweed), ERM-CE278k (mussel tissue), Tort-3 (lobster hepatopancreas) and Dorm-4 (fish protein). Precision of the method was always better than 9%, with a recovery efficiency ranging from 85.0 to 105.4% (**Supplementary Table S1**).

Organic mercury (OHg) was quantified in three randomly selected samples of each invertebrate and fish species per site (due to limitations in salt marsh root material). Each sample was prepared via duplicate digestion with a mixture of 18% KBr in 5% H₂SO₄, followed by extraction into toluene and then back-extracted into an aqueous sodium thiosulfate solution (for further details see Válega et al., 2006). Samples were analyzed in the same AMA-LECO 254 instrument, together with the reference materials mentioned above. The precision of the method was always better than 10%, and the extraction efficiency ranged from 75.1 to 86.7% (**Supplementary Table S1**).

Data Analysis

Data were first tested for normality and homogeneity of variances, and where necessary log-transformations were applied to meet these assumptions. Analysis of variance (ANOVA) were followed by *a posteriori* multiple comparison Tukey tests to assess differences in length and weight of individuals, and in sediment and organisms' mercury concentrations, as well as in abiotic conditions among estuarine sites. Comparison of organisms' mercury concentrations tested differences among all species, and differences among sites *per* species, whenever individuals occurred in all three sites. *Student's t*-tests were used to test for site differences when species occurred in only two sites (i.e., *S. senegalensis*). When data was not normally distributed, even following log-transformation (e.g., THg content in *D. labrax* muscle samples and *H. diversicolor*), equivalent non-parametric tests were applied, namely Kruskal–Wallis tests followed by *a posteriori* Dunn's test for multiple comparisons, and Mann-Whitney *U*-tests for two-sample comparisons. In addition, mercury content in fish muscle and liver tissues (*D. labrax*, *S. senegalensis*, and *H. didactylus*) were analyzed separately when assessing species and spatial differences. For simplicity, and due

to significantly high correlations between mercury concentration in liver and muscle replicates from the same fish ($r = 0.82$ and 0.71 for THg and OHg, respectively), only results based on liver Hg content are considered when testing for inter-species comparisons. Correlation analysis (Spearman) were also used to test for a relation between THg and OHg content in invertebrates and fish species.

Trophic level (TL) determination was based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data, and the methodology described in França et al. (2011), which fully characterized the differential Tejo estuarine trophic webs of ALC and VFX using carbon and nitrogen stable isotope analysis. We used both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, as their combined use enhances food web assessments (Parnell et al., 2010). Briefly, the SIAR mixing model package was used to estimate the relative importance of the primary sources of organic matter to the diet of each consumer. According to Parnell et al. (2010) this Bayesian inference approach allows for dealing with the variability of the input data (i.e., among sources and among samples). In this work, input data relied on França et al. (2011) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (and standard errors) for all sources and consumer species at the estuarine level. Additionally, trophic enrichment factors (TEF) and uncertainties in model runs followed Post (2002) and were specifically $0.4 \pm 1.3\text{‰}$ for $\delta^{13}\text{C}$ and $3.4 \pm 1.0\text{‰}$ for $\delta^{15}\text{N}$. Trophic Level for each consumer species was then calculated according to:

$$\text{TL} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{producer}_{\text{mix}}}) / 3.4 + 1$$

where 3.4‰ is the above-mentioned trophic enrichment factor, $\delta^{15}\text{N}_{\text{consumer}}$ is the $\delta^{15}\text{N}$ signature for each consumer species, and $\delta^{15}\text{N}_{\text{producer}_{\text{mix}}}$ is the proportion of the various primary producers (or mixture of sources of organic matter) that contributed to the consumer's diet (the latter attained through the SIAR model). The TL of salt marsh plants was set at 1, as they are primary producers (Borga et al., 2012). For *H. didactylus*, and in the absence of information on local $\delta^{15}\text{N}$ for this fish species, a TL of 3.95 was defined based on information from FishBase (Froese and Pauly, 2018).

Trophic magnification factors were determined for both THg and OHg at site and estuary levels. TMF considered all available measurements along the trophic web, with fish muscle and liver Hg concentrations analyzed separately. The first step was to regress the logarithm-transformed THg and OHg concentrations in organisms ($\text{Log} [\text{THg or OHg}]$) with TL, as *per* the following equation:

$$\text{Log} [\text{THg or OHg}] = a + b\text{TL}$$

TMFs or the rate of trophic transfer of THg and OHg along the food web were calculated as the antilog of the regression slopes (b):

$$\text{TMF} = 10^b$$

Slope values (b) above zero are indicative of biomagnification, with higher values or steeper slopes reflecting increased rates of accumulation over the food web ($\text{TMF} > 1$), whereas the intercept (a) of the regression reflects mercury input at the base of the food web (e.g., Borga et al., 2012). Analysis of covariance (ANCOVA), were used to test spatial variability

in mercury trophic magnification throughout food webs, by evaluating differences in either slope or intercept of multiple regressions of both THg and OHg. All statistical analyses were performed using R software, and a significance level of <0.05 was considered in all tests.

RESULTS

Total mercury concentration in sediments ranged between 0.42 and 0.62 mg kg⁻¹ dry weight (dw), and varied among sites ($F = 28.6$, $p < 0.001$), with significantly lower THg levels in ALC (Table 1). Likewise, salinity differed among sites ($F = 11.7$, $p < 0.01$, Table 1) but not temperature. Length and weight of collected organisms was similar among sites (Table 2) (ANOVA and Student's t , $p > 0.05$), hence no size-adjustments were applied.

Total mercury concentration along the estuarine trophic web varied between 0.01 mg kg⁻¹ dry weight in the roots of saltmarsh plants (*S. fruticosa*) and 1.51 mg kg⁻¹ dry weight in fish liver (*D. labrax*). For fish species, we converted dry weight contamination to mg/kg wet weight, to allow comparison of our results with EU regulation EC 1881/2006 (regarding the maximum levels of contaminants in foodstuffs, set in mg kg⁻¹ wet weight), with maximum THg values of 0.27 and 0.34 mg kg⁻¹ wet weight measured in muscle and liver samples of *D. labrax*.

Significant differences in THg load among species were observed ($F = 35.16$ $p < 0.001$), yet THg did not vary among primary producers (Tukey tests, $p > 0.05$), nor among

macroinvertebrate species (Tukey tests, $p > 0.05$). THg was also similar across fish species, except for *S. senegalensis*, which had significantly lower THg concentrations than remaining fish and invertebrate species (Tukey tests, $p < 0.05$). Notwithstanding, an increasing trend in THg from primary producers to consumers was evident (Figure 2), with mean THg ranging from 0.03 to 0.15 mg kg⁻¹ dw in primary producers; from 0.20 to 0.47 mg kg⁻¹ dw in primary consumers; and from 0.15 to 1.03 mg kg⁻¹ dw in fish tissues (Figure 2).

Regarding spatial variation of THg per species, highest concentrations were generally observed in organisms from ALC and VFX. In fact, organisms from SXL presented significantly lower THg values when compared to individuals from ALC and VFX [specifically, invertebrates *H. diversicolor* ($H = 11.68$ $p < 0.001$), *C. maenas* ($F = 10.56$, $p < 0.01$), and fish *P. microps* ($F = 25.28$, $p < 0.01$) and *D. labrax* muscle samples ($H = 7.58$ $p < 0.05$)]. However, no significant differences among sites were observed for THg in saltmarsh plants (*H. portulacoides* and *S. fruticosa*, $F < 2.27$, $p > 0.05$), in the bivalve *S. plana* ($F = 1.94$, $p > 0.05$), in *D. labrax* liver samples ($F = 0.957$, $p > 0.05$), and in liver and muscle of *S. senegalensis* (Student's $t < 2.04$, $p > 0.05$).

Mean organic mercury concentrations ranged from 0.01 to 0.23 mg kg⁻¹ dry weight in invertebrates and from 0.11 to 0.70 mg kg⁻¹ dry weight in fish (Figure 3). OHg content also differed among species ($F = 24.54$, $p < 0.01$), with significantly lower values in *H. diversicolor* (Tukey tests, $p < 0.05$), whilst highest values were observed in *D. labrax* (Tukey tests, $p < 0.05$), although with OHg levels comparable to *P. microps* and *H. didactylus* (Figure 3). An increasing trend in OHg concentrations along trophic levels was evident, despite some overlap between OHg values in invertebrate and fish species. OHg burden in *C. maenas* was comparable to all fish species, except *D. labrax* (Tukey tests, $p > 0.05$), whilst OHg content in *S. senegalensis* was comparable to *S. plana* (Tukey tests, $p > 0.05$). Likewise, the organic mercury fraction (% of OHg relative to THg) increased with trophic level, with mean values for invertebrate species ranging from 8.87 to 48.7% in invertebrate species (*H. diversicolor* and *C. maenas*, respectively), and from 63.7 to 94.9% in fish (*H. didactylus*).

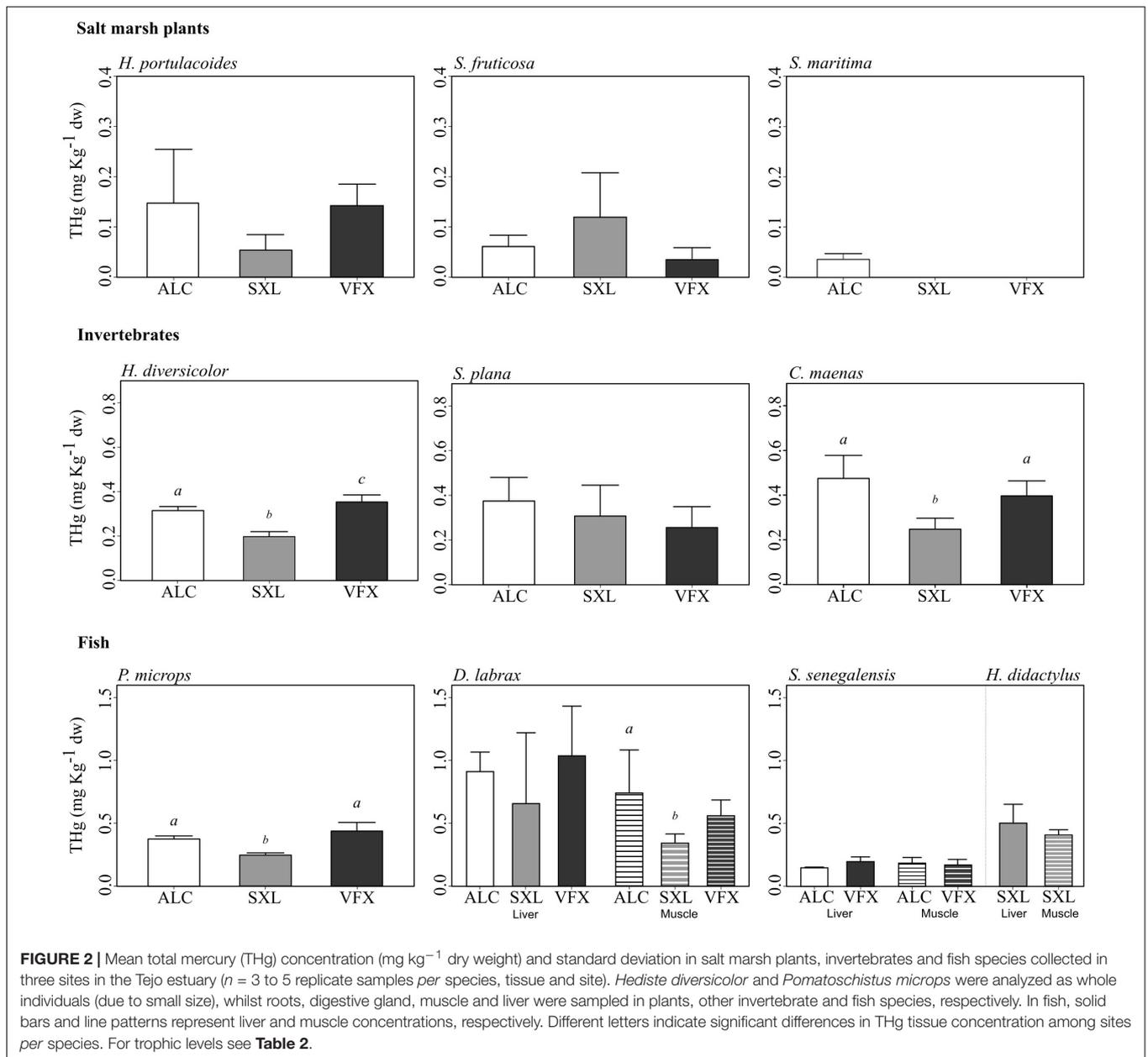
TABLE 1 | Mean (and standard deviation) salinity and temperature measured in each site of the Tejo estuary surveys (ALC, SXL, and VFX); and total mercury concentration (and standard deviation) in the sediment (top 5–8 cm layer) (sediment THg).

	ALC	SXL	VFX
Temperature (°C)	20.9 (0.26)	21.8 (1.07)	21.58 (0.47)
Salinity	20.8 (1.64)	29.12 (0.12)	13.29 (4.54)
Sediment THg (mg Kg ⁻¹ dry weight)	0.43 (0.02)	0.55 (0.05)	0.52 (0.01)

TABLE 2 | Mean (and standard deviation) of length (Lt in cm), and weight (Wt in g) of invertebrates and fish sampled per estuarine site (ALC, SXL, and VFX).

	ALC		SXL		VFX		TL
	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	
Invertebrates							
<i>S. plana</i>	3.84 (0.18)	6.91 (1.31)	3.64 (0.45)	6.10 (2.58)	3.52 (0.31)	5.73 (1.41)	1.51
<i>H. diversicolor</i>		1.77 (0.75)		1.43 (0.88)		1.86 (0.33)	2.14
<i>C. maenas</i>	4.91 (0.51)	29.3 (7.97)	4.74 (0.35)	28.32 (6.58)	4.62 (0.65)	26.64 (9.05)	2.50
Fish							
<i>P. microps</i>	3.85 (0.47)	0.40 (0.16)	3.95 (0.63)	0.45 (0.17)	3.92 (0.39)	0.54 (0.16)	3.27
<i>S. senegalensis</i>	14.2 (2.60)	31.7 (16.1)			14.2 (3.94)	33.28 (17.64)	3.78
<i>H. didactylus</i>			16.5 (2.33)	78.4 (27.2)			3.95
<i>D. labrax</i>	12.4 (1.24)	18.4 (4.15)	12.7 (4.66)	24.4 (6.59)	10.9 (4.95)	19.35 (7.52)	3.38

Trophic level (TL) determined via SIAR mixed modeling is also shown.



Regarding spatial differences in OHg content *per* species, concentrations were significantly higher in VFX for *H. diversicolor*, *P. microps*, and *D. labrax* muscle samples ($F > 7.56$, $p < 0.05$, Tukey tests, $p < 0.05$). OHg concentrations in muscle of *D. labrax* from ALC also differed from the lower OHg levels measured in SXL (Tukey tests, $p < 0.05$) (**Figure 3**).

Overall, a significant positive correlation between THg and OHg was observed, when considering both fish liver and muscle samples ($\rho > 0.58$, $p < 0.05$).

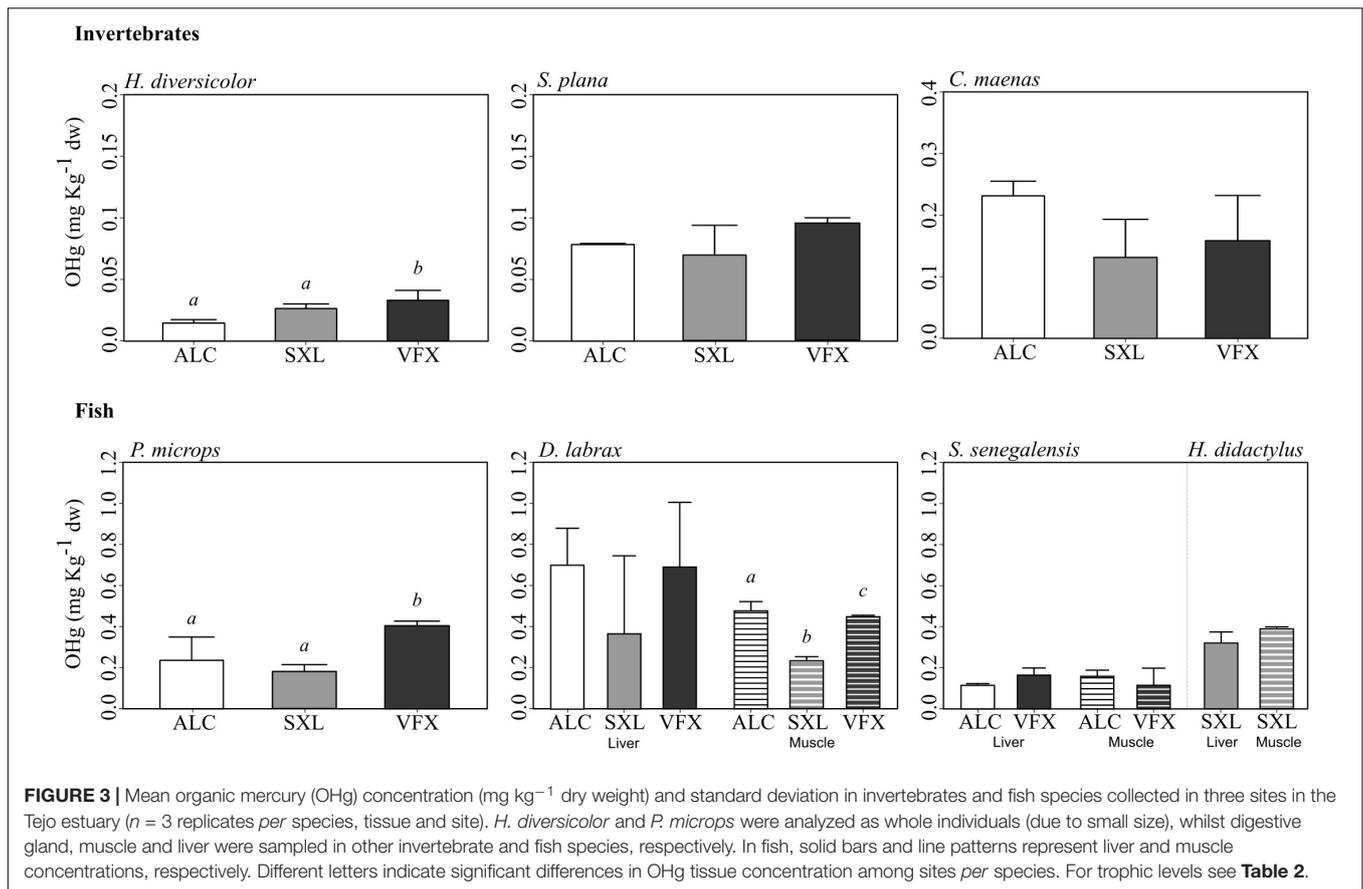
Mercury trophic magnification was evident for both mercury forms, with significant regressions of $\log[\text{Hg concentration}]$ with organisms' trophic levels ($p < 0.05$, **Table 3**). TMF values ranged between 1.56 and 1.76 for THg and between 1.78 and

2.47 for OHg, with fish muscle and liver samples analyzed separately (**Table 3**).

Analysis of covariance did not show significant differences among regressions, in either slopes or intercepts, for both THg and OHg (ANCOVA, $p > 0.05$).

DISCUSSION

Spatial variability was observed in sediment THg and reflected the expected gradient across sites, with lower values in ALC, the sampling site located furthest from known Hg hotspots and well within the Tejo Natural Reserve. Nonetheless, the range of THg sediment concentrations was not as wide



as could have been expected given the significant distance between sites (12 to 24 km). This may result from natural attenuation of Hg concentration of surface sediments in Hg hotspots or by high Hg transport occurring within the Tejo estuary (Cesário et al., 2018). Tidal influence (which reaches c.80 km landward from the entrance of the estuary) and river discharge (which occurs near the historic Hg hotspot of VFX) determine the hydrology of the Tejo system (Vaz and Dias, 2014) and therefore influence the anthropogenic Hg signal throughout the estuary, and explain Hg signals near ALC (Cesário et al., 2016). Overall, present values of THg concentration in sediments were within the range of historic anthropogenic Hg values measured within the Tejo estuary, although considerably higher THg concentrations (up to 125 mg kg^{-1}) have been measured in deeper sediment ($>10 \text{ cm}$) (Cesário et al., 2016, 2017).

Spatial variability in mercury content of estuarine organisms was also observed for some species, namely invertebrates *H. diversicolor* and *C. maenas*, and fish species *P. microps* and *D. labrax* (muscle samples). The variation pattern was similar between THg and OHg across invertebrate and fish species, with generally higher values in the area located near an Hg hotspot (VFX). Nevertheless, lower Hg levels were observed in organisms collected from SXL, where maximum THg values were determined. Differences in mercury bioavailability among sites, in association to the different local abiotic conditions

(e.g., salinity, pH, dissolved organic matter) may be the cause for the observed mismatch between mercury concentration in organisms and sediments from SXL (e.g., Mason et al., 1993; Schartup et al., 2015). However, baseline biogeochemical data and knowledge on local mercury fluxes and behavior will be required to further resolve this issue. In addition to varying environmental Hg concentrations, dietary plasticity can also influence inter-site variation in Hg accumulation if individuals are able to explore different prey items or temporary peaks in abundance of prey that differ from one area to another (e.g., Ley et al., 1994). This could be the case for predator species *P. microps*, *D. labrax*, and *H. didactylus* (Thorman and Wiederholm, 1986; Cabral and Costa, 2001; Pereira et al., 2011), or omnivorous species *C. maenas* (Baeta et al., 2006) and *H. diversicolor*, although the latter may act both as a predator and a deposit-feeder (Scaps, 2002). Nonetheless, considering that ALC and VFX have similar benthic trophic web structures (França et al., 2011), it is more likely that variations in Hg concentration and bioavailability underpin the observed inter-site differences in Hg bioaccumulation.

Regarding other analyzed species, mercury load did not vary across sites in salt marsh plants, in the bivalve *S. plana*, or in the senegalese sole *S. senegalensis*. Halophytes are known to retain metals and metalloids, particularly in roots (Duarte et al., 2010), with recent studies evidencing high tolerance and regulation of mercury content in salt marsh species (e.g., Anjum et al., 2016;

TABLE 3 | Regression analyses of log-transformed total (THg) and organic mercury (OHg) concentrations in estuarine organisms with species trophic level, per site (ALC, SXL, and VFX) and in the Tejo estuary.

	<i>n</i>	intercept	slope	<i>F</i>	<i>p</i>	TMF
THg_{Liver}						
Estuary	112	1.822	0.241	67.1	<0.001	1.74
ALC	38	1.851	0.246	19.6	<0.001	1.76
SXL	37	1.756	0.240	34.6	<0.001	1.74
VFX	37	1.860	0.234	16.8	<0.001	1.71
THg_{Muscle}						
Estuary	112	1.875	0.203	57.3	<0.001	1.60
ALC	38	1.913	0.205	16.1	<0.001	1.60
SXL	37	1.799	0.211	34.8	<0.001	1.63
VFX	37	1.920	0.192	13.0	<0.001	1.56
OHg_{Liver}						
Estuary	54	1.156	0.347	25.9	<0.001	2.22
ALC	18	1.169	0.335	4.8	<0.05	2.16
SXL	18	1.042	0.369	18.5	<0.001	2.34
VFX	18	1.253	0.345	10.0	<0.01	2.21
OHg_{Muscle}						
Estuary	54	1.209	0.317	21.7	<0.001	2.07
ALC	18	1.146	0.331	5.1	<0.05	2.14
SXL	18	0.988	0.393	32.1	<0.001	2.47
VFX	18	1.510	0.251	4.6	<0.05	1.78

Trophic magnification analyses considered all available measurements (*n*, number of samples) of THg (including saltmarsh plants, invertebrates and fish species), and OHg (invertebrates and fish species), with separate analyses considering mercury determination in liver and muscle tissues of larger fish species.

Cabrita et al., 2018). This could also be related to widespread Hg transport throughout the estuary, coupled with species-specific ecological and physiological traits. At lower trophic levels, species could be reflecting the small gradient in Hg surface sediment levels, whilst species at higher trophic positions evidenced higher spatial differences due to trophic transfer processes. *S. plana* and *H. diversicolor* are both sediment-dwelling invertebrates that can behave as deposit feeders (Hughes, 1969; Scaps, 2002), yet showed different THg and OHg accumulation patterns. As such, *H. diversicolor* may be using diverse feeding strategies to explore resources as an omnivore or a predator. On the other hand, dissolved and particulate fractions are likely the main driver of Hg accumulation in *S. plana*. Coelho et al. (2013) has made similar inferences regarding observed differences in trophic level and Hg burden between these two species in another coastal system with significant mercury contamination. Lack of occurrence of juvenile *S. senegalensis* in SXL limited inter-site comparison for this species, given that this site was key to unravel contamination patterns in other fish species.

In terms of mercury trophic transfer, both THg and OHg concentrations increased with trophic level, although the rate of OHg biomagnification was higher, as could be expected (Lawson and Mason, 1998), and evident in the increased proportion of OHg (% of THg) with increasing trophic level. The slopes or rates of Hg trophic transfer (i.e., TMF) were within range of a recent meta-analysis on Hg biomagnification in aquatic food

webs worldwide (Lavoie et al., 2013). These authors described spatial differences in trophic magnification slopes with latitude, yet lack of environmental data hindered analysis on the effects of physical and chemical variation on Hg biomagnification at the ecosystem level. Present results do not allow to address this caveat, since the number of sites was also reduced. In addition, trophic magnification factors did not vary among sites, despite differences in local abiotic features, Hg input, and observed inter-site variation in Hg content of some invertebrate and fish species. The most likely cause is widespread transport and potential homogenization of the distribution of Hg throughout the Tejo estuary, despite the existence of known Hg hotspots (Cesário et al., 2018). Furthermore, continuous dredging and other port activities occurring in the estuary can promote the resuspension of sediments and the potential increase in transport and availability of mercury throughout the system (Eggleton and Thomas, 2004).

In hindsight, spatial gradient in Hg concentrations between sampled sites might not be strong enough to influence TMFs. Though recent modeling approaches have explored the potential bias associated with spatial concentration gradients on chemical biomagnification and TMF in aquatic ecosystems (Kim et al., 2016; Mackay et al., 2016), reported spatial variation in TMFs refer only to wider geographical areas (e.g., Baeyens et al., 2003; Borga et al., 2012).

Uncertainties associated with stable isotopes analysis and trophic level determination could also influence biomagnification assessment and TMF calculations (Borga et al., 2012). Nevertheless, trophic levels were similar to those reported elsewhere for the same species (e.g., Pasquaud et al., 2010; Coelho et al., 2013), and the SIAR mixed-modeling approach enabled the adequate characterization of food web baselines, as emphasized by Lavoie et al. (2013). Though all sampling took place in consecutive days, spatial and seasonal differences in isotopic signatures are known to occur within estuaries (e.g., França et al., 2011; Green et al., 2012) and could influence the consistency of TMF. Accordingly, we encourage future work exploring seasonal variability in TMFs, given such information will be crucial to underpin potential sources of variability in field biomagnifications assessments (Lavoie et al., 2013).

Overall, results show that Hg transport and uptake by individuals is ubiquitous in the Tejo estuary, regardless of the low connectivity among nursery areas, and the distance among sites and to Hg hotspots. Noteworthy, mercury concentrations in juvenile fish life stages did not exceed regulatory standards set for contaminants in foodstuffs (0.5 mg kg⁻¹ wet weight following EC 1881/2006). Nevertheless, observed sediment and individual Hg concentrations fall within the range of concentrations previously linked with deleterious biological effects on vertebrate and invertebrate species (e.g., Long et al., 1995; Depew et al., 2012; Mieiro et al., 2014). Ultimately, anthropogenic emissions of Hg have been significantly reduced in the last decades (Pacyna et al., 2001), yet ongoing contamination and bioaccumulation across ecosystems persists. In the context of nursery areas, which play a key role in the replenishment of coastal fishery stocks, exploring small-scale variation in estuarine Hg contamination is key to

assess potential effects on juvenile biota, and consequent potential long-term implications on the adult coastal fishery stocks targeted for human consumption (e.g., Rochet, 2000; Fonseca et al., 2015).

AUTHOR CONTRIBUTIONS

VF wrote the main draft of the manuscript, based on ideas conceived by VF and PR-S. All authors contributed ideas and to writing the manuscript. PR-S, BD, and VF designed and implemented the field collections. CM and JC performed the extraction and chemical analyses. SF and VF conducted data analysis. All authors reviewed and approved the final submitted version.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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