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Tracking organic mercury bioaccumulation by the brown mussel *Perna perna* (Linnaeus, 1758) in subtropical bays: Environmental exposure and seasonal effects

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ABSTRACT

The brown mussel (*Perna perna*) is a key aquaculture species and sentinel for coastal pollution. We investigated the bioaccumulation of total mercury (THg), total organic mercury (TotOrgHg), and monomethylmercury (MMHg) in *P. perna* from aquaculture farms in three subtropical Brazilian bays with contrasting anthropogenic pressures. Over eight months, mercury species were quantified in mussel tissue, sediments, and suspended solids. Environmental parameters (sediment organic matter) and mussel physiological traits (lipid content, condition index) were assessed. Stable carbon and nitrogen isotope ratios ($\delta^{13}C$, $\delta^{15}N$) were analysed to evaluate trophic influences. Median THg in mussels (41.9 $\mu g \cdot kg^{-1}$) was approximately half that in sediments (96.3 $\mu g \cdot kg^{-1}$) and suspended solids (73.8 $\mu g \cdot kg^{-1}$), showing lower THg_Muss medians than Sed and SS. Lipid content and condition index negatively correlated with TotOrgHg and THg, indicating a biodilution effect. A moderate correlation was found between TotOrgHg and MMHg, highlighting limitations of TotOrgHg as a proxy for MMHg. $\delta^{15}N$ correlated with %MMHg only in the least impacted bay, suggesting trophic modulation of methylation under lower contamination. The human health risk assessment showed that a 60 kg adult could exceed the tolerable weekly intake for MMHg by consuming fewer than five mussels weekly. These findings confirm *P. perna* as an effective biomonitor and provide new insights into the drivers of mercury speciation in tropical coastal aquaculture.

1. Introduction

Nearly 75 years after the Minamata disaster, mercury remains the third most hazardous substance on the ATSDR's priority list due to its persistence, global transport, and bioaccumulation in food webs (ATSDR, 2022). Despite global efforts to reduce its environmental presence, mercury pollution continues to pose serious challenges, particularly in developing countries such as Brazil, where limited resources hinder monitoring and mitigation efforts. As a signatory to the Minamata Convention, Brazil is committed to monitoring mercury as one of its six priority pollutants (MMA, 2024). Given the widespread use of mercury in artisanal gold mining in Brazil, mercury pollution hotspots are typically associated with gold-mining regions in the Amazon basin and with industrialised/urbanised estuaries and bays receiving long-term contaminant inputs (Lacerda and Malm, 2008; Selin and Selin, 2022). Contaminated effluents from mining are often inadequately treated, resulting in significant mercury releases to soils and water bodies, especially from illegal operations (Castilhos and Domingos, 2024). Considering its persistence in aquatic systems, mercury from gold extraction in drainage basins can be transported to coastal zones, where

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it accumulates in sediments over time. In Brazil, coastal pollution is further intensified by limited or poorly maintained sewage treatment infrastructure and the uncontrolled discharge of urban and industrial effluents, compounding legacy contamination in historically impacted bays. In light of this, evaluating human exposure through food webs is critical. In the United States, for instance, an extensive survey reported in utero exposures to methylmercury (MMHg) above safety thresholds in hundreds of thousands of cases (Mahaffey, 2005).

Although mussels are widely recognised as effective bioindicators of metal contamination, data on the brown mussel *Perna perna*—a species of growing aquaculture and ecological importance in tropical and subtropical regions—remain scarce, limiting its application in large-scale biomonitoring programmes. This gap contrasts sharply with the species' wide biogeographic range, its increasing relevance in tropical aquaculture, and the existence of long-standing biomonitoring initiatives such as the U.S. EPA's Mussel Watch program, which has tracked contaminant trends in bivalves to assess coastal pollution since 1986 (O'Connor, 1998). The brown mussel *P. perna* spans a distribution from Sri Lanka to the Atlantic coasts of the Americas, including the Caribbean (Fernandes et al., 2008), supporting its use as a sentinel organism and facilitating comparisons across sites. In Brazil, marine farms produce approximately 5301.84 tons annually (MPA, 2024). Notably, Latin America and the Caribbean experienced the second-highest growth in animal aquaculture between 2000 and 2020 (FAO, 2024). Despite its nutritional value and economic relevance, *P. perna* and other filter-feeding shellfish can serve as vectors for environmental contaminants, including microplastics (Gündogdu et al., 2023).

Concerns are especially acute regarding mercury, especially its most toxic and bioavailable form monomethylmercury (MMHg), which is subject to biomagnification (ATSDR, 1999). In aquatic environments, MMHg is predominantly formed in situ via microbial methylation of inorganic Hg under favourable biogeochemical conditions, linking environmental Hg inputs to food-web exposure (Correia and Guimarães, 2017, 2016). While recent food regulations increasingly target MMHg rather than total mercury (THg), many research laboratories - particularly in low- and middle-income countries—still rely on simpler, less selective methods. To bridge this analytical gap—particularly where advanced speciation techniques are unavailable, some studies have explored total organic mercury (TotOrgHg) as a potential proxy for MMHg, with varying levels of success (Ackerman et al., 2013; Wagemann et al., 1997). Previous studies indicate that TotOrgHg may serve as a pragmatic screening indicator for methylmercury (MMHg) in aquatic organisms, although its performance is strongly species- and context-dependent. While good agreement between mercury speciation and organic mercury fractions has been reported in aquatic invertebrates, the proportion of MMHg relative to total mercury varies widely across trophic levels and environmental conditions, underscoring the need for cautious and site-specific application of this proxy (Kehrig et al., 2010; Taylor et al., 2008; Watras et al., 1998).

Along Brazil's 8000 km coastline, the proximity of major urban centers to coastal waters exacerbates environmental pressures through untreated sewage and industrial discharge. Despite these threats, data on MMHg concentrations—and their seasonal and spatial dynamics—in Brazilian coastal ecosystems remain scarce. Previous studies conducted in Brazilian coastal environments indicate that mercury bioaccumulation in the brown mussel *P. perna* does not follow a simple linear relationship with environmental contamination levels. Reported mercury concentrations in mussel tissues range widely, even among sites with comparable degrees of anthropogenic pressure (Kehrig et al., 2001, 2002). Such variability suggests that local environmental conditions play a critical role in modulating mercury uptake by mussels. Transplant experiments further demonstrate that *P. perna* can rapidly respond to changes in environmental mercury exposure, while still exhibiting site-specific bioaccumulation patterns governed by local biogeochemical conditions (Longo et al., 2018). Seasonal variations are known to influence Hg dynamics, with runoff during the rainy season enhancing particulate-bound Hg transport and promoting microbial methylation under anoxic conditions (Molina et al., 2023). Several studies have also shown that seasonal rainfall influences heavy metal uptake in filter-feeding bivalves, including mussels, by altering suspended particulate loads, organic matter availability, and metal bioavailability in coastal waters (Nguyen Thanh Kim et al., 2025; Shenai-Tirodkar et al., 2018).

In tropical and subtropical Brazilian coastal systems, field studies have consistently shown that the rainy season enhances mercury transport through increased river discharge and suspended particulate matter, with most mercury being exported in particulate form and strongly correlated with total suspended solids (Lacerda et al., 2013; Molisani et al., 2007; Paraquetti et al., 2007, 2004). In tropical aquatic systems, seasonal hydrology can also modulate net MMHg production by shifting microbial methylator pathways and increasing organic-carbon availability (e.g., DOC inputs), thereby potentially favouring methylation during high-water/rainy periods (Lázaro et al., 2018). Physiological traits such as lipid content (Lip), tissue mass, and condition index (CI) can modulate mercury bioaccumulation in mussels, consistent with biodilution/growth-dilution mechanisms reported in field biomonitoring studies (Rogers et al., 2024). Accordingly, CI and Lip may help explain variability in both THg and organic-Hg metrics (TotOrgHg and MMHg) via condition- and growth-related dilution effects, rather than by assuming preferential lipid partitioning of organic Hg. Nevertheless, field studies report inconsistent and species-specific responses, particularly for MMHg, indicating that such effects cannot be assumed a priori and require site- and species-specific evaluation (Casas et al., 2008; Rogers et al., 2024). In filter-feeding bivalves, stable isotope (SI) ratios ($\delta^{13}C$ and $\delta^{15}N$) primarily reflect variability in organic matter sources and baseline nitrogen signatures rather than trophic position. Previous studies have shown that isotopic variability in mussels is linked to anthropogenic inputs, particulate organic matter composition, and mercury exposure pathways in coastal systems, supporting the use of SIs as exploratory indicators of MMHg dynamics rather than direct trophic proxies (Briant et al., 2018; Kim et al., 2024).

This study aims to (1) assess the bioaccumulation patterns of THg, TotOrgHg, and MMHg in the brown mussel *P. perna* cultivated in three subtropical bays with contrasting anthropogenic pressures; (2) evaluate the influence of environmental parameters (e.g. sediment organic matter, suspended solids, sediment-bound mercury) and mussel physiological traits (Lip, CI) on Hg accumulation. Furthermore, we examined SI ratios to explore whether spatial and seasonal variability in organic matter sources is associated with differences in MMHg accumulation in mussels, and we assessed the toxicological risk associated with human consumption of *P. perna*. Finally, we addressed the methodological reliability of TotOrgHg as a proxy for MMHg, with implications for monitoring strategies in data-poor regions.

Based on these objectives, this study hypothesises that mussels from more contaminated bays will tend to exhibit higher mercury concentrations, particularly during the rainy season when runoff enhances Hg transport and may favour conditions conducive to methylation (e.g., OM/DOC inputs and redox shifts). It is further expected that higher Lip and CI will be associated with lower bioaccumulation, reflecting a biodilution effect. A positive relationship is also anticipated between SI values and MMHg levels, consistent with site-specific food-web processes and baseline variability that may influence MMHg exposure. Finally, TotOrgHg is hypothesised to correlate with MMHg concentrations, supporting its use as a practical proxy in resource-limited settings.

2. Materials and methods

2.1. Study area

The samples were collected from three coastal bays along the southeastern Brazilian shoreline (Fig. 1), covering approximately 120 km. These bays—Guanabara Bay (GUA), Sepetiba Bay (SEP), and Ilha Grande Bay (ILH)—present contrasting levels of anthropogenic

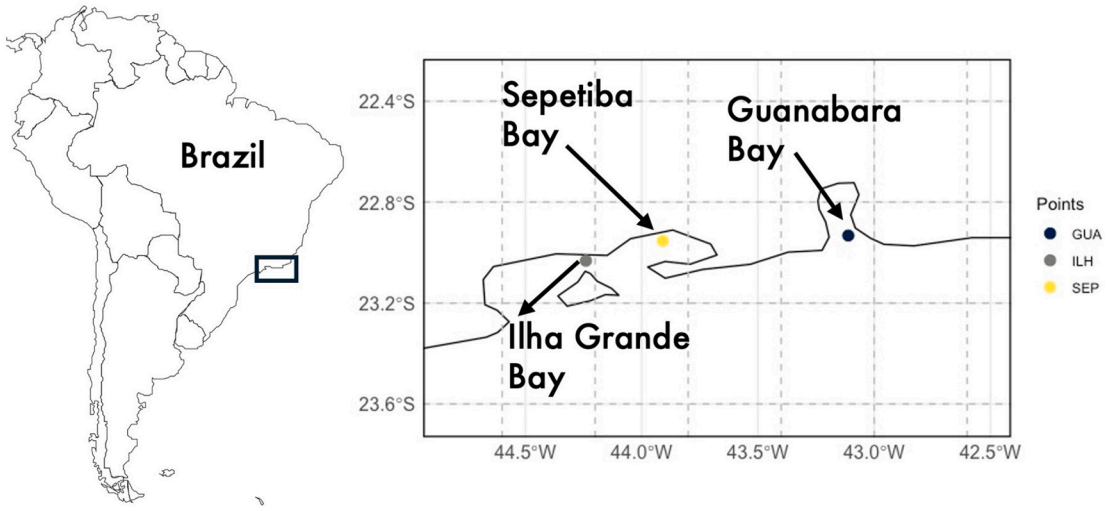

Fig. 1. Sampling locations along the coastline of Rio de Janeiro State (southeastern Brazil). Sites include Guanabara Bay (GUA), Sepetiba Bay (SEP), and Ilha Grande Bay (ILH).

influence, offering a natural gradient to investigate mercury bioaccumulation in marine environments. Guanabara Bay (GUA) is widely recognised as one of Brazil's most polluted coastal ecosystems, with extensive inputs of untreated domestic sewage, industrial discharges, hydrocarbons, nutrients from agricultural activities, and legacy contaminants such as pesticides and toxic metals (Costa et al., 2007; Galvao et al., 2012; Lailson-Brito et al., 2010; Neto et al., 2006; Soares-Gomes et al., 2010). This high level of contamination makes GUA a critical site for investigating environmental mercury exposure. Sepetiba Bay (SEP) is a semi-enclosed estuarine lagoon with a surface area of approximately 519 km^2. Historically impacted by port and shipyard activities, the bay also receives effluents from metallurgical and chemical industries. From the 1950s until 2010, wastewaters enriched with zinc (Zn) and cadmium (Cd) were discharged in the bay. In addition, the São Francisco Channel drains the highly contaminated Guandu River into SEP, further exacerbating the pollution (Rodrigues and Machado, 2023). Ilha Grande Bay (ILH) is comparatively less impacted and serves as a reference site in this study. However, it is not pristine; the bay hosts a major oil terminal that handles nearly 40% of Brazil's oil exports (TRANSPETRO, 2024). A recent sediment quality assessment based on 66 surface sediment samples classified ILH as moderately polluted (de Souza et al., 2021). Despite this, its limited fluvial input and reduced levels of suspended solids—owing to the absence of large riverine discharges—suggest a higher bioavailability of mercury (Longo et al., 2018). Previous studies have demonstrated frequent intrusions of South Atlantic Central Water (SACW), a cold-water mass that can reach the innermost regions of the bay. These events, with recorded temperatures as low as 16.4 °C, are believed to enhance the input of offshore water masses and may influence mercury dynamics in this system (Kjerfve et al., 2021; Longo et al., 2018). Although no pristine reference site was available, ILH represents the least anthropogenically impacted system among the studied bays and was therefore used as a relative reference for background mercury levels in mussels.

Sampling points were chosen to be representative of mussel farming in each bay. At GUA (Jurujuba Beach; 22°55′59″S, 046°06′40″W), there is a traditional fishing community where a substantial commercial production of *P. perna* is carried out. In SEP, sampling was conducted at an experimental mariculture farm used for academic purposes (Itacuruçá Island; 22°57′04″S, 043°54′28″W), where mussels were collected naturally attached to the longline structures. In ILH, sampling was performed at a farm associated with a reproductive laboratory for marine bivalves (IEDBIG; Biscaia Embayment; 23°01′38″S, 044°14′14″W), where mussels were collected following the same approach used in SEP.

2.2. Experiment design

The three bays investigated, GUA, SEP, and ILH, were considered as experimental replicates in multivariate analyses to identify broad bioaccumulation patterns across contrasting environmental settings. While acknowledging the inherent environmental differences among the bays, such as the level of anthropogenic contamination and continental inputs, the authors adopted this integrated approach to detect bioaccumulation patterns across varying ecological contexts. These site-specific contrasts, previously documented in terms of mercury exposure (Bisi et al., 2012), are leveraged here to reveal robust biotic and abiotic drivers of Hg bioaccumulation in *P. perna*.

2.2.1. Monthly monitoring

To assess the seasonal influence of Hg bioaccumulation in *P. perna*, sampling was conducted over eight months, encompassing both rainy period (summer months, December to March) and the dry period (winter months, June to September). Each month, thirty commercially sized mussels (5–8 cm shell length) were collected from each bay (total of 90 individuals per month). To reduce intra-sample variability and ensure sufficient analytical mass for speciation analyses, mussels were grouped into six composite samples per site, each composed of five individuals. In parallel, six surface sediment samples were collected monthly at each site (a total of 18 samples per month). Suspended solids (SS) were also collected with the mussels.

2.3. Sediment and suspended solid sampling

The sediment (Sed) and suspended solids (SS) samples were collected following previously established protocols (Galvao et al., 2014). An Eckman dredge was used to collect surface sediment samples from the uppermost 10 cm. Within each bay, samples were collected across multiple positions along the mussel longlines (i.e., spatially distributed deployments), providing within-site replication (18 sediment samples per month across the three bays). SS were collected via sediment traps deployed at 15-day intervals. The SS samplers consisted of modified PET (polyethylene terephthalate) Cylinders fitted with a glass tube to form a sedimentation funnel. The traps were installed along mussel longlines at depths of 1.5–2.0 m. Three to five trap tubes were pooled to form one SS sample. The number of recovered SS samples varied among bays and months (GUA: median 6, range 2–6; SEP: median 6, range 3–6; ILH:

median 3, range 1–5), due to adverse weather and oceanographic conditions.

2.4. Sample treatment

Mussels were manually collected from aquaculture ropes at marine farms. The specimens were kept cool during transport to the laboratory, where they were frozen at −20 °C until processing. After thawing, individuals were grouped into six pooled samples per site, each containing five mussels, resulting in a total of 144 pooled samples. Mussels, sediment (SED), and suspended solids (SS) were stored at −80 °C before freeze-drying. After lyophilisation, all samples were homogenized using an aluminium grinding vessel (Marconi®/MA-345H). The vessel was decontaminated between samples with detergent and ethanol to prevent cross-contamination. SED and SS samples were sieved, and the <74 μm fraction was retained to ensure a conservative estimate of mercury content, as this grain size has a high surface-to-volume ratio and is known to accumulate higher concentration of mercury (Forstner, 1981). All processed samples were stored in screw-capped glass flasks until analysis.

2.4.1. Physiological parameters

Two parameters were chosen to assess the physiological status of the sampled mussels: the condition index (CI_Muss) and the lipid content (Lip_Muss). Several methods for calculating the CI_Muss are available in the literature. A previous study using the same set of samples as the present study evaluated the difference between ten distinct equations proposed in the literature and suggested the following as the best fit to reflect the environmental variations (Galvao et al., 2015; Kagley et al., 2003).

$$\mathrm{CI} : [\text{soft tissue wet weight (g)}] \times [\text{shell length (mm)} \times 100]^{-1}$$

The Lip_Muss (%) was quantified gravimetrically following accelerated solvent extraction with n-hexane:acetone (75:25 v/v) at 120 °C and 120 bar, as previously described (Galvao et al., 2012).

2.5. Analytical procedures

2.5.1. Total mercury

Total mercury concentrations in mussels (THg_Muss) were determined following the protocol described by Bastos et al. (1998). Aliquots of approximately 0.05 g of lyophilised mussel tissue and 0.5 g of SS or Sed were digested using sulfonitric acid mixture (H_2SO_4:HNO_3 1:1 v/v) in combination with hydrogen peroxide (H_2O_2, 30%). The samples were heated in a water bath at 60 °C for 2 h. After cooling for 15 min, 5 mL of $KMnO_4$ (5%) (Merck®-purity level 99%) was added to each sample. One mL of 12% hydroxylamine hydrochloride (NH_2OH. HCl + NaCl 12% - Vetec®-purity level 99%) was added to each sample, with subsequent homogenisation of the extract. The extracts were subsequently heated in a water bath (60 °C) for 15 min and left to cool overnight. The SS and Sed extracts were filtered through Whatman® filter paper n° 42 before being taken to the final volume. The final extract was made up to 12 mL with high-purity deionised water (Direct 8 – Merck Millipore®). Total mercury determination was performed via cold vapour atomic absorption spectroscopy (CVAAS) via a flow-injection mercury system (FIMS-400, Perkin Elmer®) equipped with an AS-90 autosampler. NaBH4 (0.2%) and NaOH (0.05%) were used as the reducing agents.

2.5.2. Total organic mercury in mussel soft tissue

TotOrgHg in mussel tissue was determined via acid-assisted extraction followed by liquid-liquid partitioning. 0.2 g of dry tissue was subjected to acid extraction (KBr 30%: $CuSO_4$; 1:1:v:v) in a 50 mL plastic flask with a screw cap. The mixture was manually shaken for one minute, after which organic mercury species were partitioned into the non-polar phase by adding 5 mL of dichloromethane:hexane (3:2). The sample was then shaken for an additional two minutes (final volume: 15 mL) and centrifuged at 3200 rpm for 5 min (LS-3PLUS, CELM®) (Wagemann et al., 2000). For acid digestion, 1 mL of the organic phase was added to 5 mL of HNO_3:H_2SO_4 (1:4 - v:v) for 30 min in a hot bath (60 °C). After cooling at room temperature, 4 mL of an oxidative reagent was added (5% KMnO4) for 15 min in a hot bath. The samples were left overnight before the addition of the reducing reagent (1 mL of $HONH_2$·HCl + NaCl 12%), and the final volume was adjusted to 12 mL with Milli-Q® water. The samples are then immediately injected into the CVAAS (Bastos et al., 1998).

2.5.3. Monomethylmercury in mussel soft tissue

Due to the high analytical cost of MMHg determination, this compound was quantified only during four representative months—two from the rainy season (February and March) and two from the dry season (August and September). Basic extraction was performed in a 3 mL solution of KOH:CH_3. OH 25% (Sigma® and TEDIA®, purity level 85% and HPLC, respectively) was added to ±0.03 g of dry tissue at 68 °C for six h, and the mixture was shaken every hour by using a vortex mixer. The samples were incubated for 48 h. Centrifugation (3200 rpm for 10 min) was required before an aliquot of 30 μL to 40 mL amber vial partially filled with Milli-Q water and acetate buffer (300 μL) (Aldrich®, purity level 97%) was taken. The ethylation reaction starts when 50 μL of NaBEt4 is added (Brooks Rand Instruments®, 1% $NaBEt_4$ in 2% KOH). The vial volume is then immediately taken up to the inverted meniscus with Milli-Q water and capped. The samples were injected into an automated methylmercury system (MERX – Brooks Labs®) and detected via an atomic fluorescence spectrophotometer (AFS) (Taylor et al., 2011; USEPA, 2001a). Nitrogen (99.998% purity) was used for purging and as the drying gas. Argon (99.999% purity) was used as a carrier gas.

2.5.4. $\delta^{13}C$ and $\delta^{15}N$ in mussel soft tissues

To investigate the trophic ecology of *P. perna* and identify potential sources of organic matter influencing mercury bioaccumulation, SI ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were analysed in mussel soft tissues. Analyses were performed on the same pooled samples used for MMHg determination, corresponding to February, March, August, and September. The isotopic values were used to infer differences in dietary sources and trophic positions across the three bays.

Following freeze-drying, mussel tissue samples were ground to a fine powder using a mortar and pestle. SI ratios were determined using an isotope ratio mass spectrometer (IsoPrime100, Isoprime, Cheadle, UK) operated in continuous-flow mode, coupled to an elemental analyser (vario MICRO cube, Elementar Analysensysteme GmbH, Hanau, Germany). Isotope ratios of carbon and nitrogen were expressed in delta (δ) notation and expressed in per mille (‰) relative to Vienna PeeDee Belemnite (vPDB) for carbon and atmospheric N_2 for nitrogen. Analytical precision and accuracy were ensured using certified reference materials from the International Atomic Energy Agency (IAEA, Vienna, Austria), including sucrose (IAEA-C6, $\delta^{13}C = -10.8 \pm 0.5$‰), ammonium sulfate (IAEA-N2, $\delta^{15}N = 20.3 \pm 0.2$‰), and silver sulfide (IAEA-S1, $\delta^{34}S = -0.3$‰). Sulfanilic acid ($\delta^{13}C = -25.6 \pm 0.4$‰; $\delta^{15}N = -0.1 \pm 0.5$‰; $\delta^{34}S = 5.9 \pm 0.5$‰) was used as a secondary laboratory standard for quality assurance.

2.6. Quality control

All glassware used during the sampling and analytical procedures was decontaminated in two steps: two separate baths with (1) a detergent solution and (2) an acid solution. In each bath, the flasks were immersed for 24 h. The baths consisted of (1) a 5% neutral detergent solution (Detertex®) and (2) a 5% HNO_3 solution. The flasks were rinsed with distilled and deionised water between each other and after the second bath. All glassware was then dried in an oven at 100 °C.

This study determined THg_Muss in duplicate, ensuring a coefficient of variation of ≤15%. The analysis included processing analytical blanks

alongside the samples, with three blanks for each batch of twenty samples. A coefficient of variation of ≤15% between duplicate samples served as the reference for valid results. This study analysed a certified reference material (CRM) in the same manner as the samples, using NIST-2976 (mussel soft tissues) as the reference material. The recovery rates ranged from 85% to 115%. To calculate the instrument detection limit (DL_i, in ng/mL), this approach uses three times the standard deviation of ten blank solution runs divided by the calibration curve slope (α). The method detection limit (DL_m) was then calculated by multiplying DL_i (in ng/mL) by the final extract volume (in mL) and dividing by the mean sample mass (in grams).

2.7. Data treatment

When matrices were analysed separately (e.g., ANOVA within a given matrix), all available replicates were retained. However, for analyses requiring direct comparability across matrices and variables (PCA/PERMANOVA and correlation matrices), we aggregated replicates by computing the mean of the available samples within each Site × Month × Matrix combination. These monthly values were then used to calculate Site × Season summaries for the multivariate analyses. All statistical analyses were conducted in R (R_Core_Team, 2023). Site-specific effects were evaluated explicitly using ANOVA and PERMANOVA, and further explored using PCA-based contrasts. Results are presented as boxplots showing median values and interquartile ranges (Q1–Q3) due to non-normality. Complementary descriptive statistics (n, mean ± standard deviation, median, and range [minimum–maximum]) are provided in Supplementary Table S1, unless otherwise stated. All graphical outputs were produced using the ggplot2 package (Wickham, 2016). The significance level was set at $p < 0.05$. For p-values below 0.01, results are reported as $p < 0.01$. Organic matter (OM) content in sediments (OM_Sed) was treated as a contextual environmental variable and reported descriptively to support the interpretation of mercury results. OM was determined by loss-on-ignition, following oven-drying at 105 °C for 16 h and combustion at 400 °C for 2 h (USEPA, 2002). Samples were weighed using an analytical balance with 0.0001 g precision and allowed to cool in a desiccator containing silica gel prior to weighing.

2.7.1. Univariate analyses (ANOVA and post hoc tests)

Residual normality (Shapiro–Wilk) was not fully met, and variance homogeneity was also violated according to Levene's test (Fox and Weisberg, 2019). Because one-way ANOVA is generally robust to small-to-moderate departures from variance homogeneity—particularly when sample sizes are similar (Quinn and Keough, 2002)—particularly under balanced sample sizes—we treated one-way ANOVA as an exploratory tool and interpreted it alongside non-parametric correlations and multivariate analyses. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test was used to assess spatial and matrix-level differences in mercury metrics and selected biological variables. To evaluate differences among environmental matrices, THg concentrations were compared using Matriz (mussel tissue, sediment, and suspended solids) as the explanatory factor (THg ~ Matriz). Spatial differences among bays were subsequently tested separately within each matrix using one-way ANOVA with Site as the factor. These analyses included THg in mussel tissue (THg_Muss ~ Site), THg in sediments (THg_Sed ~ Site), and THg in suspended solids (THg_SS ~ Site). The TotOrgHg was expressed as percentage of total mercury. Spatial differences in TotOrgHg were tested using one-way ANOVA with Site as factor. Regarding the MMHg analysis, only a subset of samples (four months, comprising two rainy and two dry months) was quantified due to analytical constraints. For this subset, spatial differences in MMHg concentration were tested using one-way ANOVA (MMHg ~ Site). Seasonal patterns were evaluated primarily in multivariate space (PCA/PERMANOVA), while univariate variability was visualised using month-by-site boxplots. In figures, MMHg is also expressed as a percentage of THg for a direct comparison with TotOrgHg_Muss.

2.7.2. Pairwise associations and proxy evaluation

Relationships among continuous variables were initially explored using a Spearman correlation matrix and plotted as a heatmap to aid visualisation of major patterns and potential collinearity. Because MMHg was analysed over a shorter temporal window (four months) than TotOrgHg_Muss (eight months), correlations involving MMHg were restricted to the MMHg subset. For correlation analyses, replicate aggregation was applied only to ensure comparability across matrices by computing mean values within each Site × Month × Matrix combination (thereby standardising the replicate structure prior to integration); the same procedure was used for the PCA/PERMANOVA workflow (Sections 2.7.4–2.7.5). In contrast, matrix-specific analyses retained the full replicate structure and therefore were not based on monthly averages. To evaluate the suitability of TotOrgHg_Muss as a screening proxy for MMHg, Kendall's tau correlation was applied due to the presence of tied values. This analysis excluded the extreme TotOrgHg_Muss values (>100%), representing approximately 2% of the dataset.

2.7.3. SI analyses in mussel soft tissues (exploratory approach)

$\delta^{13}C$ and $\delta^{15}N$ were determined in the same pooled mussel tissue samples used for MMHg analysis. Isotopic variability among sites was summarised using medians and ranges and visualised in $\delta^{13}C$–$\delta^{15}N$ space to characterise differences in organic matter sources. Associations between $\delta^{15}N$ and MMHg were explored using Spearman rank correlations on log-transformed data, both for the complete dataset and within each site (ILH, SEP, and GUA). These analyses were conducted in an exploratory framework to evaluate whether isotopic variability was associated with patterns of MMHg accumulation, rather than to infer trophic position.

2.7.4. Multivariate analyses (PCA): data preprocessing and structure

Principal component analysis (PCA) was applied to explore multivariate structure and identify the main drivers of variability in organic mercury bioaccumulation. The PCA dataset comprised the following numeric variables: OM_Sed, CI_Muss, Lip_Muss, THg_Muss, TotOrgHg_Muss, and THg_SS. The variable THg_Sed was excluded to avoid multicollinearity, as it showed a strong correlation with THg_SS ($r = 0.94$) and a higher association with TotOrgHg_Muss ($r = 0.42$) than THg_SS ($r = 0.39$). Missing values (<5% of the dataset) were imputed using the k-nearest neighbours method ($k = 5$) to preserve matrix integrity (Kowarik and Templ, 2016). All numeric variables were standardized (z-scores), shifted to ensure positive values, and log-transformed to reduce skewness and improve comparability across scales. PCA was conducted using the FactoMineR package (Le et al., 2008) and visualised using factoextra (Kassambara and Mundt, 2020). The categorical variables Site, Month, and Season were included as supplementary qualitative variables to aid interpretation of multivariate patterns.

2.7.5. Multivariate hypothesis testing (PERMANOVA)

To formally test for group-level differences in multivariate structure, a permutational multivariate analysis of variance (PERMANOVA) performed using the vegan package in R (Oksanen et al., 2024). The analysis was conducted on the Euclidean distance matrix derived from the standardized and log-transformed PCA dataset, which included the following numeric variables: OM_Sed, CI_Muss, Lip_Muss, THg_Muss, TotOrgHg_Muss, and THg_SS. A two-factor additive design was applied, with Site (GUA, SEP, ILH) and Season (Rainy, Dry) as fixed factors. No interaction term was included, as preliminary exploratory analyses indicated that interaction effects were negligible for the purposes of this study. Significance was assessed using 999 permutations under a reduced model. When a significant main effect of Site was detected, pairwise PERMANOVA tests were conducted using the pairwise.perm.manova function from the RVAideMemoire package (Hervé, 2023) to

identify which specific bays differed from each other. This approach allowed us to test whether the multivariate centroids of mercury-related variables differed significantly among sites and seasons, while accounting for the non-parametric nature of the distance-based data.

2.8. Toxicological risk assessment

For toxicological risk assessment, mercury concentrations in *P. perna* mussels were expressed on a wet-weight basis. The provisional tolerable weekly intake (PTWI) for MMHg, established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at 1.6 μg/kg body weight week^{-1}, was used to estimate maximum allowable weekly intake and is considered sufficiently protective for developing fetuses, the most sensitive subgroup of the population (FAO/WHO, 2004). In parallel, the United States Environmental Protection Agency (USEPA) reference dose (RfD) for chronic oral exposure to MMHg (0.1 μg/kg day^{-1}) was applied to derive daily intake limits (USEPA, 2001b). Since no quantitative statistics are available on average mussel consumption in Brazilian populations, the authors applied a reverse-calculation approach. PTWI and RfD reference values were used to compute the maximum allowable mussel intake (mussels per week/day) that would not exceed MMHg thresholds, based on observed THg_Muss concentrations and the mean edible tissue mass. We assume the average body weights of 60 kg for adults and 30 kg for children. We used an edible soft-tissue mass of 11.7 g per mussel, corresponding to the mean wet weight of soft tissues measured in the analysed organisms, for all calculations. We calculated the maximum number of mussels that could be safely consumed per week (PTWI-based) or per day (RfD-based) by dividing the allowable MMHg intake (μg per person per week or day) by the estimated MMHg content per mussel. We estimated MMHg content per mussel by applying the mean MMHg proportion (%) to THg_Muss (wet weight basis) and multiplying the resulting MMHg concentration by the mean edible soft-tissue mass per individual (11.7 g). To provide conservative exposure estimates, we used both the median and maximum MMHg concentrations estimated in this study.

3. Results

The concentrations of THg_Muss, THg_Sed, and THg_SS are reported on a dry weight basis, whereas values used for toxicological risk assessment are expressed on a wet weight basis. Organic mercury species (TotOrgHg_Muss and MMHg) are presented as percentages relative to THg. Unless otherwise stated, results are reported as median (Q1–Q3).

3.1. Total mercury concentrations

The median THg_Muss concentration was 41.9 μg·kg^{-1} (18.1–57.9) (Fig. 2A), approximately half the median THg_Sed (96.3 μg·kg^{-1}; 57.5–441.0) and THg_SS (73.8 μg·kg^{-1}; 48.7–377.0) (Fig. 3A and B). However, the upper quartile for THg_Muss (57.9 μg·kg^{-1}) was nearly one order of magnitude lower than the upper quartiles for THg_Sed (441.0 μg·kg^{-1}) and THg_SS (377.0 μg·kg^{-1}). Regarding abiotic compartments, GUA exhibited the highest THg levels in both sediments (median 461.0 μg·kg^{-1}; 440.0–522.0) and suspended solids (median 396.0 μg·kg^{-1}; 339.0–418.0), with significant differences among bays (one-way ANOVA, Sed: $F_{2,\ 313} = 2677$, $p < 0.01$; SS: $F_{2,\ 191} = 911.3$, $p < 0.01$) (Fig. 3). Sediment THg was lower in ILH (94.8 μg·kg^{-1}; 81.1–105.0) and SEP (50.8 μg·kg^{-1}; 44.6–57.2), while THg_SS medians were 67.2 μg·kg^{-1} (60.8–76.4) in ILH and 41.3 μg·kg^{-1} (33.2–52.1) in SEP (Fig. 3). Across bays, THg_Muss differed significantly (one-way ANOVA, $F_{2,\ 310} = 313.9$, $p < 0.01$), with higher median values in ILH (61.2 μg·kg^{-1}; 51.1–90.3) than in GUA (45.3 μg·kg^{-1}; 39.2–51.4) and SEP (14.2 μg·kg^{-1}; 10.1–18.4) (Fig. 2A).

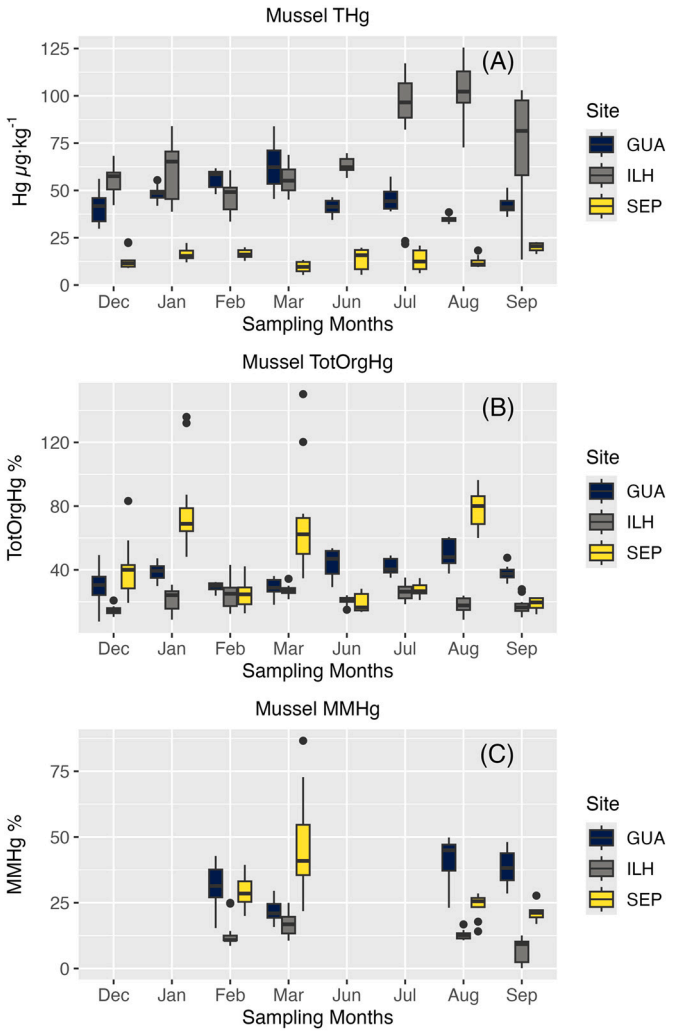

Fig. 2. Mercury concentrations in *Perna perna* soft tissue from Guanabara Bay (GUA), Sepetiba Bay (SEP), and Ilha Grande Bay (ILH): total mercury (THg; A) total organic mercury (TotOrgHg; B), and monomethylmercury (MMHg; C). Boxplots show the 25th and 75th percentiles (box limits), the median (center line), whiskers as ±1 standard deviation, and outliers as black dots.

3.2. Organic fraction of mercury in mussels

The median TotOrgHg_Muss proportion relative to THg was 28.9% (21.3–42.2) (Fig. 2B), with 2% of samples exceeding 100%. Site-specific medians differed significantly among bays (one-way ANOVA, $F_{2,\ 272} = 41.45$, $p < 0.01$), with higher %TotOrgHg in GUA (36.8%; 31.3–44.7) and SEP (34.6%; 22.3–64.9) than in ILH (21.2%; 15.2–25.9). For MMHg/THg proportions, the overall median was 9.03% (4.6–13.6) (Fig. 2C). Site-specific medians were 16.2% (12.9–16.9) in GUA, 9.2% (6.5–12.2) in ILH, and 4.1% (3.1–4.7) in SEP (one-way ANOVA, $F_{2,\ 121} = 147.4$, $p < 0.01$). A moderate positive association was observed between %TotOrgHg and %MMHg (Spearman's $\rho = 0.39$, $p < 0.01$) (Fig. 4).

3.3. Seasonal variation

Significant seasonal variation was observed in both THg_Sed concentrations (one-way ANOVA, $F_{1,\ 314} = 8.34$, $p < 0.01$) and THg_SS (one-way ANOVA, $F_{1,\ 192} = 6.53$, $p < 0.05$). In contrast, seasonal differences in THg_Muss concentrations were not statistically significant (one-way ANOVA, $F_{1,\ 311} = 2.92$, $p = 0.09$). Mussel physiological parameters also varied between seasons. Both CI_Muss and Lip_Muss differed

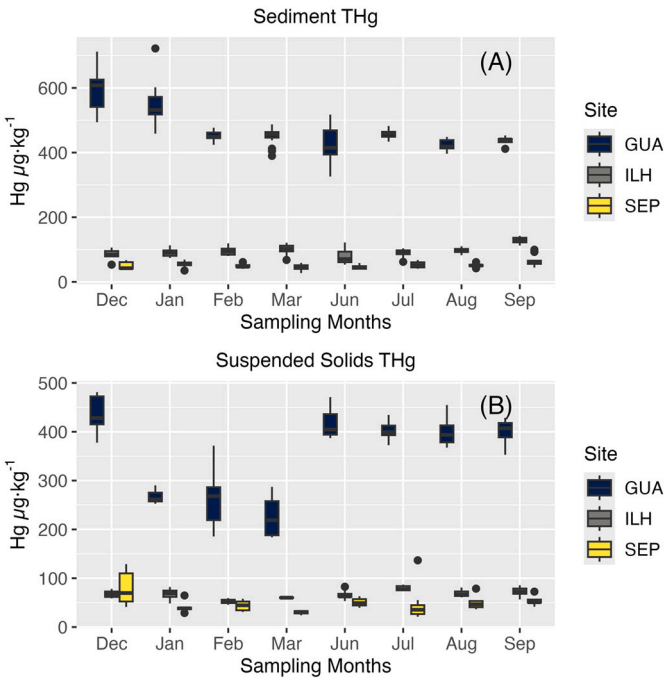

Fig. 3. Total mercury (THg) concentrations in suspended solids (SS; A) and surface sediments (Sed; B) from Guanabara Bay (GUA), Sepetiba Bay (SEP), and Ilha Grande Bay (ILH). Boxplots show the 25th and 75th percentiles (box limits), the median (center line), whiskers as ±1 standard deviation, and outliers as black dots.

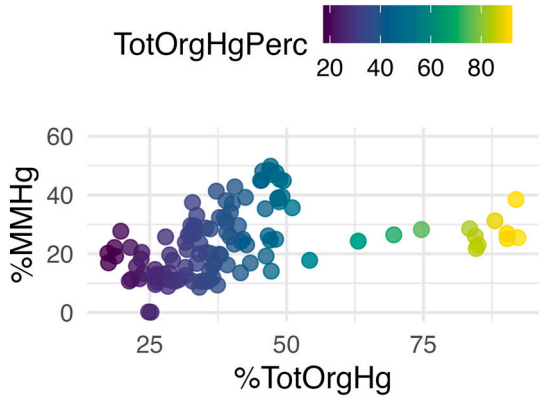

Fig. 4. Relationship between the percentage of total organic mercury (% TotOrgHg) and the percentage of monomethylmercury (%MMHg), both expressed relative to total mercury, in soft tissues of mussels collected in February, March, August, and September.

significantly between seasons, with lower median values observed during the rainy period (one-way ANOVA, CI_Muss: $F_{1,\ 69} = 12.84$, $p < 0.01$; Lip_Muss: $F_{1,\ 68} = 8.16$, $p < 0.01$). Descriptive statistics indicate that median THg_Sed and THg_Muss were higher during the rainy season (100.2 vs 95.3 μg·kg^{-1} and 46.1 vs 39.2 μg·kg^{-1}, respectively), whereas THg_SS was higher during the dry season (79.1 vs 60.1 μg·kg^{-1}) (Table 1).

3.4. Associations among biotic and abiotic parameters

CI_Muss differed among sites (one-way ANOVA, $F_{2,\ 69} = 12.8$, $p < 0.01$), with medians of 13.1 (9.8–14.9) in GUA, 14.7 (12.8–18.3) in SEP, and 10.9 (8.6–12.3) in ILH. Lip_Muss also varied significantly among bays (one-way ANOVA, $F_{2,\ 68} = 8.16$, $p < 0.01$), with medians of 10.4 (9.0–11.2) in GUA, 13.7 (11.7–16.0) in SEP, and 9.11 (7.84–10.4) in ILH. OM_Sed differed among bays, with medians of 12.9 (9.0–15.3) in GUA, 8.7 (2.9–9.7) in ILH, and 6.7 (6.1–8.8) in SEP. Spearman rank correlations among biotic (CI_Muss, Lip_Muss) and abiotic variables (OM_Sed, THg_Sed, THg_SS) and mussel Hg metrics (THg_Muss, TotOrgHg_Muss) are summarised in Fig. 5. The strongest positive associations were observed between Lip_Muss and CI_Muss ($\rho = 0.78$) and between THg_Sed and THg_SS ($\rho = 0.83$), whereas THg_Muss was negatively correlated with CI_Muss ($\rho = -0.53$) and Lip_Muss ($\rho = -0.63$). Non-significant correlations ($p \geq 0.05$) are marked with an "X" in Fig. 5.

Table 1
Summary of median values for sediment, suspended solids and mussels regarding mercury concentrations (total - THg; total organic - TotOrgHg; monomethylmercury - MMHg) in rainy (November, December, January and February) and dry (June, July, August and September) seasons.

Matriz	Season	Hg	Value
Mussel	THg (μg·kg^{-1})	Rainy	46,1
		Dry	39,2
	TotOrg (%)	Rainy	29,0
		Dry	28,4
	MMHg (%)	Rainy	24,4
		Dry	23,0
Sediment	THg (μg·kg^{-1})	Rainy	100,2
		Dry	95,3
Suspended solids	THg (μg·kg^{-1})	Rainy	60,1
		Dry	79,1

3.5. Stable isotopes of the mussels soft tissues

$\delta^{13}C$ values ranged from −19.7‰ to −13.8‰ (median = −17.6‰ ± 1.3), while $\delta^{15}N$ values varied between 6.2‰ and 11.8‰ (median = 8.5‰ ± 1.2). Among sites, ILH presented lower median $\delta^{13}C$ values (−18.3‰ ± 0.5), whereas GUA showed higher median $\delta^{15}N$ values (9.8‰ ± 0.6). When the pooled dataset was evaluated, the Spearman correlation between $\delta^{15}N$ and MMHg was not significant ($\rho = 0.10$, $p = 0.446$). In site-stratified analyses, a significant positive association was observed in ILH ($\rho = 0.52$, $p = 0.016$), whereas no significant correlations were observed in SEP ($\rho = -0.20$, $p = 0.449$) or GUA ($\rho = 0.05$, $p = 0.825$) (Fig. 6).

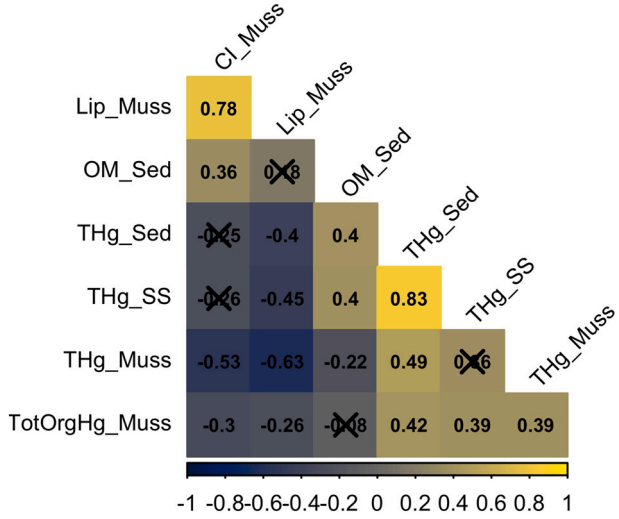

Fig. 5. Spearman correlation matrix for abiotic variables (OM_Sed, THg_Sed, THg_SS) and biotic variables (THg_Muss, TotOrgHg_Muss, CI_Muss, Lip_Muss). Correlation coefficients (ρ) are shown in the cells; non-significant correlations ($p \geq 0.05$) are marked with an "X".

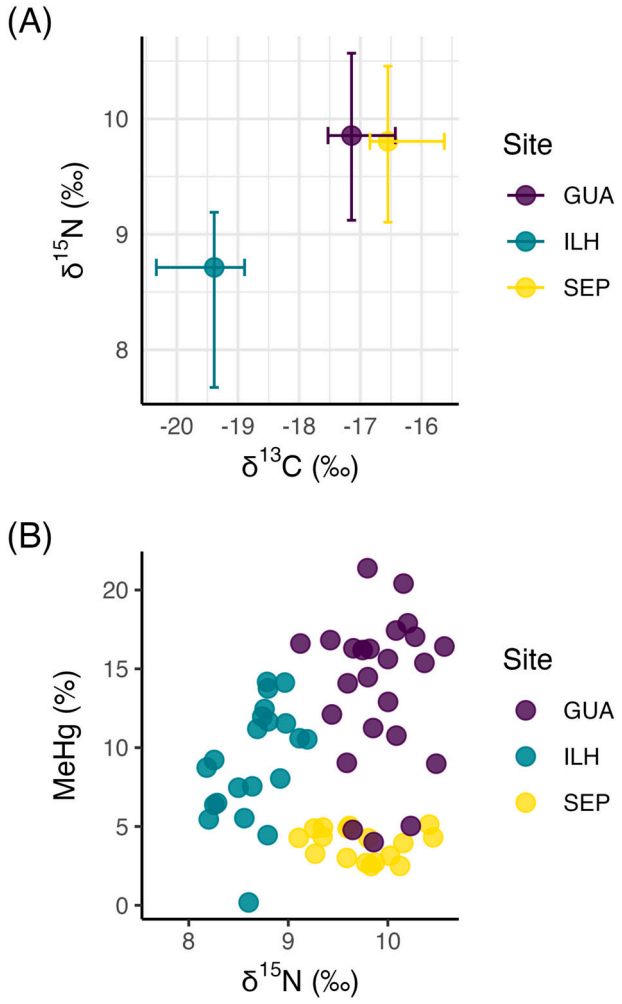

Fig. 6. Stable isotope results for mussel soft tissues collected in February, March, August, and September. (A) $\delta^{13}C$ versus $\delta^{15}N$ by site (GUA, SEP, ILH). (B) D15N versus %MMHg (MMHg/THg × 100). Points represent medians and error bars indicate ±1 standard deviation.

3.6. Key drivers of organic mercury bioaccumulation in mussel soft tissues

To explore multivariate patterns related to organic mercury bioaccumulation in mussel soft tissues across sites and seasons, a PCA was applied, integrating biotic condition, organic matter, and mercury variables. Dimensions 1 and 2 of the PCA explained 72.6% of the total variance in the dataset (Fig. 7A). All variables were projected within the correlation circle. The highest contributions to Dimension 1 were observed for THg_SS (53.6%), OM_Sed (50.8%), and THg_Muss (28.6%). Lip_Muss showed a negative loading relative to TotOrgHg_Muss, while CI_Muss was positively associated with THg_Muss. When site was included as a supplementary qualitative variable (Fig. 7B), samples clustered according to bay, with a statistically significant separation among sites (PERMANOVA, Site: $p = 0.001$). In this ordination, samples from SEP were more closely associated with CI_Muss and Lip_Muss, whereas samples from GUA were associated with higher values of THg_SS and OM_Sed, while samples from ILH tended to plot opposite to the OM_Sed vector.

The inclusion of season as a supplementary variable (Fig. 7C) also resulted in a statistically significant separation (PERMANOVA, Season: $p = 0.001$), although clustering was less distinct than that observed for site. Consistent with the seasonal pattern, THg_SS showed higher median values in the dry season (79.1 vs 60.1 $\mu g \cdot kg^{-1}$), whereas median THg_Sed and THg_Muss were higher in the rainy season (100.2 vs 95.3

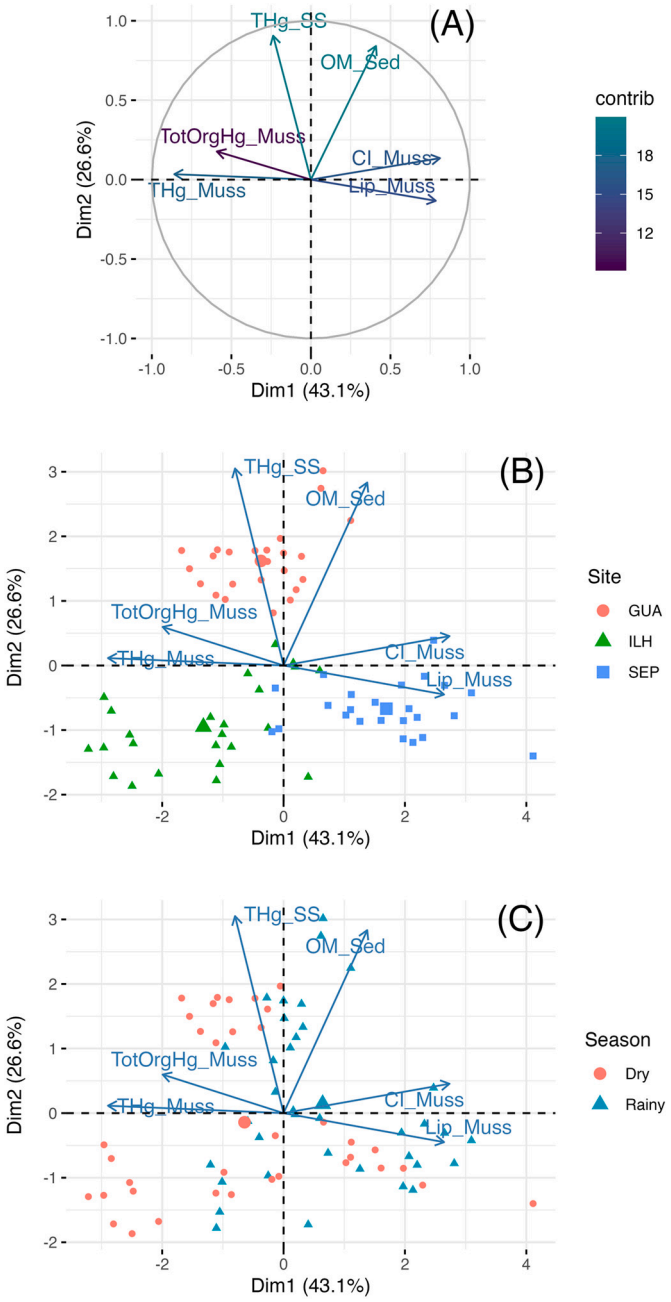

Fig. 7. Principal component analysis (PCA) biplot for Dimensions 1 and 2. (A) Variable loadings (vectors). (B) Scores coloured by site (GUA, SEP, ILH). (C) Scores coloured by season (Rainy: December–March; Dry: June–September). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$\mu g \cdot kg^{-1}$; 46.1 vs 39.2 $\mu g \cdot kg^{-1}$) (Table 1).

3.7. Toxicological assessment

Table 2 summarises the toxicological assessment of maximum safe mussel consumption for adults and children, based on the MMHg concentrations measured in this study. Using median THg and %MMHg values, the estimated safe intake for a 60 kg adult corresponds to a maximum of five mussels per week or less than half a mussel per day. For children (30 kg), the estimated intake limit is reached at approximately half a mussel per day when considering the 25th percentile of MMHg concentrations.

Table 2
Toxicological assessment of mussel consumption in terms of the monomethylmercury (MMHg) content in edible tissue. The estimated MMHg percentage is calculated by applying the proportion of MMHg applied to the total mercury concentration (THg). The evaluation of the number of mussels that reach the threshold limit is based on the provisional tolerable weekly intake (PTWI) and the reference dose for chronic oral exposure (RfD).

	MMHg (μg/kg)	MMHg (%)	THg (μg·kg^{-1})	Estimated MMHg (μg·kg^{-1})	The number of mussels to reach the threshold limit (average edible tissue weighs: 11.7 g in w.w.)			
					PTWI = 1.6 μg·kg^{-1} body weight per week		RfD = 0.1 μg·kg^{-1}·day^{-1}	
					Adult = 60 kg	Child = 30 kg	Adult = 60 kg	Child = 30 kg
Min	0,03	0,14	1,03	0,00	5635,6	2817,8	352,2	176,1
25% quart	0,86	14,56	3,44	0,50	16,4	8,2	1,0	0,5
MED	1,58	24,04	7,23	1,74	4,7	2,4	0,3	0,1
75% quart	2,20	33,36	10,63	3,55	2,3	1,2	0,1	0,1
Max	5,09	86,61	18,58	16,09	0,5	0,3	0,0	0,0

Min – minimum; 25% quartile – 1st quartile; MED – median; 75% quartile – 3rd quartile; Max - maximum.

4. Discussion

This study provides an integrated assessment of Hg accumulation in the brown mussel *P. perna* across three subtropical bays with contrasting levels of anthropogenic pressure. By quantifying THg, TotOrgHg, and MMHg in mussel tissues and environmental matrices, and by incorporating physiological and isotopic indicators, we offer new insights into the biogeochemical and biological processes governing Hg bioaccumulation in tropical coastal aquaculture systems.

4.1. Hg accumulation in mussels and environmental compartments

The sessile filter-feeding bivalves generally accumulate metals to levels exceeding those of surrounding environmental compartments (Zuykov et al., 2013). However, *P. perna*, in this study displayed lower THg levels than both Sed and SS. This pattern was particularly pronounced in GUA, the most contaminated site. Such observations have been reported in other heavily impacted systems, where a large fraction of sedimentary Hg is bound to sulfides or refractory organic matter, reducing its bioavailability (Benoit et al., 2001; Bloom et al., 2003). In contrast, mussels from ILH, the least anthropogenically impacted site, showed the highest THg levels in soft tissue despite lower environmental concentrations, a pattern previously reported for *P. perna* in upwelling-influenced systems (Longo et al., 2018). Together, these observations indicate that environmental Hg concentrations alone may be insufficient to predict bioaccumulation and that local bioavailability may modulate bioaccumulation.

Comparing the present results with those of previous studies, it is worth highlighting that mussels from the French coast (Briant et al., 2017) presented MMHg concentrations approximately five times higher than those in the most polluted bay assessed in the present study (GUA). However, the present data are comparable to those reported for *M. galloprovincialis* from the Turkish coast (Gedik and Koral, 2023) and *P. perna* from the Ghana coast (Otchere, 2003). Another species from the genus *Perna*, *Perna viridis*, bioaccumulated up to 3 μg.g^{-1} of THg (Rojas et al., 2009), which is one order of magnitude greater than the results from the most polluted bay in the present study (GUA). Table 3 summarises the data from previous studies and the current investigation.

Although the present study did not measure monomethylmercury (MMHg) in sediments or suspended solids, the observed MMHg proportions in *P. perna* tissues (median 9.0% of THg) provide indirect insight into methylation dynamics within these bays. Future studies quantifying MMHg in water and sediments across these bays would help clarify the sources and transfer pathways of this toxic species to cultivated bivalves, improving risk assessments for human consumption.

4.2. Importance of suspended solids and seasonal variability

Suspended solids were most closely aligned with THg_Muss than sTHg_Sed, supporting the hypothesis that SS represents a more direct exposure pathway for filter-feeders. This is consistent with previous observations that particulate-bound contaminants are efficiently intercepted during bivalve filtration (Baudrimont et al., 2005). Seasonal dynamics further modulated Hg distribution across compartments: THg_Muss and THg_Sed were higher during the rainy season, whereas THg_SS was higher during the dry season. Similar matrix-dependent seasonal patterns in Hg dynamics have been reported in coastal systems subject to hydrological variability (Mason et al., 2006). Seasonal variability in metal burdens has been widely reported in mussel biomonitoring and may be driven by changes in freshwater/riverine runoff, hydrochemical shifts (e.g., temperature and salinity), and the gametogenic cycle (Azizi et al., 2018; Regoli and Orlando, 1994; Szefer et al., 2004). Previous studies have also associated lower CI_Muss and Lip_Muss during the rainy season with higher THg_Muss, in agreement with the biodilution effect described for mussels (Mubiana et al., 2006).

4.3. TotOrgHg vs MMHg: methodological and ecological considerations

This study evaluated whether TotOrgHg can be used as a proxy for MMHg, in contexts where dedicated Hg speciation is not available. While a statistically significant correlation was observed between TotOrgHg and MMHg, the relationship was moderate and became more dispersed at higher TotOrgHg values. This suggests that TotOrgHg should be interpreted with caution as a proxy for MMHg in *P. perna*. Multi-step extraction procedures can reduce yields and may lack species specificity, and acid extraction can promote interconversion among

Table 3
Concentrations (μg/kg) of total mercury (THg) and methyl mercury (MMHg) in mussels from coastal areas around the world.

Location	Species	THg	MMHg	Author
South Atlantic coast (Brazil)	*P. perna*	5.35–125.51	0.14–22.23	This study
Ghana coast	*P. perna*	220–370	–	Otchere et al. (2003)
Sardinia Island (Italy)	*M. galloprovincialis*	35–830	15–116	Ipolyi et al. (2004)
Guanabara Bay (Brazil)	*P. perna*	58.2–227.2	21–105	Kehrig et al. (2006)
Sepetiba Bay (Brazil)	*P. perna*	75.5 ± 7.1	48.1 ± 5.1	Kehrig et al. (2006)
Venezuela coast	*P. viridis*	432–2990	–	Rojas et al. (2009)
Mediterranean Sea (French coast)	*M. galloprovincialis*	60–670	18–132	Briant et al. (2017)
Black Sea (Turkish coast)	*M. galloprovincialis*	1.10–130.70	0.22–37.90	Gedik and Koral (2023)

methylated Hg species (Bloom, 1992). Thus, the ecological heterogeneity may affect Hg speciation and partitioning across particulate, dissolved, and tissue pools, potentially adding context-dependent variability to the TotOrgHg–MMHg relationship (Mason et al., 2006; Pauly et al., 2023; Watras et al., 1998). Because food-safety reference values are defined for MMHg, direct MMHg quantification remains preferable whenever feasible, particularly for dietary risk assessment (FAO/WHO, 2004; USEPA, 2001b).

4.4. Trophic modulation and insights from stable isotopes

SI approaches are widely used to infer trophic structure in aquatic food webs, where $\delta^{15}N$ typically increases with trophic position, and they provide useful tools for assessing MMHg trophic transfer and trophic magnification metrics (Lavoie et al., 2013; Post, 2002). $\delta^{15}N$ values in mussel tissues correlated positively with MMHg only in ILH (Fig. 7), the least anthropogenically impacted bay in the study area (GUA > SEP > ILH; Section 2.1) and the one with the lowest fluvial input. In contrast, the $\delta^{15}N$–MMHg correlation was not significant in GUA or SEP. Anthropogenic N inputs may confound the assessment of $\delta^{15}N$ trophic structure in nutrient-impacted coastal systems, due to the organisms' $\delta^{15}N$ signatures, resulting in decoupling the consumer–trophic position relationships and potentially biasing $\delta^{15}N$-based biomagnification metrics (Boquete et al., 2023). Therefore, the lack of a clear relationship in GUA and SEP may reflect baseline distortion associated with nutrient loading rather than the absence of trophic transfer processes. Regarding $\delta^{13}C$, it is widely used to trace carbon sources across adjacent water masses and to differentiate offshore pelagic food webs from coastal-shelf ones (France, 1995; Perry et al., 1999). More enriched (less depleted) $\delta^{13}C$ values in GUA and SEP may be consistent with a greater contribution of coastal/benthic-associated carbon sources to the basal carbon pool in these systems, whereas ILH shows more depleted signatures, consistent with a stronger offshore/pelagic influence (Section 3.6). This pattern may also be consistent with the site-level distribution in the PCA biplot (Fig. 6B), where ILH clusters in the region opposite to the OM_Sed vector, while GUA and SEP are more distributed towards the direction associated with higher OM_Sed and THg_SS contributions. More depleted $\delta^{13}C$ values in ILH are also potentially influenced by episodic upwelling of South Atlantic Central Water (Kjerfve et al., 2021). Upwelling systems can naturally enhance mercury concentrations in coastal waters by bringing Hg-enriched deeper waters into the euphotic zone (Bowman et al., 2016; Cossa et al., 2004; Mason and Fitzgerald, 1993). It is also worth highlighting that bacterial activity can play a crucial role in energy flow through the microbial loop (Reynolds, 2008). Based on these observations, this scenario may provide a plausible ecological context for higher MMHg in ILH. However, the specific role of microbial processes in Hg methylation remains to be tested in this system.

4.5. Drivers of Hg bioaccumulation: integrating biotic and abiotic controls

PCA highlighted THg_SS, THg_Muss and OM_Sed as the main contributors to the multivariate structure (Fig. 6), suggesting a balance between (i) particle/organic-matter controls on Hg partitioning and (ii) biotic modulation of tissue concentrations. Based on the mussels' feeding habits, we initially hypothesised that particulate-bound Hg in suspended solids would be more closely related to bioaccumulation than Hg in sediments. However, in shallow farming areas, the water column and bottom sediments are often tightly coupled. Sediments integrate temporal variability and local hydrodynamic conditions (Burton, 2010). Resuspension can transfer sediment-bound Hg to the particulate pool captured by suspension-feeding bivalves (Wang et al., 2022). In this context, SS more directly reflects the particulate exposure pathway, while sediments act as an integrating reservoir that can intermittently supply particulate-bound Hg to the water column through episodic sediment–water exchange (Fig. 5).

Regarding the OM_Sed vector in the PCA, biodeposition in bivalve farms and associated biofoulers can enrich sedimentary organic matter (Chamberlain et al., 2001). This organic phase can act as an important sorbent for metals, including Hg, and thereby modulate their bioavailability (Wu et al., 2017; Zhong and Wang, 2006). Our results support the interpretation that organic-rich sediments and particles may retain a substantial fraction of Hg within solids, thereby constraining the fraction that is effectively transferred to mussel soft tissues. This highlights the potential for carbon-rich organic matter to modulate Hg bioavailability in farm-influenced areas, although the direction and magnitude of this effect will depend on hydrodynamics and particle dynamics.

Physiological features act as an additional driver of Hg bioaccumulation, as they are not only seasonally modulated (maturation cycle) but also locally influenced (food availability). The negative associations involving CI_Muss/Lip_Muss and mussel Hg metrics (Figs. 5–6) are consistent with biodilution/growth-dilution effects described for metals in mussels (Mubiana et al., 2006). Although size-related 'growth dilution' of THg has been reported for *P. perna* (Otchere, 2003), for mercury bioaccumulation CI-related patterns may be clearer for MMHg than for THg (Rogers et al., 2024). Additionally, the CI–THg relationship may be context-dependent across systems (Rogers et al., 2024). Accordingly, CI and Lip should be considered when interpreting bioaccumulation endpoints, as they influence tissue mass and energy reserves, and should not be interpreted as evidence of preferential lipid partitioning of organic Hg.

Finally, the relationship between TotOrgHg and MMHg indicates that TotOrgHg can support screening but cannot be assumed to track methylmercury uniformly. The increasing dispersion at higher TotOrgHg values (Fig. 4) suggests that the organic-Hg pool captured by TotOrgHg may reflect a mixture of compounds and transformation pathways, while methylmercury levels may be sensitive to local biogeochemical conditions and biological turnover. Taken together, these results support the interpretation that Hg bioaccumulation in *P. perna* is driven by the interaction between particle/OM-driven partitioning in the environment and physiological regulation of tissue concentrations, with site-specific hydrodynamics and farm-related organic enrichment shaping the dominant exposure pathways.

4.6. Implications for biomonitoring and environmental management

The present data support the use of *P. perna* as a biomonitor and reinforce that Hg speciation and bioavailability should be considered in monitoring programs. Because the proportion of MMHg relative to THg in mussel soft tissues appears to be modulated by site-dependent factors, assessments focused only on THg may underestimate toxicological risk when the MMHg fraction is elevated despite moderate THg concentrations. Accordingly, our data caution against substituting TotOrgHg for MMHg without prior validation.

Based on the main drivers of Hg bioaccumulation identified here, mitigation and monitoring can be discussed in terms of practical management recommendations. Strategies that reduce sediment–water coupling and resuspension in farming areas (e.g., favouring deeper and lower-energy settings, and adjusting farm layout/density) may help limit the transfer of sediment-bound Hg back to the water column and suspended particles. Because organic-rich particles can retain Hg in solids, management may aim to enhance retention in the solid phase and reduce sediment–water exchange; however, as changes in particulate/OM dynamics may also alter Hg bioavailability, these actions should be accompanied by monitoring to confirm their net effect on tissue concentrations. For biomonitoring, an integrated scheme should track the key compartments of the pathway, including SS and Sed (together with OM), and mussel soft tissues with Hg speciation whenever feasible. In line with the seasonal patterns observed here, THg_SS was higher during the dry season, whereas THg_Sed and mussel tissue Hg measurements were higher during the rainy season. Accordingly, sampling should prioritise the end of the dry season and the rainy period, complemented

by targeted surveys after resuspension events. Finally, simple physiological metrics (e.g., CI, and when feasible lipid proxies) should be reported alongside Hg to improve interpretability and to support harvest planning by prioritising periods of optimal CI.

The toxicological assessment draws attention to a potential public health concern, as even low consumption rates, particularly of mussels from the most contaminated bay (GUA), may be sufficient to reach MMHg exposure thresholds. The brown mussel *P. perna* has particular cultural and nutritional importance in artisanal fisheries and aquaculture in Brazil; therefore, these results point to the need for specific policy strategies targeting these populations. In this context, although cooking has been suggested to reduce metal concentrations in some foods (Perello et al., 2008), available evidence for Hg in mussels does not consistently support this reduction (Costa et al., 2021), and risk estimates should therefore remain based on measured concentrations.

The observed spatial variability supports the need for shoreline-focused monitoring and management in areas where mussel farms are installed. Although THg_Muss values in GUA are within the range of earlier studies, the maximum measured in this study is lower than that reported by Kehrig et al. (2006). This difference likely reflects marked spatial heterogeneity within the bay due to contrasting sampling locations (e.g., farmed mussels from an embayment versus central-channel sites), highlighting that Hg exposure and bioaccumulation can vary substantially even within a single bay. Similar spatial variability in MMHg fractions has been reported for *Mytilus galloprovincialis* along the French Mediterranean coast (Briant et al., 2017) and in other coastal systems (Ipolyi et al., 2004; Mancini et al., 2022), indicating that MMHg fractions may vary markedly even when THg occurs within similar ranges. More broadly, mussel-farm environments can modify P and N effluxes (Nizzoli et al., 2011) and sediment biogeochemistry, potentially shifting redox conditions and altering dissolved Hg speciation and the balance between methylation and demethylation processes (Driscoll et al., 2012). However, to the best of our knowledge, no study has explicitly quantified mercury methylation and demethylation dynamics in sediments and the water column within longline farming systems to resolve the role of high-density bivalves and farm-driven organic enrichment. This knowledge gap is particularly relevant given the continued growth of marine bivalve aquaculture worldwide as a key protein source for human diets (FAO, 2023). A better understanding of the methylmercury biogeochemical cycle in farm-influenced coastal systems is strategically important for managing Hg-related toxicological risk and for developing evidence-based guidance for marine farmers (e.g., site selection, farm density, site rotation, harvest timing). From a risk-management perspective, post-harvest approaches warrant investigation. Although depuration is widely used to reduce microbiological contamination in bivalves, and recent work has evaluated depuration systems (e.g., ozone/UV-based approaches) in *P. perna* for sanitary purposes (Guimarães Filho et al., 2025), future studies should assess whether depuration (or relaying) can reduce THg and/or MMHg in mussels as a complementary risk-management option.

5. Conclusion

This study evaluated mercury bioaccumulation in *Perna perna* across contrasting coastal settings and examined key drivers of Hg exposure and speciation by monitoring Sed and SS in parallel with mussel soft tissues during rainy and dry seasons. Overall, the expectation of a straightforward increase in tissue Hg with higher environmental contamination was not consistently supported, indicating that bioaccumulation may not track contamination gradients due to site-specific processes affecting Hg partitioning and bioavailability. The hypothesis of biodilution was supported, reinforcing the relevance of accounting for CI when interpreting bioaccumulation endpoints and potentially guiding harvest timing in routine farming practices. The SI approach added site-specific context: $\delta^{15}N$ tracked MMHg only where baseline signals were less affected by anthropogenic N inputs, whereas $\delta^{13}C$ differentiated predominant carbon sources (more coastal/benthic vs more offshore/pelagic), framing MMHg exposure in terms of contrasting baseline sources. Finally, TotOrgHg showed potential as a screening metric for organic Hg but should not replace MMHg determination when risk assessment or mechanistic inference is required. Taken together, *P. perna* remains a valuable sentinel species, and the results underscore the importance of integrating Hg speciation, environmental context, and physiological metrics in monitoring strategies, as well as the need for further work on biogeochemical controls of methylation in farm-influenced coastal systems.

CRediT authorship contribution statement

Petrus Galvao: Writing – original draft, Data curation, Conceptualization. **Renan L. Longo:** Methodology, Formal analysis. **Adan S. Lino:** Methodology. **Loïc N. Michel:** Formal analysis. **Valquiria M.C. Aguiar:** Writing – review & editing, Formal analysis. **Daniel F. Araújo:** Writing – review & editing. **João P.M. Torres:** Writing – review & editing, Resources. **Olaf Malm:** Supervision, Resources. **Humberto Marotta Ribeiro:** Resources. **Paulo R. Dorneles:** Supervision. **Krishna Das:** Writing – review & editing, Resources.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used Jenni.ai to improve English-language fluency. The authors reviewed and edited the content as needed and take full responsibility for the final content of the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.119510.

Data availability

Data will be made available on request.

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