Marine Pollution Bulletin 192 (2023) 115095

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

# Revealing the environmental pollution of two estuaries through histopathological biomarkers in five fishes from different trophic guilds of northeastern Brazil

Ítala Gabriela Sobral dos Santos [a,*], Alex Souza Lira [c], Caroline da Silva Montes [b], David Point [e], Anaïs Médieu [f], Clístenes Williams Araújo do Nascimento [g], Flávia Lucena-Frédou [a], Rossineide Martins da Rocha [d]

[a] *Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manuel de Medeiros, s/n, Dois Irmãos, CEP: 52171-900 Recife, Pernambuco, Brazil*
[b] *University of Concepción, Chile*
[c] *Universidade Federal de Sergipe (UFS), Cidade Univ. Prof. José Aloísio de Campos Av. Marechal Rondon, s/n, Jd. Rosa Elze São Cristóvão/SE, CEP 49100-000, Brazil*
[d] *Laboratory of Cellular Ultrastructure and Immunohistochemistry, Institute of Biological Sciences, Federal University of Pará (UFPA), Rua Augusto Correa n° 01, Guamá, CEP 66075-110 Belém, Pará, Brazil*
[e] *Observatoire Midi-Pyrénées, Géosciences Environnement Toulouse, UMR CNRS 5563/IRD 234/Université Paul Sabatier Toulouse 3, 14 avenue Edouard Belin, 31400 Toulouse, France*
[f] *Universite de Bretagne Occidentale (UBO), Institut de Recherche pour le Développement (IRD), Centre National de la Recherche Scientifique (CNRS), Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer), LEMAR, Plouzane F-29280, France*
[g] *Department of Agronomy, Federal Rural University of Pernambuco, Dom Manuel de Medeiros street, s/n - Dois Irmãos, 52171-900 Recife, PE, Brazil*

ARTICLE INFO

*Keywords:*
Gill
Liver
Sentinel
Histology
Anthropic impacts
Brazilian coast

ABSTRACT

Estuaries in Brazil are mostly anthropically affected due to the discharge of industrial and domestic effluents. In two of them, the Santa Cruz Channel Estuary (ITAP) and Sirinhaém River Estuary (SIR), historically affected by mercury pollution and sugarcane industry in Northeast Brazil, we assessed environmental pollution using liver and gill histopathological biomarkers in fish from different trophic levels. Liver samples exhibited serious damages such as hepatic steatosis, necrosis, and infiltration. The gills showed moderate to severe changes, such as lifting of epithelial cells, lamellar aneurysm, and rupture of lamellar epithelium. Most of the changes in the liver and gills were reported for species *Centropomus undecimalis* and the *Gobionellus stomatus*, which were considered as good sentinels of pollution. The combination of biomarker methodologies was efficient in diagnosing the serious damage to the species, reinforcing the need for monitoring the health of the ecosystems evaluated.

## 1. Introduction

Discharges of domestic, industrial and agriculture effluents are among the activities with the greatest potential for contamination in tropical coastal ecosystems (Saldarriaga-Hernandez et al., 2020). Industries of leather tanning, production of cement, chlor-alkali, mining, anticorrosive agent, and fertilizer production (Aslam and Yousafzai, 2017; Becker et al., 2018; Chellaiah, 2018; Cipurkovic et al., 2014; Pacyna et al., 2010; Vieira et al., 2019) are the main industrial activities threatening the balance of ecosystems. Emergent pollutants, such as pesticides and herbicides, used in the cultivation of sugar cane, are also of great concern given their impact on estuaries, consequently on biota (Chavan and Muley, 2014; Coelho et al., 2018).

Estuaries are transitional ecosystems characterized by ecological cycles that receive high entry of organic and inorganic contaminants, such as pesticides and trace metals (Salas et al., 2017) from multiple coastal anthropogenic activities. These contaminants are discharged into the water and accumulate mainly in the sediment (Kameli et al., 2017; Willacker et al., 2017) posing a risk to human health and biota (Carr et al., 2017; Dellamatrice and Monteiro, 2014).

* Corresponding author.
*E-mail addresses:* italagsobral@hotmail.com (Í.G.S. dos Santos), aliraufs@academico.ufs.br (A.S. Lira), david.point@ird.fr (D. Point), anais.medieu@ird.fr (A. Médieu), clistenes.nascimento@ufrpe.br (C.W.A. do Nascimento).

https://doi.org/10.1016/j.marpolbul.2023.115095
Received 8 December 2022; Received in revised form 19 May 2023; Accepted 21 May 2023
Available online 8 June 2023

Fish are considered good sentinels for assessing the biological effects of contaminants in aquatic environments since they bioconcentrate and bioaccumulate contaminants by direct absorption from water or, in some cases, indirectly by consumption of contaminated preys (Kroon et al., 2017), consequently, can reach the upper trophic levels used as food by humans (Wang, 2002). The choice of species to be used as a monitors of contamination is essential (Oost et al., 2003), and those from different trophic guilds and with distinct trophic position are recommended because they use different absorption/accumulation routes, directly related to the type of food consumed (Terra et al., 2008).

Histopathological biomarkers are widely used to investigate the effects of contamination in organisms, due to the possibility of identifying lesions in cells, tissues, and organs (Hook et al., 2014; Oost et al., 2003; Viana et al., 2013). Among the organs used for histopathological studies, the liver is often chosen since it is essential for the physiological functioning of the animal, by detoxifying and excreting toxic substances, and bioaccumulating pollutants (Borges et al., 2018). Gills, the first organ in contact with pollutants, play a key role in gas exchange and osmotic regulation in fish, similarly recommended for environmental biomonitoring (Ameur et al., 2015). This approach is widely used to assess the health of aquatic environments (Dalzochio et al., 2016).

The use of histopathological biomarker gives support through the responses of biological effects to the analysis of the physical-chemical variables of abiotic matrices, water and sediments, favoring the elaboration of the diagnosis of the environmental quality of ecosystems with a holistic and efficient vision (Maggioni et al., 2012; Tashla et al., 2018; Viana et al., 2013).

This study aims, for the first time in Northeast Brazil, use liver and gills as histopathological biomarkers to assess environmental pollution of five fish species with different trophic levels in two historically impacted estuaries of Pernambuco, the Canal de Santa Cruz Estuary (ITAP) and Sirinhaém Estuary of the River (SIR). Even though the studied areas are impacted by different anthropic sources and different severities, such as the launch of 22 to 35 tons of Hg in the ITAP for 24 years and the sugar industry established in SIR since the 19th century (Meyer, 1996; Pelage et al., 2019).

## 2. Materials and methods

### 2.1. Study area

The study area comprises two estuarine systems positioned along the coast of Pernambuco state, northeastern Brazil (Fig. 1), which exhibits distinct morphologic and physicochemical characteristics subject to different anthropogenic pressures (Table 1).

The Santa Cruz Channel Estuary (ITAP) is the largest and most productive estuary in Pernambuco coast (Macêdo et al., 2000) (Fig. 1a). It is a 22 km long U-shaped channel with width ranging from 0.6 to 1.5 km, located within the Santa Cruz Environmental Preservation Area (APA Santa Cruz). A chloro-alkali industry was responsible for launching 22 to 35 tons of inorganic mercury in an important tributary of the ITAP complex for approximately 24 years of operation (Meyer, 1996). These impacts such as shrimp farm, discharge industry and domestic may have contributed for the reduction of up 10 % of mangrove coverage in this area (Pelage et al., 2019).

The Sirinhaém River Estuary (SIR) is a small, shallow, coastal plain estuary, 9.5 km long, with maximum depth ranging from 1.2 to 4.5 m (Da Silva et al., 2011). Located between the Guadalupe Marine Protected Area and the Sirinhaém Marine Protected Area, SIR is characterized by a high density of mangroves, occupying 18.7 km$^2$ (Maia et al., 2006) (Fig. 1b). Spatial land cover is predominantly urbanization, vegetation, mangrove and bare soil (Pelage et al., 2019). Although it is of extreme biological importance, multiple impacts are reported in the SIR estuary such as domestic and industry effluent, and the sugar industry established since the 19th century (CPRH, 2011; Pelage et al., 2019).

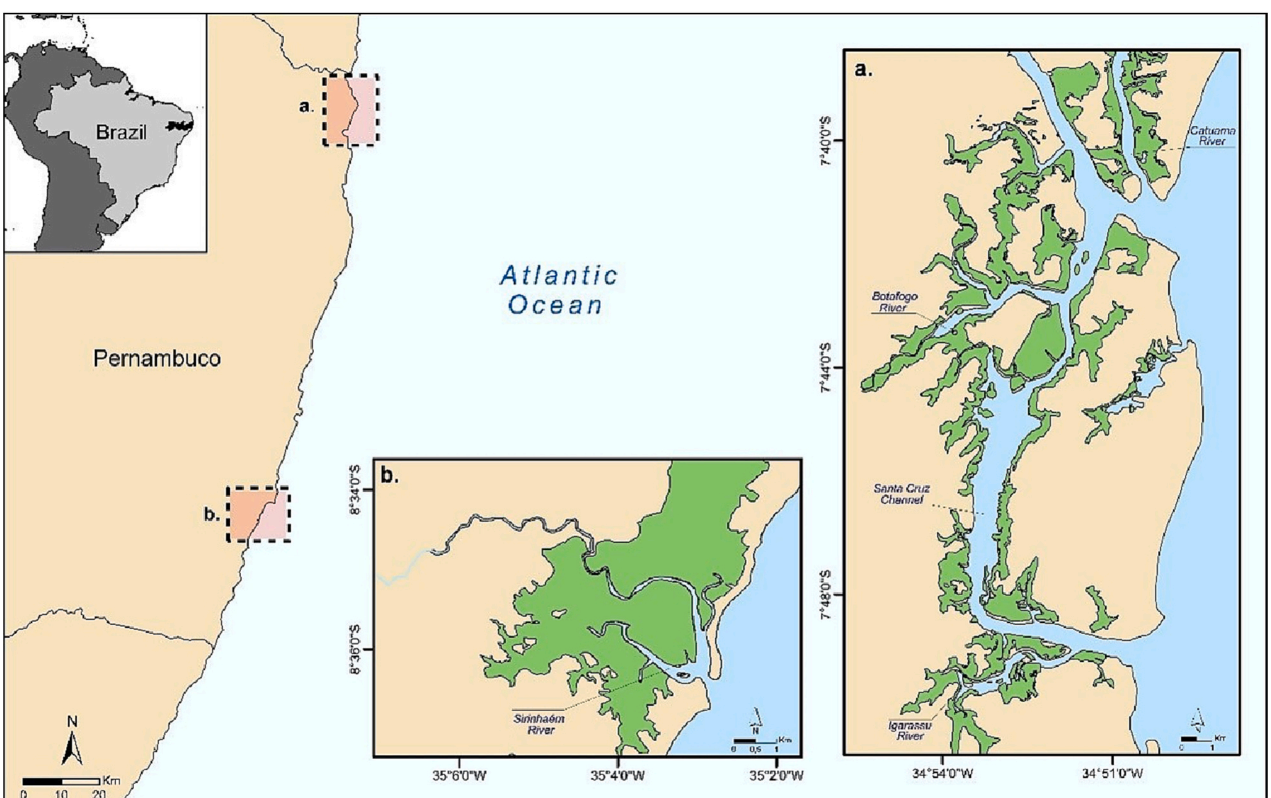


**Fig. 1.** Study area (Pernambuco, northeast Brazil. a: Santa Cruz Channel Estuary (ITAP), b: Sirinhaém River Estuary (SIR).

 

**Table 1**
Summary of morphological characteristics, environmental settings and anthropogenic activities of the two study areas (Santa Cruz Channel — ITAP and Sirinhaém — SIR estuaries) along the coast of Pernambuco, northeastern Brazil.

| Characteristics | Estuary | |
|---|---|---|
| | Santa Cruz Channel (ITAP) | Sirinhaém (SIR) |
| Type | Ria | Coastal plain |
| Human pressures | Aquaculture, agricultural, chemical industry, and domestic waste | Sugar cane industry and domestic waste |
| Vegetated area ($km^2$) | 48.0 | 17.0 |
| Water surface area ($km^2$) | 25.5 | 1.7 |
| Mean depth (m) | 3.0 | 2.6 |
| Max. depth (m) | 20 | 5 |
| N of marine entrances | 2 | 1 |
| Width of marine entrances (km, mean and range) | 0.9 (0.5–1.3) | 0.4 |
| Pelagic productivity (mg C $m^{-3}$ $h^{-1}$) | 14.7 | 34.2 |
| Water surface temperature (°C, mean ± SD) | 28.5 ± 1.1 | 27.2 ± 2.4 |
| Salinity (mean ± SD) | 31.1 ± 2.9 | 9.5 ± 3.6 |
| Pluviometry (mm, monthly mean ± SD) | 126 ± 24 | 170 ± 25 |
| References | Medeiros and Kjerfve (1993); Medeiros et al. (2001); Neumann-Leitão et al., 2001; CPRH, 2003, 2003a; Borges (2011); da Silva (2009); Guimarães et al. (2010); Da Silva et al. (2011); APAC, 2019; Gonzalez et al. (2019); Silva et al. (2019) | |

### 2.2. Fish samples

The fish species were chosen considering their importance for the local community as a source of food and income, ecological role as key species for the ecosystem (Lira et al., 2018, 2022) and are from the different trophic guilds. The trophic guild of each species was classified according to Elliott et al. (2007) and Mourão et al. (2014) as Detritivore (DV), Piscivore/Zoobenthivore (PVZB) and Piscivore (PV). In ITAP *Bairdiella ronchus* (n = 24, PV) and *Gobionellus stomatus* (n = 34, DV) were sampled, and *Caranx latus* (n = 35, PVZB), *Centropomus undecimalis* (n = 24, PV) and *Centropomus parallelus* (n = 29, PVZB) were collected in SIR.

Fishes were collected using block nets set close to mangrove creeks (350 × 2.9 m, 70 mm mesh) and beach seine trawls (20 × 1.9 m, 20 mm mesh), in 2018 and 2019. The specimens analyzed were obtained from artisanal fishermen, refrigerated immediately after collection. As soon as they returned from fishing, they were kept on ice until the organs were removed in the shortest possible time. All fishes were measured (standard length — cm) and weighed (g). Posteriorly, an abdominal incision was made to dissect the liver, and the second branchial arch from the right side was removed. The liver was weighted, and its median portion removed.

### 2.3. Histology

The collected tissues were fixed in 10 % formalin for 24 h, preserved in 70 % ethanol for histopathological analysis and subjected to routine histological processing for paraffin embedding (Prophet et al., 1995). Samples were subsequently sectioned into 5-μm-thick sections using a RM2245 microtome (Leica Microsystems, Germany). For each organ 6 sections were analyzed in all species. The liver was stained with hematoxylin and eosin (HE), hematoxylin and eosin-phloxine B (HE-P), and Mallory Trichrome. The gills were stained with (HE) and (HE-P). Stained sections were analyzed, and photomicrographs were obtained using an Eclipse Ci-S light microscope (Nikon, Japan) connected to a Nikon DS-Ri1 digital camera.

### 2.4. Histopathological indicators

#### 2.4.1. Histopathological index of the liver (HIL)

The prevalence of histopathological alterations (% = [number of changes / total changes analyzed] × 100) was estimated per fish species for liver and gills. Liver changes were semi-quantitatively assessed according to the adapted protocol described by Bernet et al. (1999). The Histopathological Index of the Liver (HIL) was calculated for each fish by multiplying the importance factor by the score value of each alteration (see Table 2 for the-List of alterations). According to this index, an importance factor was attributed to each change by taking into consideration the following tissue injury levels: (1) minimum — easily reversible change; (2) moderate — reversible if the stressor is neutralized; and (3) severe — often irreversible, thus leading to decreased liver function. It was possible to assign a score value to the changes after they were identified. This value corresponds to the level and extent of the tissue changes, namely: (0) unchanged; (2) occasional; (4) moderate and (6) severe (diffuse lesion). Given the reaction pattern, these alterations are divided into four categories: (1) circulatory disturbances (pathological condition of blood and tissue flow), including hemorrhage and aneurysm; (2) regressive changes (causes reduction or loss or the organ function) which involve cytoplasm alteration, hepatic steatosis, hepatic parenchyma, necrosis, nuclear alterations, and vacuolar degeneration; (3) progressive changes (increased activity of cells or tissues) represented by hypertrophy and hyperplasia in hepatic parenchyma; and, (4) inflammation (consequence of other reaction patterns), such as infiltration and parasites.

The Hepatosomatic Index (HSI) (Eq. 1) (Slooff et al., 1983) and Condition Factor (K) (Eq. 2) (Smolders et al., 2002) were also used to

**Table 2**
Classification of histopathological changes of gill and liver in relation to the type, location and stage of lesions in which they occur.

| Gill/liver histopathology | Stage/importance factor |
|---|---|
| Hypertrophy and hyperplasia of gill epithelium | |
| Hypertrophy of respiratory epithelium | I |
| Lifting of epithelial cells | I |
| Lamellar epithelial hyperplasia | I |
| Focal hyperplasia of epithelial cells | I |
| Leukocyte infiltration of gill epithelium | I |
| Derangement lamellar | I |
| Incomplete fusion of some lamellae | I |
| Parasites | I |
| Complete fusion of all lamellae | II |
| Rupture of the lamellar epithelium | II |
| Uncontrolled proliferation of tissue | II |
| Necrosis | III |
| Changes in mucous cells | |
| Hypertrophy and hyperplasia of mucous cells | I |
| Changes in blood vessels | |
| Filament blood vessel enlargement | I |
| Vessel blood congestion | II |
| Hemorrhages with rupture of epithelium | II |
| Lamellar telangiectasis | II |
| Aneurysm lamellar | II |
| Changes in hepatocytes | |
| Cytoplasm alteration | I |
| Hepatic steatosis | I |
| Hepatic parenchyma | I |
| Nuclear alterations | II |
| Vacuolar degeneration | III |
| Necrosis | III |
| Cell hypertrophy | I |
| Cell hyperplasia | II |
| Infiltration of leucocytes | II |
| Parasites | III |
| Wall proliferation of bile ducts | I |
| Changes in blood vessels | |
| Haemorrhage/aneurysm | I |

Modified from Poleksic and Mitrovic-Tutundzic (1994) for gills (stage) and Bernet et al. (1999) for liver (importance factor).

assess the status of the fish health, since the variation in the weight of the organ in relation to body weight is a response of the individual when exposed to contaminants (Pavione et al., 2019). The HSI was calculated as:

$$\mathrm{HSI} = 100 \times \frac{\text{liver weight (g)}}{\text{body weight (g)}} \tag{1}$$

While the condition factor calculated by Eq. 2:

$$\mathrm{K} = 100 \times \frac{\mathrm{W}}{\mathrm{L}^3} \tag{2}$$

Where W = body weight (g) and L = total length of fish (cm).

#### 2.4.2. Fish Health Index (FHI)

The Fish Health Index (FHI) (Zimmerli et al., 2007) was used to classify liver injury severity. This index is divided into five classes, as follows: Class I (<10), normal/healthy tissue structure without impairments or pathological alterations; Class II (11−20), normal tissue structure with slight histological alterations; Class III (21−30), moderate modifications of normal tissue and morphology; Class IV (31–40), pronounced histological changes (in the liver); and Class V (>40), severe histological alterations of normal tissue and morphology.

#### 2.4.3. Histopathological Index of the Gill (HIG)

The gill histopathological alterations were calculated from the adaptation of the Poleksic and Mitrovic-Tutundzic (1994) classification. The type and location of the damaged gill tissue are divided into five groups: *a.* hypertrophy and hyperplasia of gill epithelia and related changes; *b.* changes in the mucous cells; *c.* gill parasites; *d.* blood vessel changes, and *e.* last terminal stages. After that, the method determines the stage of each group of alteration (Table 2). The HIG was calculated as Eq. 3:

$$\mathrm{HIG} = \sum \mathrm{I} + \left(10 \times \sum \mathrm{II}\right) + (100 \times \Sigma\, \mathrm{III}) \tag{3}$$

where I, II, and III are the number of stages for lesions. An overall score is used to classify the organ, being 0–10: functioning normally, 11–20: slightly to moderately damaged, 21–50: moderately to heavily damaged and > 100 irreparably damaged.

### 2.5. Trace metal in fish samples

Some individuals were selected from those collected for histopathological analysis, for the analysis of trace metals in the muscle, such as *Bairdiella ronchus* (n = 20) and *Gobionellus stomatus* (n = 23) were sampled at ITAP, while *Caranx latus* (n = 21), *Centropomus undecimalis* (n = 24) and *Centropomus parallelus* (n = 22) were collected at SIR. Subsequently, for each fish, a muscle sample was taken from the dorsal part, washed with distilled water and dried in an oven at 60 °C for 48 h. In each sample, we measured total concentrations of six trace elements: cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), zinc (Zn), and mercury (Hg). Total Hg concentrations (were measured in samples dried by thermal decomposition, gold amalgamation, and atomic adsorption (sediments: DMA-80, Milestone, Italy, and fish: AMA-254 (Altec, Czech Republic) at GET (Toulouse, France). For fish samples, Hg concentrations expressed on a dry weight basis were converted to a wet weight basis (ww) by applying a species-specific moisture factor, previously determined for each sample. To investigate the robustness, accuracy and precision of the measurements, blanks and two biological reference materials and one certified sediment reference material were used for every ten samples, covering the range of our Hg concentrations: IAEA-436 (tuna tissue), SRM2976 (mussel tissue) and Mess-3 (marine sediment).

The fish samples for the other trace elements, i.e. Cd, Cr, Cu, Pb, and Zn. were subjected to 3051A acid digestion (U.S. EPA, 2007). These samples (~500 mg) were weighed and pre-digested with nitric acid (8 mL) and hydrogen peroxide (2 mL), and then digested in a microwave oven for 10 min at 180 °C. The extracts were filtered and diluted in 25 mL vials with the addition of 5 % potassium permanganate and stored in borosilicate vials until the time of determination. The levels of trace metals (Pb, Cd and Hg) in biological samples were compared with the guidelines of the National Health Surveillance Agency (ANVISA, 2013).

### 2.6. Statistical analysis

The Kruskal-Wallis non-parametric test, followed by Nemenyi test for multiples comparisons (post-hoc), was performed to compare the HIL and HIG values among fish species and trophic guild. These tests were also used for comparisons between the lesion classes HIL (4 stages) and HIG (3 stages) for each species.

To assess the degree of resemblance in the histopathological alterations among species for liver and gills, multidimensional scaling (MDS) based on a Euclidian distance matrix was applied using the frequency of occurrence of histopathological alterations (%) as input, with the specimens considered as the sampling units. Wisconsin double standardization was applied to improve the gradient detection ability of the dissimilarity indices (Bray et al., 1957). To compare the whole set of histopathological alterations among sites and fish species, a two-way PERMANOVA was applied (Anderson, 2001). All analyses were performed in the R environment (R Core Team, 2020), using "vegan" (Oksanen et al., 2017) and "rrcov" packages (Todorov and Filzmoser, 2009). For all analyses, a significance level of 0.05 % was considered.

## 3. Results

### 3.1. Fish analysis

A total of 146 individuals were analyzed. *Gobionellus stomatus* (3.82) and *B. ronchus* (1.13) in Santa Cruz Channel Estuary (ITAP) had the highest HSI values (Table 3). In terms of condition factor (K), the highest values were observed in *C. latus* (2.68) and *B. ronchus* (1.97), and the lowest in *G. stomatus* (1.01).

Concentration of Cd, Cr, Cu, Hg, Pb, and Zn in the analyzed fish samples are presented in Table 4. For all species (i.e., *B. ronchus*, *C. latus*, *C. undecimalis*, *C. parallelus* and *G. stomatus*), Cd (<0.05 mg·kg$^{-1}$, ww) and Pb (<0.3 mg·kg$^{-1}$, ww) concentrations were below the tolerance limit reported by the Brazilian health regulatory agency (ANVISA), meaning that their consumption do not pose serious risk for human health.

### 3.2. Histopathology of liver

A total of 13 types of alterations in the liver were observed: circulatory (1), inflammatory (3), progressive (1) and regressive (8), summarized by the Histopathological Index of Liver (HIL — Fig. 2). The

**Table 3**
Morphometric data and gross index. Mean ± standard deviation. n: number of individuals analyzed, HSI: hepatosomatic index, K: condition factor, ITAP: Santa Cruz Channel Estuary, SIR: Sirinhaém River Estuary.

| Species | n | Area | Standard Length (cm) | Body mass (g) | HSI | K |
|---|---|---|---|---|---|---|
| *Bairdiella ronchus* | 24 | ITAP | 14.17 ± 1.38 | 34.74 ± 11.66 | 1.13 ± 0.42 | 1.97 ± 0.15 |
| *Gobionellus stomatus* | 34 | ITAP | 14.36 ± 3.18 | 11.96 ± 5.91 | 3.82 ± 3.31 | 1.01 ± 0.25 |
| *Centropomus undecimalis* | 24 | SIR | 24.5 ± 3.05 | 101.9 ± 37.38 | 0.91 ± 0.28 | 1.23 ± 0.17 |
| *Centropomus parallelus* | 29 | SIR | 17.9 ± 2.1 | 55.18 ± 21.85 | 0.90 ± 0.17 | 1.85 ± 0.16 |
| *Caranx latus* | 35 | SIR | 12.49 ± 1.1 | 27.32 ± 8.8 | 1.06 ± 0.35 | 2.68 ± 0.22 |

**Table 4**
Concentration of trace metals in mg. $Kg^{-1}$. Mean ± standard deviation. n: number of individuals analyzed, ITAP: Santa Cruz Channel Estuary, SIR: Sirinhaém River Estuary.

| Species | n | Area | Cd | Cr | Cu | Pb | Zn | Hg |
|---|---|---|---|---|---|---|---|---|
| *Bairdiella ronchus* | 20 | ITAP | 0.00 ± 0.01 | 0.11 ± 0.26 | 0.19 ± 0.04 | 0.02 ± 0.02 | 2.52 ± 0.28 | 0.26 ± 0.13 |
| *Gobionellus stomatus* | 23 | ITAP | 0.00 ± 0.00 | 0.02 ± 0.03 | 0.13 ± 0.04 | 0.01 ± 0.02 | 3.78 ± 0.75 | 0.05 ± 0.09 |
| *Centropomus undecimalis* | 24 | SIR | 0.00 ± 0.00 | 0.09 ± 0.12 | 0.14 ± 0.04 | 0.00 ± 0.00 | 2.96 ± 0.21 | 0.02 ± 0.00 |
| *Centropomus parallelus* | 22 | SIR | 0.00 ± 0.00 | 0.07 ± 0.14 | 0.16 ± 0.03 | 0.01 ± 0.01 | 2.98 ± 0.45 | 0.01 ± 0.02 |
| *Caranx latus* | 21 | SIR | 0.00 ± 0.00 | 0.05 ± 0.09 | 0.40 ± 0.05 | 0.06 ± 0.07 | 3.26 ± 0.41 | 0.01 ± 0.01 |

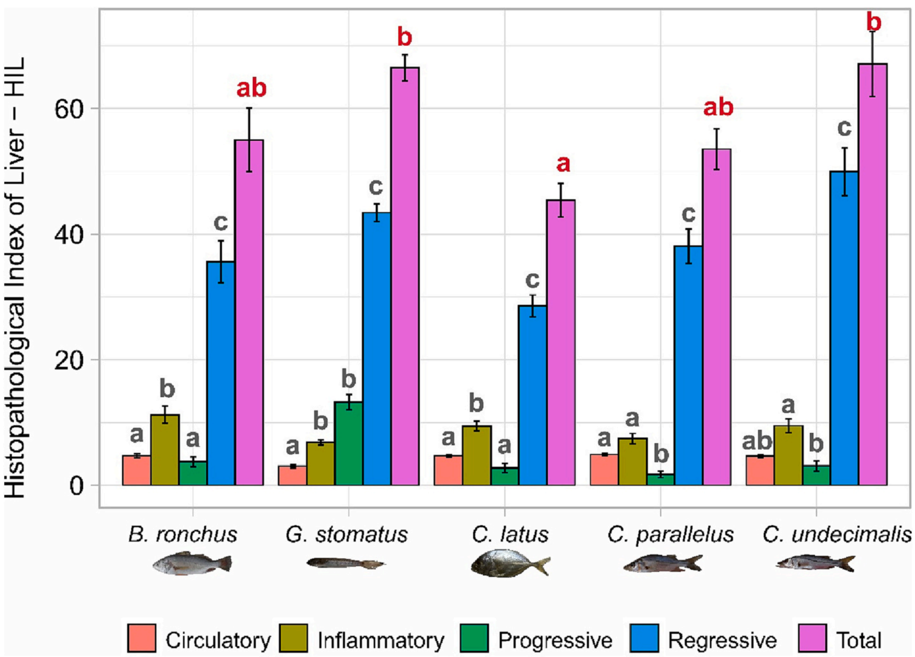


**Fig. 2.** Histopathological index of liver (mean ± SD) by represented species. Grey and red letters indicate the difference lesions within each species and total alterations among the species respectively. *B. ronchus* and *G. stomatus* from Santa Cruz Channel Estuary (ITAP), *C. latus*, *C. parallelus* and *C. undecimalis* from Sirinhaém River Estuary (SIR). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

more relevant alterations are regressive changes. The six most important alterations, present in all fishes with different magnitudes were: hemorrhage (97.26 %), structural alterations in liver tissue (100 %), hepatic steatosis (92.46 %), necrosis (72.60 %), vacuolar degeneration (95.89 %), and infiltration (94.52 %).

The alterations were significantly different between the species (Fig. 2 — HIL total and Table S1). *Centropomus undecimalis* (67.08 ± 5.97) in SIR and *G. stomatus* (66.47 ± 3.32) in ITAP had the most severe changes i.e., higher values of HIL (Fig. 2 — HIL total). These species were followed by *B. ronchus* (55.21 ± 5.85) caught in ITAP, *C. parallelus* (52.10 ± 4.26) and *C. latus* (45.4 ± 3.44) caught in SIR. Significant differences were observed between the types of alterations within each species, being the regressive-type the most frequent (Fig. 2 and Table S2). Based on Fish Health Index (FHI), all species were considered with severe alterations given the morphology and hepatic parenchyma.

The rank of the histopathological index of liver (HIL), in severity order, was *C. undecimalis* > *G. stomatus* > *B. ronchus* > *C. parallelus* > *C. latus*. Although *C. undecimalis* was more impacted in absolute values of HIL, *G. stomatus* had more severe histological changes, such as fatty degeneration. In general, the HIL values were significant different between guilds (Table S1), being piscivores (*C. undecimalis* and *B. ronchus*) and detritivores (*G. stomatus*) were more affected.

In the MDS plot based on liver alterations, it was possible to observe a clear separation of *G. stomatus* from the other species (Fig. 3a). There were significant alterations in all histopathological alterations between *G. stomatus* and other species (pseudo-F = 9.89; $p < 0.001$), mainly due to the high occurrence of alterations (Fig. 3b) as the vacuolar degeneration (Fig. 4g), architectural and structural alteration, hyperplasia, and hepatic steatosis, in comparison with the other species. A high frequency of some alterations by species was observed, such as necrosis in *C. undecimalis* (Fig. 4e), inflammation by leukocyte infiltrate in *C. latus* (Fig. 4d), and parasites in *B. ronchus*.

Hepatic steatosis (Fig. 4b–c) was noted with a varying degree of intensity, indicating accumulation of lipids. However, particularly to *G. stomatus*, the liver parenchyma completely filled with fat accumulation was observed (Fig. 4g).

Parasites were present in 12.32 % of the individuals of all species, except in *G. stomatus*, that have no parasites. The most affected species were *B. ronchus* and *C. latus*, with 29.16 % and 17.14 % of livers infected (Fig. 3b). Parasites were identified as metacercariae of digenetic trematodes (Fig. 4m–o) and myxozoan parasite (Fig. 4j). Metacercaries were found within the exocrine pancreas and hepatic parenchyma. Its presence has a clear separation within the blood congestion (Fig. 4k) and presents a central portion of indefinite shape, with a circular tendency in pink and branches in yellow (Fig. 4m). These pathogens were surrounded by a capsule of connective tissue (Fig. 4l). Myxozoan parasite (Fig. 4j) were observed in the bile ducts of *B. ronchus* that presented granulomas with inflammatory response, the parasite's morphology is mainly composed of collagen fibers, blue in color stained with Mallory trichrome. It occupies the entire bile duct and multiplies within it, increasing the size of this inflammatory capsule.

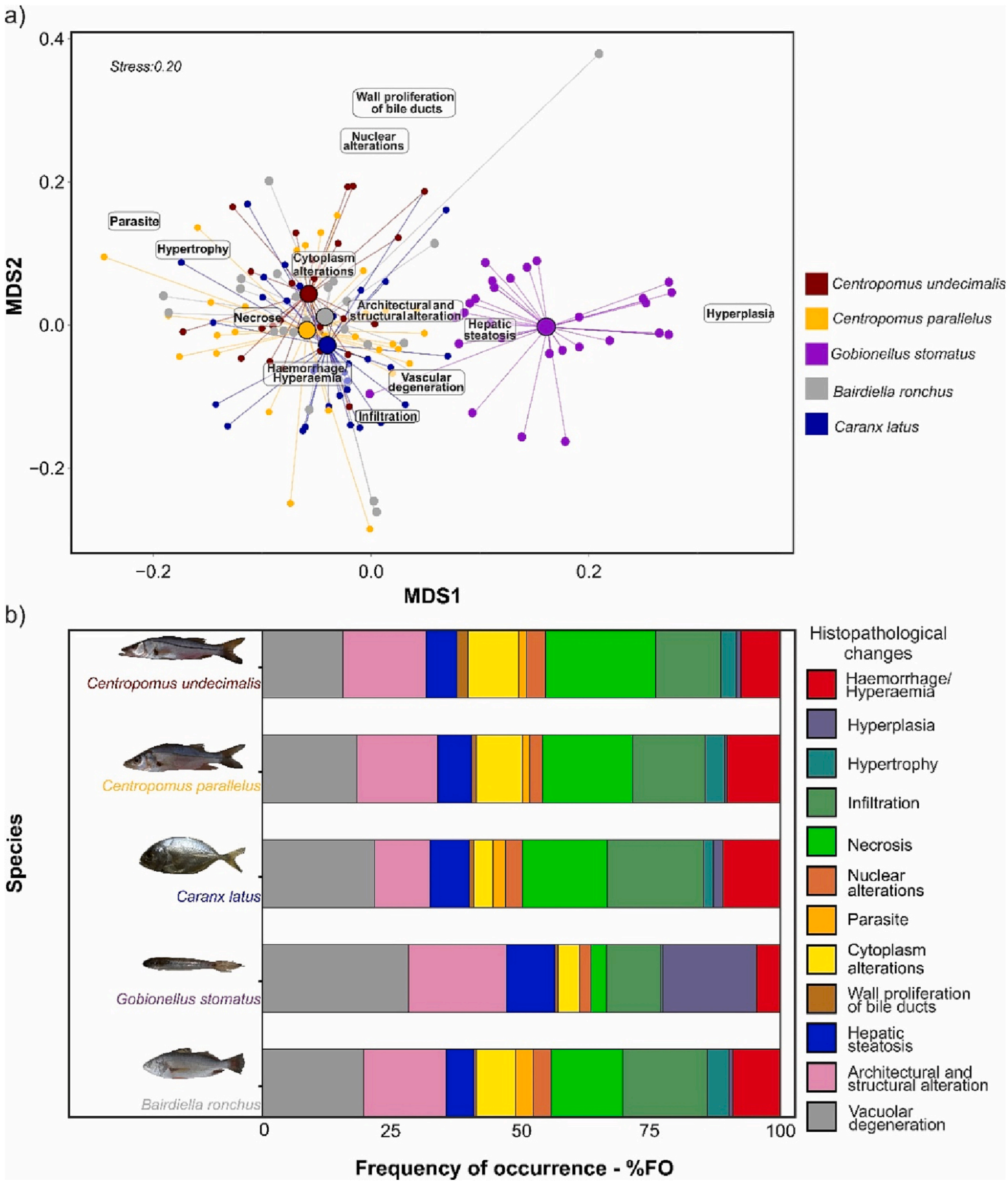


**Fig. 3.** Plot of multidimensional scaling (MDS) with histopathological changes of the liver (a) and the frequency of occurrence of changes (b).

### 3.3. *Histopathology of gills*

There were 18 types of alterations in the gills, distributed in 3 stages: stage I (10), stage II (7) and stage III (1) (Fig. 6). The most abundant changes were lifting of epithelial cells (65.06 %) and fusion of several secondary lamellae (33.56 %) in stage I, lamellar aneurysm (63.01 %), vessel blood congestion (46.57 %) and rupture of the lamellar epithelium (56.16 %) in stage II, and necrosis (34.93 %) in stage III.

The alterations were significantly different between the species (Fig. 5 — HIG total and Table S3). The higher values of HIG were observed in *G. stomatus* (93.55 ± 10.36) and *B. ronchus* (66.95 ± 12.21), both caught in ITAP. Significant differences were observed between the

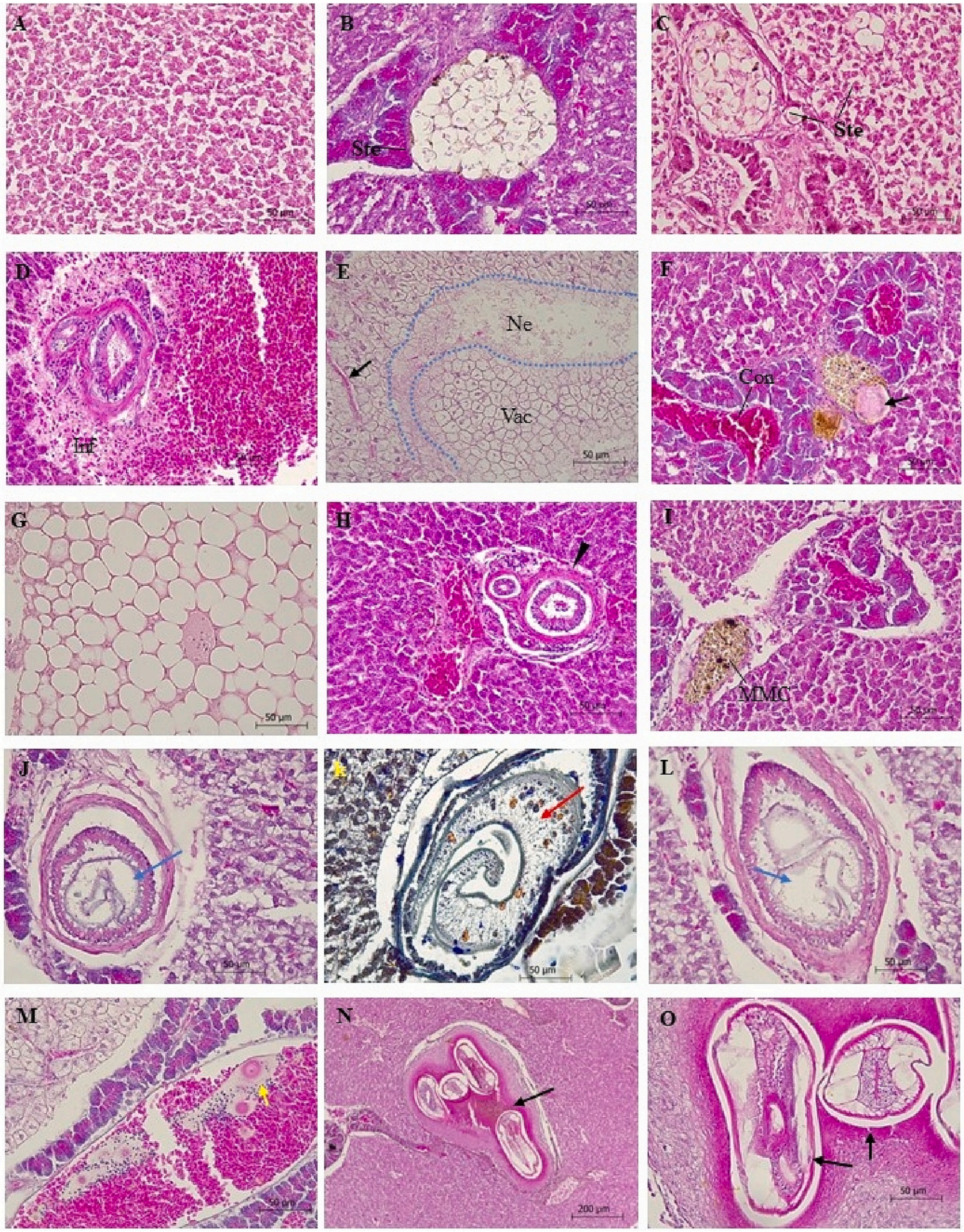


**Fig. 4.** Histopathological changes in the liver of the analyzed species, *C. parallelus* (A, C), *B. ronchus* (E), *C. undecimalis* (B, F, I), *C. latus* (D, H), *G. stomatus* (G), Bar = 50 µm; H & E-P stain. Photomicrograph hepatic tissue [A] Normal structural architecture; [B] extensive hepatic steatosis (Ste); [C] hepatic steatosis (Ste) allocated to exocrine tissue and dispersed in the hepatic parenchyma,; [D] Inflammation by leukocyte infiltrate (Inf); [E] leukocyte infiltrate (arrow), vacuolated hepatocytes (Vac) and necrosis (Ne); [F] granuloma (black arrow), blood cell congestion within the exocrine pancreas (Con); [G] severe fatty degeneration; [H] hypertrophy of bile duct; [I] melano-macrophage centers; *B. ronchus* (J, K, L), *C. undecimalis* (M, N, O), Bar = 50 µm (J, K, L, M, O); 200 µm (N), H & E-P stain, mallory of trichrome stain (K), [J] sporoplasms of a myxozoan parasite into the bile ducts (blue arrow); [K] sporoplasms of a myxozoan paratise into the bile ducts (red arrow); [L] initial of sporoplasms of a myxozoan paratise into the bile ducts (blue arrow); [M] metacercariae of digenetic trematodes within the blood vessel (yellow arrow); [N] metacercariae of digenetic trematodes inside an envelope capsule (black arrow); [O] metacercariae of digenetic trematodes inside an envelope capsule (black arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

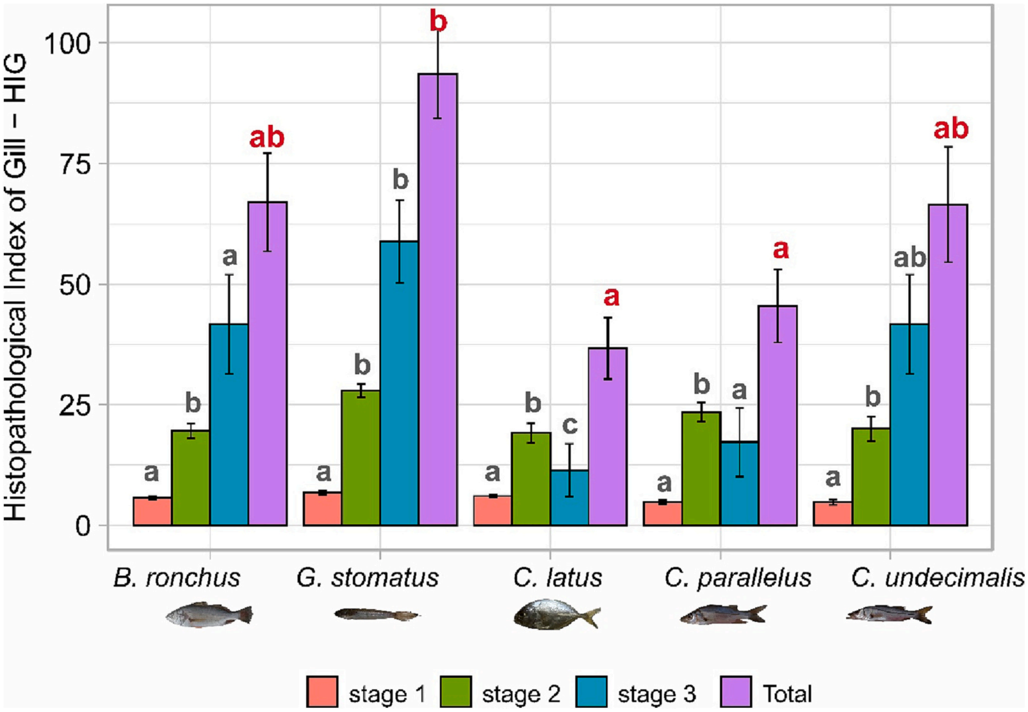


**Fig. 5.** Histopathological index of gill (mean ± SD) by represented species. Grey and red letters indicate the difference lesions within each species and total alterations among the species respectively. *B. ronchus* and *G. stomatus* from Santa Cruz Channel Estuary (ITAP), *C. latus*, *C. parallelus* and *C. undecimalis* from Sirinhaém River Estuary (SIR). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lesion stage within each species, being the stage 3 (necrosis presence) the most frequent (Fig. 5 and Table S4).

In terms of severity, the rank of HIG was *G. stomatus* > *B. ronchus* > *Centropomus undecimalis* > *C. parallelus* > *Caranx latus*. Although all species had moderate to heavy damaged gills, *C. undecimalis* in SIR and *G. stomatus* in ITAP had more damages. Additionally, significant differences were observed among the trophic guilds (Table S3), being the detritivores (*G. stomatus*) the more affected.

The frequency of changes was generally divided into four classes, 6.16 % of fishes had normally functioning gills (HIG: 0–10) and 13.1 % had slightly to moderately damaged gills (HIG: 11–20). The third class (HIG: 21–50) was the most abundant (45.89 % of fishes), and corresponds to moderately to heavily damaged gills, with more severe changes that can affect normal functioning. In the latter class (33.56 % of fishes) (HIG values > 100), there were irreparably damaged gills.

In the MDS plot based on gills damage (Fig. 6a), was observed significant difference between the species (pseudo-F = 2.23; $p < 0.05$). There was predominance of specific alterations in some species that were responsible for these differences. For example, *B. ronchus* had mostly necrosis, in *C. parallelus* hemorrhage with rupture of epithelium was most abundant, and complete fusion of all secondary lamellae was most common in *C. undecimalis* (Fig. 7c). In general, changes such as necrosis, lamellar aneurysm, rupture of the lamellar epithelium and hemorrhages with rupture of epithelium were present in all fishes in significant proportions (Figs. 6b and 7).

## 4. Discussion

The evaluation of anthropic impacts on biota can be much widely, for example, from trace metals has interference in the bioavailability of metals, because its bioavailable fraction is directly influenced by environmental variables (Adams et al., 2019). Especially in fish, these different available chemical species of contaminants that bioaccumulate, directly affect the vital functions of the organisms, such as respiration and nutrient metabolization, being accumulated in the gills and liver, respectively, and through biomagnification, for example MeHg (Filote et al., 2021; Savoca and Pace, 2021). The application of histopathological biomarkers are widely used to assess the health status of fish in anthropized environments, as they allow the detection of sublethal effects, being a low-cost and easily applicable tool (Bernet et al., 1999; Fonseca et al., 2016). Our findings obtained using liver and gills as biomonitors, the species showed severe degree of histological damage in the organs and structures observed, possibly reflecting a high degree of anthropogenic impacts in the areas where they are inhabiting, while for the trace metals in muscle tissue are below the limit of current legislation (ANVISA, 2013), but does not prevent cellular damage is from acute exposure and/or point of anthropogenic impacts of both estuaries.

High values of hepatosomatic index (HSI) were observed in all species. The liver is an organ commonly used to assess contaminant effects and anthropic impact in fish, mainly through morphometric indices such as HSI and condition factor (K). Several studies have already evaluated the positive correlation between liver enlargement and the exposure to chemical pollutants, which can be a metabolic activity during the process of detoxifying contaminants (Schmitt and Dethloff, 2000; Oost et al., 2003). The size of the liver can be used as a tool to monitor the health of fish in impacted aquatic environments (Oost et al., 2003). Higher HSI values are associated to the presence of toxic compounds and, the increase of liver size is a result of hypertrophy and hyperplasia processes, as well as the increasing ability to metabolize xenobiotics (Araújo et al., 2018; Pavione et al., 2019). The relationship between increased HSI and lipid accumulation in fish liver was also reported by Fåhraeus-Van Ree and Spurrell (2003) for *Limanda ferruginea* from Witless Bay, Newfoundland and Labrador.

As for the HSI, the lowest K value among the analyzed species was observed in *G. stomatus*. In general, K is often associated to physical and biological condition of the species (e.g., growth and reproductive pattern) taking in account interaction among feeding conditions, parasitic infections and physiological factors (Cren, 1951). It can vary among populations (Kaufman et al., 2007), seasons (Freitas et al., 2011), life

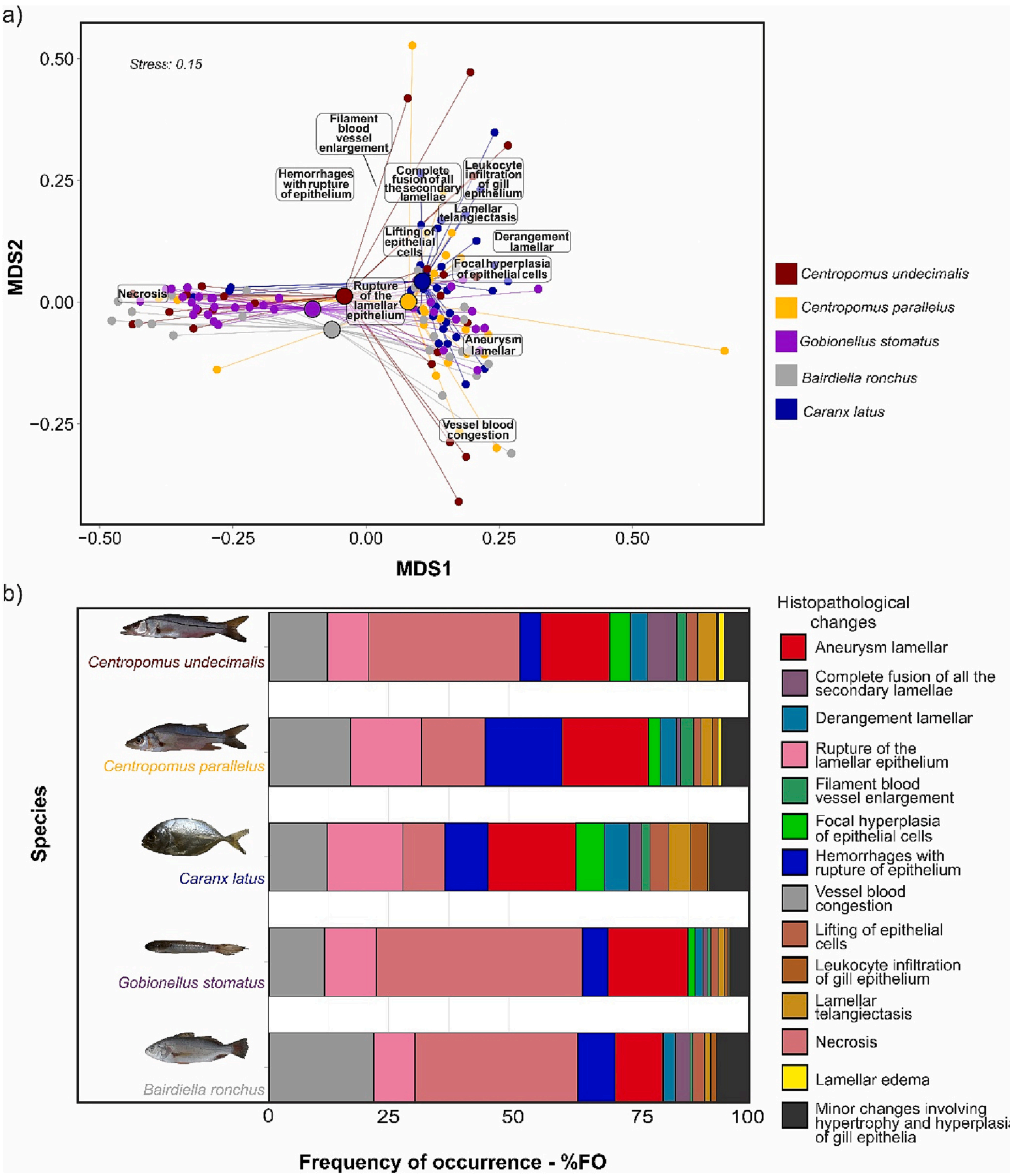


**Fig. 6.** Plot of multidimensional scaling (MDS) with histopathological changes of the gills (a) and the frequency of occurrence of changes (b).

stage (Ontogeny) (da Costa and Araújo, 2003), and change with sexual maturity (McPherson et al., 2011). However, it may also be related to water quality and food availability and is therefore considered an indicator of fish health (Rossi et al., 2020; Oost et al., 2003). For example, this index may be reduced by the presence of pollutants given the increased metabolic activity and demand of energy for detoxification process, altering the fish feeding rate (Heath, 1995). In contrast, high HSI is commonly associated to sublethal impacts of effluents, such as retention zones for contaminants, exposure time and bioavailability of contaminants (Pavione et al., 2019).

All species had high values of histopathological alteration index of the liver (HIL) (HIL = 45.44–67.8). Several authors have stated the efficiency of using HIL in fishes for environmental monitoring (Borges et al., 2018; Kostić et al., 2017; Viana et al., 2013). The HIL values in ITAP and SIR were higher than those recorded in other estuarine and coastal areas submitted to anthropic impacts using the same method, for

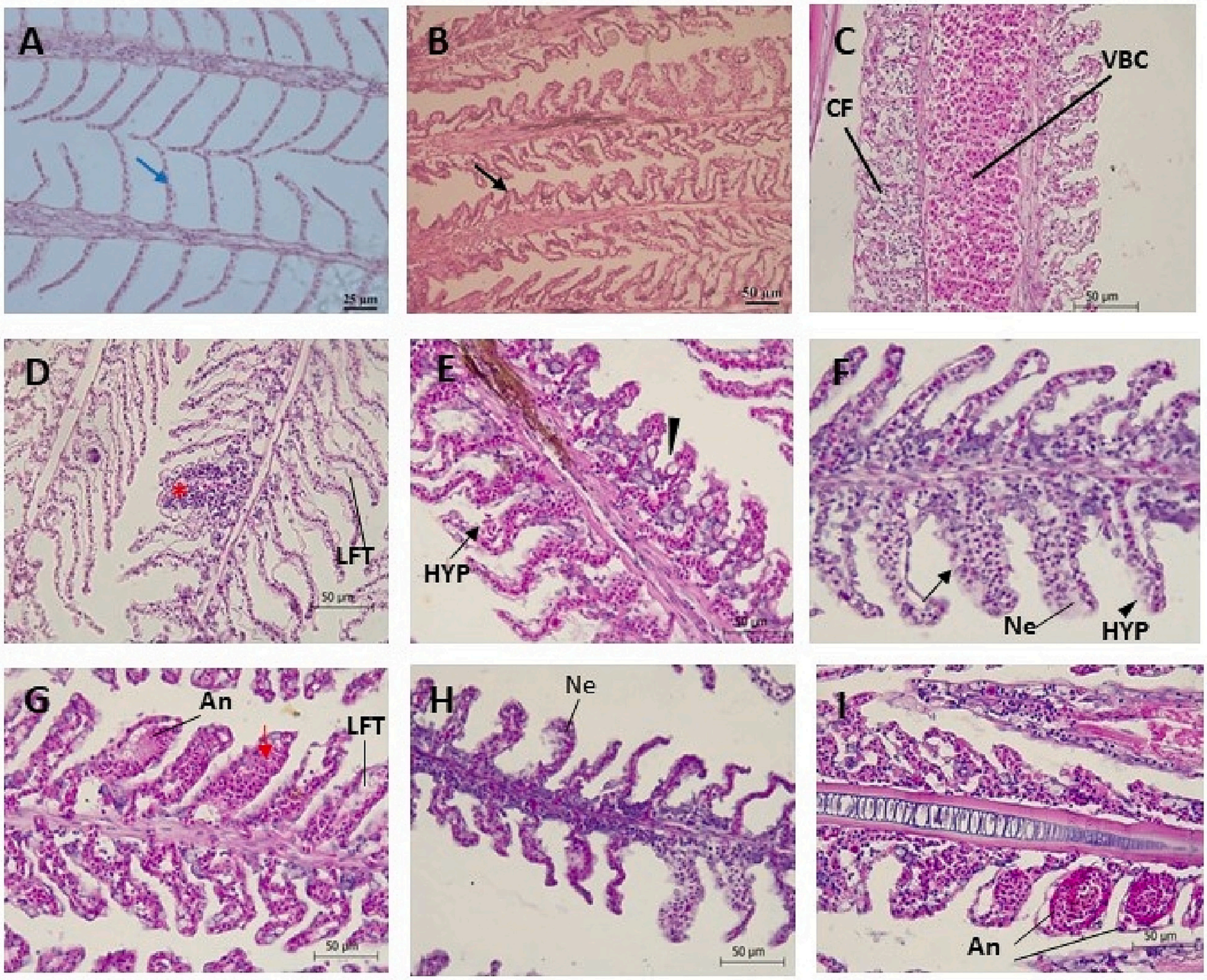


**Fig. 7.** Histopathological gills of analyzed fish, *Bairdiella ronchus* (A); *Caranx latus* (D), *Centropomus undecimalis* (F, H), *Gobionellus stomatus* (E, G, I). [A] normal structure for teleost gills with little lamellar derangement, filament (F) and secondary lamella (blue arrow); [B] Fusion of several secondary lamellae (black arrow); [C] complete fusion of several lamellae (CF), vessel blood congestion (VBC); [D] Lifting of respiratory epithelial cells (LFT), focal hyperplasia of epithelial cells (*), [E] hypertrophy of mucus cell (head arrow), lamellar hyperemia (HYP), [F] epithelial rupture (arrow), necrosis (Ne), lamellar hyperemia (HYP), [G] aneurysm (An), hyperplasia of epithelial cells (red arrow), epithelial lifting (LFT) with some intraepithelial edema, [H] necrosis (Ne), [I] aneurysm lamellar (An), H & *E*-P stain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

example: *Solea senegalensis* had HIL < 10 in contaminated sediments from south of the Gulf of Gabes, Tunisia (Ghribi et al., 2019); *Solea senegalensis* (HIL < 12) in sediment from Sado estuary (Costa et al., 2009); and *Solea* spp. (HIL < 12) in Bilbao estuary (Bay of Biscay) (Briaudeau et al., 2019); *Jenynsia multidentate* (HIL < 36) in Suquiá River Basin (Córdoba) (Amé et al., 2019); and the species *Engraulis encrasicolu* (HIL < 30), *Sardinella aurita* (HIL < 20), *Sardina pilchardus* (HIL < 20) from Algiers Bay, Bou Ismail Bay, and Zemmouri Bay in Algerian coastline (Bencheikh et al., 2022). In Brazilian estuaries, our indices were much higher than those reported for *Atherinella brasiliensis* (HIL < 30) in the Estuarine-Lagoon Complex of Iguape-Cananéia, São Paulo (Salgado et al., 2018) and *Cathorops spixii* (HIL < 12) in Paranaguá Bay, Brazil (dos Santos et al., 2014). It is important to highlight that although they have similar levels of anthropogenic impacts, these studies use different species from ours, limiting a more robust comparison. However, this shows that the use of fish as bioindicators of ecosystem health is widely used.

Severe histological changes in the tissue (HIL > 40) may be categorized as irreparable, like necrosis, that can lead to mortality due to the loss of vital tissue function or increase of susceptibility to disease (Zimmerli et al., 2007). The HLI and the HSI are directly related, and possibly correlated - high HSI values leading to high HIL, and vice-versa.

The liver changes observed ranged from mild to severe, with necrosis in the liver tissue and bile duct being the most severe lesions in 72.60 % of the fish samples. Santos et al. (2014) observed necrosis alteration in 100 % of liver of *Cathorops spixii* when evaluating the effects of bioaccumulation of butyltins in Paranaguá Bay. Khoshnood (2017) reported that different sources of pollution can result in moderate to severe damage to liver cells. Our findings were similar to those obtained by Oliva et al. (2013) with *Solea senegalensis* in Huelva, Spain; *Cathorops spixii* in Paranaguá Bay (dos Santos et al., 2014), and *Arius thalassinus* in Hodeida, Yemen Republic (Saleh and Marie, 2016); *Odonthestes bonariensis* in Chasicó Lake, Buenos Aires Province (Puntoriero et al., 2018). In general, all the studies presented above reported alterations as observed in our study, the liver had an increased vacuolar degeneration, necrotic foci, hemorrhage, leukocyte infiltration, granulomas and parasites when submitted to an anthropified environment. Among these alterations, the granuloma is the most complex due to the origin of its formation.

Granulomas observed for all species in this study were associated with inflammatory processes, which are capsules surrounded by multiple fibrotic layers involving an inflammatory response (Fricke et al., 2012). This alteration may be a response to environmental stress, presence of parasites, or both (Araújo et al., 2019), as observed in our study. Parasites affect structure of tissue, increase granulomas, and can cause irreversible damage (Melo et al., 2014). These reaction patterns were similarly observed in all fishes of our study, associated with the presence of parasites. Highly anthropized environments directly affects the immunity of fish living in this ecosystem, making them more susceptible to parasite infestations (Byers, 2020; de Melo Souza et al., 2019; Falkenberg et al., 2019).

All fish specimens evaluated in both areas presented around 95.89 % vacuolization in the liver. It usually occurs due to the storage of lipids or glycogen as an energy source when found in low proportion or minimums, but it is possible to increase if subjected to some stressors like contaminant exposure and nutritional status of the species (Wolf et al., 2015). Oliva et al. (2013) found 100 % vacuolization in *Solea senegalensis*, from a polluted estuary in Huelva, Spain.

*Gobionellus stomatus* had a steatosis in higher degree of intensity than other species. The steatosis or fatty degeneration found in all species in this study may be derived from the toxic exposure to pollutants, such as organic compound and heavy metals (Wolf and Wolfe, 2005). The severity of this pathology is due to the direct impact on liver functions, enabling an increase in the individual's susceptibility to poison by contaminants (Roberts and Rodger, 2012). This pathology in *G. stomatus* may be associated with a specific response of this species to contaminants. Similar results were observed in degenerative processes associated with excessive fat storage in fish from heavily polluted river in *Zoarces viviparus* of the Baltic Sea (Fricke et al., 2012).

In general, the studied fishes had moderately to heavily damaged degree of gill damage. Some individuals had permanent lesions, such as necrosis, resulting in irreparable damage, indicating that these fish are being subjected to long exposure to toxic agents. The presence of these lesions indicates that a complex mixture of contaminants is present in natural environments affecting the fishes and their health condition (Montes et al., 2020). Mucous and chloride cells are the cells mostly affected by toxic substances, as they act in the defense and transport mechanism of chloride ions, respectively. These stress-inducing factors can induce pathological changes (David and Fontanetti, 2009; Nimet et al., 2020). *Odonthestes bonariensis* from Lake Chasicó, presented lesions of different levels such as epithelial displacement and necrosis, due to the high level of As and F in the gills, as these elements act directly in the osmoregulation of the fish (Puntoriero et al., 2018).

In this study, the histopathological index of gills (HIG) ranged from 36.68 to 93.55. Fish as sentinels for histopathological alterations to assess biological effects of environmental pollution are being used by several authors. Carvalho et al. (2020) evaluated *Micropogonias furnieri* and *Menticirrhus americanus* (HIG = 100) from the Bay of Sepetiba-Rio de Janeiro, an area known for the disposal of materials and contaminants and recorded higher indexes than those reported in the present study. *Centropomus undecimalis*, with an average HIG of 66.45, is frequently used as a bioindicator of water quality in impacted areas. Several authors in Maranhão (Brazil) (Cantanhêde et al., 2014, 2016, 2018; Santos et al., 2014) found lower values than those observed in our study (HIG = 19.11 to 53).

In both estuaries, all fishes had several gill alterations. In general, the histological changes observed in the liver and gills are not of specific origin, being associated to toxic agents, which can vary from trace metals, and vinasse to pesticides (Chavan and Muley, 2014; Coelho et al., 2018). Because of this, it is difficult to determine the main source that causes histopathological changes in the liver and gills. Trace metals are responsible for several deleterious effects in fish tissues leading to changes in metabolism, such as hypertrophy (Cr), hyperplasia, lamellar fusion, rupture of epithelium lamellar (Zn), hypertrophy and hyperplasia of primary and secondary lamellae; and liver hepatocytes vacuolations (Cd) (Labarrère et al., 2012; Naïja et al., 2016; Aslam and Yousafzai, 2017). Moreover, the release of vinasse in the estuary can favor the appearance of lesions, such as rupture and hemorrhage in the gills (Correia et al., 2017). The pollution of these areas was studied by Lopes (2018) which evaluated the pollution in sediments and biota, finding Hg values above the level 2 established by Brazilian environmental legislation (CONAMA, 2012), which means a high probability of harm effect to biota. The presence of this metal resulted in alteration in neurological physiology, measured by inhibition of AChE activity in *C. undecimalis*, used as sentinel species.

*Gobionellus stomatus* had fatty degeneration with a magnitude of severity, totally different from the other species. This species and *C. undecimalis* (or of similar genus) are already assigned as keystone species in these estuaries (Lira et al., 2018), and according to our findings, can also certainly be considered as efficient sentinels in tropical estuaries. The combination of methodologies pioneer in the Northeast of Brazil, using different fish species and trophic guilds, was efficient in diagnosing the health status of the species and the environment they inhabit. There is no study of how these potential damages, evident by histological changes, may impact human health, mainly resulting from trophic biomagnification, since the species studied are of great importance to the local community as a source of food and economically, mainly *C. parallellus*, *C. undecimalis* and *C. latus*. This study associated to other approaches for assessing anthropogenic impacts on species serves as a warning to the population that feeds on fish from impacted estuarine areas, as is the case here, and public policies and constant environmental monitoring are needed to investigate the effects of water pollution and thus ensure food security for the population.

## CRediT authorship contribution statement

Ítala Gabriela Sobral dos Santos: Conceptualization; Data curation; Formal analysis and Roles/Writing – original draft.
Alex Souza Lira: formal analysis and illustrations.
Caroline da Silva Montes: review & editing.
David Point: review & editing.
Anaïs Médieu: review & editing.
Clístenes Williams Araújo do Nascimento: review & editing.
Flávia Lucena-Frédou: Research concept, review & editing, Supervision.
Rossineide Martins da Rocha: review & editing, Supervision.

## 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.

## Data availability

The authors do not have permission to share data.

## Acknowledgments

This study was supported by the LMI TAPIOCA program CAPES/COFECUB (88881.142689/2017-01); and UFRPE in edital PRPPG 015/2018 (Support To Ufrpe's Institutional Research). We thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil, which provided research grants to Flávia Lucena Frédou (CNPq nº 308554/2019-1) and Rossineide Martins da Rocha (307688/2019–4). CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for granting a scholarship at doctoral level for the first author. All authors thank the members of the laboratory BIOIMPACT, Soil Science laboratory, Geosciences Environment Toulouse (GET - IRD), laboratory of Cellular Ultrastructure and Immunohistochemistry, for the support in the sampling procedures and the fishermen of Itapissuma and Sirinhaem for the sampling collect.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.115095.

## References


Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P.H., Spry, D., 2019. Bioavailability assessment of metals in freshwater environments: A historical review. Environ. Toxicol. Chem. 39 (1), 48–59. https://doi.org/10.1002/etc.4558.

Amé, M.V., Ballesteros, M.L., Bistoni, M.D.L.Á., Hued, A.C., Monferrán, M.V., Wunderlin, D.A., 2019. Effects of river pollution on its biota: results from a 20-year study in the Suquía River Basin (Córdoba, Argentina). In: Pollution of Water Bodies in Latin America. Springer, Cham, pp. 177–200.

Ameur, W. Ben, El Megdiche, Y., de Lapuente, J., Barhoumi, B., Trabelsi, S., Ennaceur, S., Camps, L., Serret, J., Ramos-López, D., Gonzalez-Linares, J., Touil, S., Driss, M.R., Borràs, M., 2015. Oxidative stress, genotoxicity and histopathology biomarker responses in *Mugil cephalus* and *Dicentrarchus labrax* gill exposed to persistent pollutants. A field study in the Bizerte Lagoon: Tunisia. Chemosphere 135, 67–74. https://doi.org/10.1016/j.chemosphere.2015.02.050.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46.

ANVISA, 2013. Technical regulation Mercosul on maximum limits of inorganic contaminants in food (in Portuguese). Resolut. 42.

APAC, 2019. Sistema de Geoinformação hidrometeorológico de Pernambuco. Agência Pernambucana de Águas e Clima available at: http://www.apac.pe.gov.br/sighpe/.

Araújo, F.G., Morado, C.N., Parente, T.T.E., Paumgartten, F.J.R., Gomes, I.D., 2018. Biomarkers and bioindicators of the environmental condition using a fish species (*Pimelodus maculatus* Lacepède, 1803) in a tropical reservoir in southeastern Brazil. Braz. J. Biol. 78, 351–359. https://doi.org/10.1590/1519-6984.167209.

Araújo, F.G., Gomes, I.D., Nascimento, A.A., dos Santos, M.A.J., Sales, A., 2019. Histopathological analysis of liver of the catfish *Pimelodus maculatus* in a tropical eutrophic reservoir from Southeastern Brazil. Acta Sci. Biol. Sci. 41, 1–11. https://doi.org/10.4025/actascibiolsci.v41i1.41039.

Aslam, S., Yousafzai, A.M., 2017. Chromium toxicity in fish: a review article. J. Entomol. Zool. Stud. 5, 1483–1488.

Becker, D.J., Chumchal, M.M., Broders, H.G., Korstian, J.M., Clare, E.L., Rainwater, T.R., Platt, S.G., Simmons, N.B., Fenton, M.B., 2018. Mercury bioaccumulation in bats reflects dietary connectivity to aquatic food webs. Environ. Pollut. 233, 1076–1085. https://doi.org/10.1016/j.envpol.2017.10.010.

Bencheikh, Z., Refes, W., Brito, P.M., Prodocimo, M.M., Gusso-Choueri, P.K., Choueri, R. B., de Oliveira Ribeiro, C.A., 2022. Chemical pollution impairs the health of fish species and fishery activities along the Algeria coastline, Mediterranean Sea. Environ. Monit. Assess. 194 https://doi.org/10.1007/s10661-022-10059-y.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22, 25–34.

Borges, A.C., Da Silva Montes, C., Barbosa, L.A., Ferreira, M.A.P., Berrêdo, J.F., Martins Rocha, R., 2018. Integrated use of histological and ultrastructural biomarkers for assessing mercury pollution in piranhas (*Serrasalmus rhombeus*) from the Amazon mining region. Chemosphere 202, 788–796. https://doi.org/10.1016/j.chemosphere.2018.02.169.

Borges, G.C.P., 2011. Comunidade Fitoplanctônica Do Estuário Do Rio Massangana (Pernambuco - Brasil). MSc Thesis.. Univ. Federal de Pernambuco, Brazil. unpublised.

Bray, J.R., Curtis, J.T., Roger, J., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349.

Briaudeau, T., Zorita, I., Cuevas, N., Franco, J., Marigómez, I., Izagirre, U., 2019. Multi-annual survey of health status disturbance in the Bilbao estuary (Bay of Biscay) based on sediment chemistry and juvenile sole (*Solea* spp.) histopathology. Mar. Pollut. Bull. 145, 126–137. https://doi.org/10.1016/j.marpolbul.2019.05.034.

Byers, J.E., 2020. Effects of climate change on parasites and disease in estuarine and nearshore environments. PLoS Biol. 18, 1–12. https://doi.org/10.1371/journal.pbio.3000743.

Cantanhêde, S.M., Medeiros, A.M., Ferreira, J.R.C., Alves, L.M.C., Santos, D.M.S., 2014. Using histopathological biomarker in gills of *Centropomus undecimalis* (Bloch, 1972) in assessing the water quality at Ecological Park Laguna da Jansen, São Luís-MA. Arq. Bras. Med. Vet. Zootec. 66, 593–601.

Cantanhêde, S.M., da Silva Castro, G., Pereira, N.J., de Pinho Campos, J.S., da Silva, J., Tchaicka, L., Neta, R.N.F.C., de Souza Torres, J.R., Santos, D.M.S., 2016. Evaluation of environmental quality of two estuaries in Ilha do Maranhão, Brazil, using histological and genotoxic biomarkers in *Centropomus undecimalis* (Pisces, Centropomidae). Environ. Sci. Pollut. Res. 23, 21058–21069. https://doi.org/10.1007/s11356-016-7294-9.

Cantanhêde, S.M., Campos, V.C.S., Pereira, D.P., Medeiros, A.M., Neta, R.N.F.C., Tchaicka, L., Santos, D.M.S., 2018. Parasitism in gills of *Centropomus undecimalis* (Pisces, centropomidae) from a protected area in São Luís, Maranhão, Brazil. Lat. Am. J. Aquat. Res. 46, 377–382. https://doi.org/10.3856/vol46-issue2-fulltext-13.

Carr, M.K., Jardine, T.D., Doig, L.E., Jones, P.D., Bharadwaj, L., Tendler, B., Chételat, J., Cott, P., Lindenschmidt, K.E., 2017. Stable sulfur isotopes identify habitat-specific foraging and mercury exposure in a highly mobile fish community. Sci. Total Environ. 586, 338–346. https://doi.org/10.1016/j.scitotenv.2017.02.013.

Carvalho, T.L.A.B., Do Nascimento, A.A., Gonçalves, C.F.D.S., Dos Santos, M.A.J., Sales, A., 2020. Assessing the histological changes in fish gills as environmental bioindicators in Paraty and Sepetiba Bays in Rio de Janeiro, Brazil. Lat. Am. J. Aquat. Res. 48, 590–601. https://doi.org/10.3856/vol48-issue4-fulltext-2351.

Chavan, V.R., Muley, D.V., 2014. Effect of heavy metals on liver and gill of fish *Cirrhinus mrigala*. Int. J. Curr. Microbiol. Appl. Sci. 3, 277–288.

Chellaiah, E.R., 2018. Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*: a minireview. Appl Water Sci 8. https://doi.org/10.1007/s13201-018-0796-5.

Cipurkovic, A., Trumic, I., Hodžic, Z., Selimbašic, V., Djozic, A., 2014. Distribution of heavy metals in Portland cement production process. Adv. Appl. Sci. Res. 5, 252–259.

Coelho, M.P.M., Correia, J.E., Vasques, L.I., Marcato, A.C.C., Guedes, T.A., Soto, M.A., Basso, J.B., Kiang, C., Fontanetti, C.S., 2018. Toxicity evaluation of leached of sugarcane vinasse: histopathology and immunostaining of cellular stress protein. Ecotoxicol. Environ. Saf. 165, 367–375. https://doi.org/10.1016/j.ecoenv.2018.08.099.

CONAMA, 2012. Conselho Nacional do Meio Ambiente, Resolução Conama N° 454.

Correia, J.E., Christofoletti, C.A., Marcato, A.C.C., Marinho, J.F.U., Fontanetti, C.S., 2017. Histopathological analysis of tilapia gills (*Oreochromis niloticus* Linnaeus, 1758) exposed to sugarcane vinasse. Ecotoxicol. Environ. Saf. 135, 319–326. https://doi.org/10.1016/j.ecoenv.2016.10.004.

da Costa, M.R., Araújo, F.G., 2003. Length-weight relationship and condition factor of *Micropogonias furnieri* (Desmarest) (Perciformes, Sciaenidae) in the Sepetiba Bay, Rio de Janeiro State, Brazil. Rev. Bras. Zool. 20, 685–690. https://doi.org/10.1590/s0101-81752003000400022.

Costa, P.M., Diniz, M.S., Caeiro, S., Lobo, J., Martins, M., Ferreira, A.M., Caetano, M., Vale, C., DelValls, T.Á., Costa, M.H., 2009. Histological biomarkers in liver and gills of juvenile *Solea senegalensis* exposed to contaminated estuarine sediments: a weighted indices approach. Aquat. Toxicol. 92, 202–212. https://doi.org/10.1016/j.aquatox.2008.12.009.

CPRH, 2003. Diagnóstico socioambiental do litoral Norte de Pernambuco. Agência Estadual do Meio Ambiente, Recife, p. 214.

CPRH, 2003a. Diagnóstico socioambiental do litoral Sul de Pernambuco. Recife, Agência Estadual do Meio Ambiente, p. 87.

CPRH, 2011. Área de Proteção Ambiental de Guadalupe, p. 87.

Cren, E.L., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the the length-weight relationship and seasonal cycle in gonad weight and condition in the perch. Br. Ecol. Soc. 20, 201–219.

Da Silva, J.B., Galvíncio, J.D., Corrêa, A.C.D.B., Da Silva, D.G., Machado, C.C.C., 2011. Classificação ~ geomorfológica dos estuários do Estado de Pernambuco (brasil) com base em imagens do LANDSAT 5/TM. Rev. Bras. Geogr. Fís. 4, 118–133.

Dalzochio, T., Rodrigues, G.Z.P., Petry, I.E., Gehlen, G., da Silva, L.B., 2016. The use of biomarkers to assess the health of aquatic ecosystems in Brazil: a review. Int. Aquat. Res. 8, 283–298. https://doi.org/10.1007/s40071-016-0147-9.

David, J.A.O., Fontanetti, C.S., 2009. The role of mucus in *Mytella falcata* (Orbigny 1842) gills from polluted environments. Water Air Soil Pollut. 203, 261–266. https://doi.org/10.1007/s11270-009-0009-9.

Dellamatrice, P.M., Monteiro, R.T.R., 2014. Main aspects of the pollution in Brazilian rivers by pesticides. Rev. Bras. Eng. Agric. e Ambient. 18, 1296–1301. https://doi.org/10.1590/1807-1929/agriambi.v18n12p1296-1301.

Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D., 2007. The guild approach to categorizing estuarine fish assemblages: a global review. Fish Fish. 8, 241–268. https://doi.org/10.1111/j.1467-2679.2007.00253.x.

Fåhraeus-Van Ree, G.E., Spurrell, D.R., 2003. Structure of and energy reserves in the liver of wild and cultured yellowtail flounder, *Limanda ferruginea*. Mar. Biol. 143, 257–265. https://doi.org/10.1007/s00227-003-1083-y.

Falkenberg, J.M., Golzio, J.E.S.A., Pessanha, A., Patrício, J., Vendel, A.L., Lacerda, A.C. F., 2019. Gill parasites of fish and their relation to host and environmental factors in two estuaries in northeastern Brazil. Aquat. Ecol. 53, 109–118. https://doi.org/10.1007/s10452-019-09676-6.

Filote, C., Roșca, M., Hlihor, R.M., Cozma, P., Simion, I.M., Apostol, M., Gavrilescu, M., 2021. Sustainable application of biosorption and bioaccumulation of persistent pollutants in wastewater treatment: current practice. Processes 9, 1–38. https://doi.org/10.3390/pr9101696.

Fonseca, A.R., Sanches Fernandes, L.F., Fontainhas-Fernandes, A., Monteiro, S.M., Pacheco, F.A.L., 2016. From catchment to fish: impact of anthropogenic pressures on gill histopathology. Sci. Total Environ. 550, 972–986. https://doi.org/10.1016/j.scitotenv.2016.01.199.

Freitas, T.M.S., Almeida, V.H.C., Montag, L.F.A., da Rocha, R.M., Fontoura, N.F., 2011. Seasonal changes in the gonadossomatic index, allometric condition factor and sex ratio of an auchenipterid catfish from eastern Amazonia. Neotrop. Ichthyol. 9, 839–847. https://doi.org/10.1590/S1679-62252011005000044.

Fricke, N.F., Stentiford, G.D., Feist, S.W., Lang, T., 2012. Liver histopathology in Baltic eelpout (*Zoarces viviparus*) — a baseline study for use in marine environmental monitoring. Mar. Environ. Res. 82, 1–14. https://doi.org/10.1016/j.marenvres.2012.08.012.

Ghribi, R., Correia, A.T., Elleuch, B., Nunes, B., 2019. Testing the impact of contaminated sediments from the southeast marine coast of Tunisia on biota: a multibiomarker approach using the flatfish Solea senegalensis. Environ. Sci. Pollut. Res. 26, 29704–29721. https://doi.org/10.1007/s11356-019-05872-x.

Gonzalez, J.G., Ménard, F., Le Loc'h, F., de Andrade, H.A., Viana, A.P., Ferreira, V., Lucena-Frédou, F., Lira, A.S., Munaron, J.M., Frédou, T., 2019. Trophic resource partitioning of two snook fish species (Centropomidae) in tropical estuaries in Brazil as evidenced by stable isotope analysis. Estuar. Coast. Shelf Sci. 226, 106287 https://doi.org/10.1016/j.ecss.2019.106287.

Guimarães, A.S., Travassos, P., Souza Filho, P.W.M.E., Gonçalves, F.D., Costa, F., 2010. Impact of aquaculture on mangrove areas in the northern Pernambuco Coast (Brazil) using remote sensing and geographic information system. Aquac. Res. 41, 828–838. https://doi.org/10.1111/j.1365-2109.2009.02360.x.

Heath, A.G., 1995. Water pollution and fish physiology. Nutr. Today. https://doi.org/10.1097/nt.0b013e3181fe1713.

Hook, S.E., Gallagher, E.P., Batley, G.E., 2014. The role of biomarkers in the assessment of aquatic ecosystem health. Integr. Environ. Assess. Manag. 10, 327–341. https://doi.org/10.1002/ieam.1530.

Kameli, M.A., Chorom, M., Jaafarzadeh, N., Janadeleh, H., 2017. Application of wastewater with high organic load for saline-sodic soil reclamation focusing on soil purification ability. Glob. J. Environ. Sci. Manag. 3, 197–206. https://doi.org/10.22034/gjesm.2017.03.02.008.

Kaufman, S.D., Johnston, T.A., Leggett, W.C., Moles, M.D., Casselman, J.M., Schulte-Hostedde, A.I., 2007. Relationships between body condition indices and proximate composition in adult walleyes. Trans. Am. Fish. Soc. 136, 1566–1576. https://doi.org/10.1577/t06-262.1.

Khoshnood, Z., 2017. Effects of environmental pollution on fish: a short review. Transylv. Rev. Syst. Ecol. Res. 19, 49–60. https://doi.org/10.1515/trser-2017-0005.

Kostić, J., Kolarević, S., Kračun-Kolarević, M., Aborgiba, M., Gačić, Z., Paunović, M., Višnjić-Jeftić, Ž., Rašković, B., Poleksić, V., Lenhardt, M., Vuković-Gačić, B., 2017. The impact of multiple stressors on the biomarkers response in gills and liver of freshwater breams during different seasons. Sci. Total Environ. 601–602, 1670–1681. https://doi.org/10.1016/j.scitotenv.2017.05.273.

Kroon, F., Streten, C., Harries, S., 2017. A protocol for identifying suitable biomarkers to assess fish health: a systematic review. PLoS One. https://doi.org/10.1371/journal.pone.0174762.

Labarrère, C.R., Menezes, B.D., Melo, M.M., 2012. Avaliação dos Teores ee Zinco Em Brânquias, Carcaça, Fígado e Musculaturade Diferentes Espécies ee Peixes Capturados No Rio São Francisco (Mg, Brasil). Geonomos 20, 86–91. https://doi.org/10.18285/geonomos.v20i1.31.

Lira, A., Angelini, R., Le Loc'h, F., Ménard, F., Lacerda, C., Frédou, T., Lucena Frédou, F., 2018. Trophic flow structure of a neotropical estuary in northeastern Brazil and the comparison of ecosystem model indicators of estuaries. J. Mar. Syst. 182, 31–45. https://doi.org/10.1016/j.jmarsys.2018.02.007.

Lira, A.S., Lucena Frédou, F., Lacerda, C., Eduardo, L.N., Ferreira, V., Frédou, T., Ménard, F., Angelini, R., Le Loc'h, F., 2022. Effect of fishing effort on the trophic functioning of tropical estuaries in Brazil. Estuar. Coast. Shelf Sci. v. 277, n. August, 108040.

Lopes, D.F.C., 2018. O uso da ictiofauna como bioindicadora de qualidade ambiental de estuários neotropicais. Federal Rural University of Pernambuco.

Macêdo, S.J., Flores Montes, M.J., Lins, I.C., 2000. Características abióticas da área. In: Barros, H.M., Eskinazi- Leça, E., Macêdo, S.J., Lima, T. (Eds.), Gerenciamento Participativo de Estuários e Manguezais, pp. 7–25.

Maggioni, T., Hued, A.C., Monferrán, M.V., Bonansea, R.I., Galanti, L.N., Amé, M.V., 2012. Bioindicators and biomarkers of environmental pollution in the middle-lower basin of the Suquía River (Córdoba, Argentina). Arch. Environ. Contam. Toxicol. 63, 337–353. https://doi.org/10.1007/s00244-012-9785-0.

Maia, L.P., Lacerda, L.D., Monteiro, L.H.U., Souza, G.M. (Eds.), 2006. Atlas dos manguezais do nordeste do Brasil.

McPherson, L.R., Slotte, A., Kvamme, C., Meier, S., Marshall, C.T., 2011. Inconsistencies in measurement of fish condition: a comparison of four indices of fat reserves for Atlantic herring (*Clupea harengus*). ICES J. Mar. Sci. 68, 52–60. https://doi.org/10.1093/icesjms/fsq148.

Medeiros, C., Kjerfve, B., 1993. Hydrology of a tropical estuarine system: Itamaracá, Brazil. Estuar. Coast. Shelf Sci. 36, 495–515.

Medeiros, C., Kjerfve, B., Araujo, M., Neumann-Leitão, S., 2001. The itamaracá estuarine ecosystem, Brazil. In: Seeliger, U., Kjerfve, B. (Eds.), Coastal Marine Ecosystem of Latin America (Ecological Studies). Springer, New York, pp. 71–82. https://doi.org/10.1007/978-3-662-04482-7.

Melo, F.T.V., Rodrigues, R.A.R., Giese, E.G., Gardner, S.L., dos Santos, J.N., 2014. Histopathologic aspects in Plagioscion squamosissimus (HECKEL, 1940) induced by *Neoechinorhynchus veropesoi*, metacestodes and anisakidae juveniles TT - Aspectos histopatológicos em Plagioscion squamosissimus induzidos por Neoechinorhynchus veropesoi, met. Rev. Bras. Parasitol. Vet. 23, 224–230.

de Melo Souza, D.C., dos Santos, M.C., Chagas, E.C., 2019. Immune response of teleost fish to helminth parasite infection. Rev. Bras. Parasitol. Vet. 28, 533–547. https://doi.org/10.1590/s1984-29612019080.

Meyer, U., 1996. On the fate of mercury in the northeastern Brazilian mangrove system Canal de Santa Cruz, Pernambuco. 105f. DD Thesis. Bremen, Germany.

Montes, C.S., Pantoja Ferreira, M.A., Giarrizzo, T., Amado, L.L., Rocha, R.M., 2020. Evaluation of metal contamination effects in piranhas through biomonitoring and multi biomarkers approach. Heliyon 6, e04666. https://doi.org/10.1016/j.heliyon.2020.e04666.

Mourão, K.R.M., Ferreira, V., Lucena-Frédou, F., 2014. Composition of functional ecological guilds of the fish fauna of the internal sector of the Amazon Estuary, Pará, Brazil. An. Acad. Bras. Cienc. 86, 1783–1800. https://doi.org/10.1590/0001-3765201420130503.

Naïja, A., Marchand, J., Kestemont, P., Haouas, Z., Blust, R., Chénais, B., Helal, A.N., 2016. Biomarkers assessment in the peacock blenny *Salaria pavo* exposed to cadmium. Environ. Sci. Pollut. Res. 23, 16296–16312. https://doi.org/10.1007/s11356-016-6754-6.

Neumann-Leitão, S., Schwamborn, R., Macêdo, S.J., Medeiros, C., Koening, M.L., Montes, M.J.F., Feitosa, F.A.N., Gusmão, L.M.O., 2001. Plankton dynamics at Itamaracá mangrove estuarine system, Pernambuco, Brazil. In: Villacampa, Y., Brebbia, C.A., Uso, J.L. (Eds.), Ecosystems and Sustainable Development III. W.I.T. Press, Southampton, pp. 435–445.

Nimet, J., Neves, M.P., Viana, N.P., de Arruda Amorim, J.P., Delariva, R.L., 2020. Histopathological alterations in gills of a fish (*Astyanax bifasciatus*) in neotropical streams: negative effects of riparian forest reduction and presence of pesticides. Environ. Monit. Assess. 192 https://doi.org/10.1007/s10661-019-8030-y.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2017. Package "vegan": Community Ecology Package. R package Version 2.4-4. Reference Manual, 297.

Oliva, M., Vicente-Martorell, J.J., Galindo-Riaño, M.D., Perales, J.A., 2013. Histopathological alterations in *Senegal sole*, *Solea Senegalensis*, from a polluted Huelva estuary (SW, Spain). Fish Physiol. Biochem. 39, 523–545. https://doi.org/10.1007/s10695-012-9717-y.

Oost, R.V.D., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.

Pacyna, J.M., Sundseth, K., Pacyna, E.G., Jozewicz, W., Munthe, J., Belhaj, M., Aström, S., 2010. An assessment of costs and benefits associated with mercury emission reductions from major anthropogenic sources. J. Air Waste Manag. Assoc. 60, 302–315. https://doi.org/10.3155/1047-3289.60.3.302.

Pavione, P.M., da Costa, K.G., Perônico, C., McMaster, M.E., Parrott, J.L., Hewitt, L.M., Munkittrick, K.R., Barreto, F.C.C., Basilo, T.H., Gomes, M.P., Reis Filho, R.W., Furley, T.H., 2019. Development of environmental effects monitoring protocol in Brazil: a fish guide study of three river estuaries. Environ. Monit. Assess. 191 https://doi.org/10.1007/s10661-019-7860-y.

Pelage, L., Domalain, G., Lira, A.S., Travassos, P., Frédou, T., 2019. Coastal land use in Northeast Brazil: mangrove coverage evolution over three decades. Trop. Conserv. Sci. 12 https://doi.org/10.1177/1940082918822411.

Poleksic, V., Mitrovic-Tutundzic, V., 1994. Fish gills as a monitor of sublethal and chronic effects of pollution. In: Sublethal and Chronic Effects of Pollutants on Freshwater Fish, pp. 339–352.

Prophet, E.B., Milis, B., Arrington, J.B., Sobin, L.H., 1995. Metodos Histotecnologicos. Instituto de Patologia de las Fuerzas Armadas de los Estados Unidos de America (AFIP), Washington (DC), p. 280.

Puntoriero, M.L., Fernández Cirell, A., Volpedo, A.V., 2018. Histopathological changes in liver and gills of Odontesthes bonariensis inhabiting a Lake with high concentrations of arsenic and fluoride (Chasicó Lake, Buenos Aires Province). Rev. Int. Contam. Ambient. 34, 69–77. https://doi.org/10.20937/RICA.2018.34.01.06.

R Core Team, 2020. R: A Language and Environment for Statistical Computing.

Roberts, R.J., Rodger, H.D., 2012. The pathophysiology and systematic pathology of teleosts. In: Roberts, R.J. (Ed.), Fish Pathology, p. 587. https://doi.org/10.2307/3280352.

Rossi, A.S., Fantón, N., Michlig, M.P., Repetti, M.R., Cazenave, J., 2020. Fish inhabiting rice fields: bioaccumulation, oxidative stress and neurotoxic effects after pesticides application. Ecol. Indic. 113, 106186 https://doi.org/10.1016/j.ecolind.2020.106186.

Salas, P.M., Sujatha, C.H., Ratheesh Kumar, C.S., Cheriyan, E., 2017. Heavy metal distribution and contamination status in the sedimentary environment of Cochin estuary. Mar. Pollut. Bull. 119, 191–203. https://doi.org/10.1016/j.marpolbul.2017.04.018.

Saldarriaga-Hernandez, S., Hernandez-Vargas, G., Iqbal, H.M.N., Barceló, D., Parra-Saldívar, R., 2020. Bioremediation potential of *Sargassum* sp. biomass to tackle pollution in coastal ecosystems: circular economy approach. Sci. Total Environ. 715 https://doi.org/10.1016/j.scitotenv.2020.136978.

Saleh, Y.S., Marie, M.A.S., 2016. Use of Arius thalassinus fish in a pollution biomonitoring study, applying combined oxidative stress, hematology, biochemical and histopathological biomarkers: a baseline field study. Mar. Pollut. Bull. 106, 308–322. https://doi.org/10.1016/j.marpolbul.2016.03.030.

Salgado, L.D., Marques, A.E.M.L., Kramer, R.D., de Oliveira, F.G., Moretto, S.L., de Lima, B.A., Prodocimo, M.M., Cestari, M.M., de Azevedo, J.C.R., Silva de Assis, H.C., 2018. Integrated assessment of sediment contaminant levels and biological responses in sentinel fish species *Atherinella brasiliensis* from a sub-tropical estuary in South Atlantic. Chemosphere 219, 15–27. https://doi.org/10.1016/j.chemosphere.2018.11.204.

dos Santos, D.M., Santos, G.S., Cestari, M.M., de Oliveira Ribeiro, C.A., de Assis, H.C.S., Yamamoto, F., Guiloski, I.C., de Marchi, M.R.R., Montone, R.C., 2014. Bioaccumulation of butyltins and liver damage in the demersal fish *Cathorops spixii* (Siluriformes, Ariidae). Environ. Sci. Pollut. Res. 21, 3166–3174. https://doi.org/10.1007/s11356-013-2280-y.

Santos, D.M.S., Melo, M.R.S., Mendes, D.C.S., Rocha, I.K.B.S., Silva, J.P.L., Cantanhêde, S.M., Meletti, P.C., 2014. Histological changes in gills of two fish species as indicators of water quality in Jansen lagoon (São Luís, Maranhão State, Brazil). Int. J. Environ. Res. Public Health 11, 12927–12937. https://doi.org/10.3390/ijerph111212927.

Savoca, D., Pace, A., 2021. Bioaccumulation, biodistribution, toxicology and biomonitoring of organofluorine compounds in aquatic organisms. Int. J. Mol. Sci. 22 https://doi.org/10.3390/ijms22126276.

Schmitt, C.J., Dethloff, G.M., 2000. Biomonitoring of Environmental Status and Trends (BEST) Program: selected methods for monitoring chemical contaminants and their effects in aquatic ecosystems. In: Information and Technology Report USGS/BRD/ITR-2000-0005.

da Silva, M.H., 2009. Estrutura e produtividade da comunidade fitoplanctônica de um estuário tropical (Sirinhaém, Pernambuco, Brasil). Ph.D Thesis. Univ. Federal de Pernambuco, Brazil (unpublised).

Silva, M.L., Feitosa, F.A.N., Flores-Montes, M.J., Otsuka, A.Y., Saldanha-Corrêa, F., Noriega, C., 2019. Phytoplankton productivity and hydrology in an impacted estuarine complex in northeastern Brazil. Open J. Ecol. 9, 458–477. https://doi.org/10.4236/oje.2019.910030.

Slooff, W., Van Kreijl, C.F., Baars, A.J., 1983. Relative liver weights and xenobiotic-metabolizing enzymes of fish from polluted surface waters in the Netherlands. Aquat. Toxicol. 4, 1–14. https://doi.org/10.1016/0166-445X(83)90057-7.

Smolders, R., Bervoets, L., De Boeck, G., Blust, R., 2002. Integrated condition indices as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). Environ. Toxicol. Chem. 21, 87. https://doi.org/10.1897/1551-5028(2002)021<0087:iciaam>2.0.co;2.

Tashla, T., Žuža, M., Kenjveš, T., Prodanović, R., Soleša, D., Bursić, V., Petrović, A., Ljubojević-Pelić, D., Bošković, J., Puvača, N., 2018. Fish as an important bio-indicator of environmental pollution with persistent organic pollutants and heavy metals. J. Agron. Technol. Eng. Manag. 1, 52–56.

Terra, B.F., Araújo, F.G., Calza, C.F., Lopes, R.T., Teixeira, T.P., 2008. Heavy metal in tissues of three fish species from different trophic levels in a tropical Brazilian river. Water Air Soil Pollut. 187, 275–284. https://doi.org/10.1007/s11270-007-9515-9.

Todorov, V., Filzmoser, P., 2009. An object-oriented framework for robust. J. Stat. Softw. 32, 1–47.

U.S. EPA, 2007. Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils, Revision 1. United States Environmental Protection Agency, Washington, DC, USA.

Viana, A.P., Frédou, F.L., Montes, C.S., Rocha, R.M., 2013. Fish histopathology and catalase activity as biomarkers of the environmental quality of the industrial district on the Amazon estuary, Brazil. Acta Sci. Biol. Sci. 35, 395–401. https://doi.org/10.4025/actascibiolsci.v35i3.18032.

Vieira, C.E.D., Costa, P.G., Caldas, S.S., Tesser, M.E., Risso, W.E., Escarrone, A.L.V., Primel, E.G., Bianchini, A., dos Reis Martinez, C.B., 2019. An integrated approach in subtropical agro-ecosystems: active biomonitoring, environmental contaminants, bioaccumulation, and multiple biomarkers in fish. Sci. Total Environ. 666, 508–524. https://doi.org/10.1016/j.scitotenv.2019.02.209.

Wang, W.X., 2002. Interactions of trace metals and different marine food chains. Mar. Ecol. Prog. Ser. 243, 295–309. https://doi.org/10.3354/meps243295.

Willacker, J.J., Eagles-Smith, C.A., Ackerman, J.T., 2017. Mercury bioaccumulation in estuarine fishes: novel insights from sulfur stable isotopes. Environ. Sci. Technol. 51, 2131–2139. https://doi.org/10.1021/acs.est.6b05325.

Wolf, J.C., Wolfe, M.J., 2005. A brief overview of nonneoplastic hepatic toxicity in fish. Toxicol. Pathol. 33, 75–85. https://doi.org/10.1080/01926230590890187.

Wolf, J.C., Baumgartner, W.A., Blazer, V.S., Camus, A.C., Engelhardt, J.A., Fournie, J.W., Frasca, S., Groman, D.B., Kent, M.L., Khoo, L.H., Law, J.M., Lombardini, E.D., Ruehl-Fehlert, C., Segner, H.E., Smith, S.A., Spitsbergen, J.M., Weber, K., Wolfe, M.J., 2015. Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: a guide for investigators, authors, reviewers, and readers. Toxicol. Pathol. 43, 297–325. https://doi.org/10.1177/0192623314540229.

Zimmerli, S., Bernet, D., Burkhardt-Holm, P., Schmidt-Posthaus, H., Vonlanthen, P., Wahli, T., Segner, H., 2007. Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. Aquat. Sci. 69, 11–25. https://doi.org/10.1007/s00027-006-0844-3.