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Histological changes in fish hepatopancreas and kidney as indicators of environmental quality in tropical bays

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Abstract Biomarkers are used to assess health conditions of organisms and consequently to indicate environmental hazard, being important tools to address the effects of pollutants on environmental quality. We analyzed the hepatopancreas and kidney of two sciaenid fishes, the southern kingcroaker *Menticirrhus americanus* and the whitemouth croaker *Micropogonias furnieri* in the Paraty Bay (control system) and in the Sepetiba Bay (altered system) in southeast Brazilian coast. The altered bay is heavily affected by human activities, compared to the control bay, where the human interference is much reduced. The tested hypothesis was that these species respond to environmental changes with fishes from the altered bay having more histological changes in the hepatopancreas and kidney compared with those from the control bay. The fish hepatopancreas and kidneys from the altered system had high 50–100% incidence of changes and the histopathological alteration index reached values above 100 in the hepatopancreas and kidney, indicating irreversible damage to the organs. In contrast, in the control system, those values ranged between 20 and 100, indicating moderate and severe organ damages. These findings suggest that these organs and these two fish species can be considered useful biomarkers to assess environmental contamination in tropical bays.

Keywords Estuarine fish · Histological biomarkers · Pollution effects · Tissue lesions · Tropical systems

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Introduction

Human activities such as discharges of wastewaters from agricultural, industrial, and urban activities into aquatic ecosystems result in depletion of water quality with negative impacts on biotic communities. Such discharges have increased in the last years as consequence of unplanned human activities, especially in shorelines of coastal areas and semi-enclosed systems such bays and other transitional environments. Severe morphological and physiological changes in aquatic organisms have been associated to pollution elsewhere (Mazon and Fernandes 1999; Thophon et al. 2003; Zhou et al. 2008; Wanick et al. 2011) with potential to reduce the biodiversity and

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the performance of their biological functions (Knapp et al. 2017; Gomes-Gonçalves et al. 2020).

Organisms can withstand changes in environmental conditions within certain limits, and the balance between organisms and abiotic conditions is named ecological balance (Verma 2018). Effects of contamination on ecological balance of the aquatic organisms result in severe population impacts (Farombi et al. 2007; Dietz et al. 2021). Fish species that are exposed to harmful substances in different ecosystem compartments (e.g., water, sediment, and food) can accumulate amounts of pollutants that are higher than those in the aquatic environment (Ambedkar and Muniyan 2011; Kostić et al. 2017). Hazards due to heavy metals can result in toxicity and bioaccumulation in fish tissues (Elnaggar et al. 2009). Therefore, fishes may play an important role as bioindicators of contamination caused by pollutants (Hemmadi 2017). The availability of pollutants in the environment and pathways of entry such as feeding can result in the accumulation and bioavailability of different pollutants (Monperrus et al. 2005).

Histological changes have been used as biomarkers to assess health condition of fish exposed to contaminants and to depict the effects of exposure to a series of anthropogenic pollutants (Hinton et al. 1992; Zagatto and Bertoletti 2008; Nascimento et al. 2012). This is a quick and efficient method to assess the effects of contaminants on different tissues and organs, mainly the chronic ones (Johnson et al. 1993; Murugan et al. 2009).

The vertebrate liver, formed by a primary matrix of hepatocytes, bile canaliculi, and sinusoids, plays important roles in physiological processes of these organisms. Among these functions, we highlight the assimilation of nutrients, alcohol production, detoxification, and maintenance of the body's metabolic homeostasis, thus contributing to the processing of carbohydrates, proteins, lipids, and vitamins. Fish, in general, differ with respect to the three-dimensional organizations of the stroma and the liver parenchyma (Roberts 1978). However, some features are common to this group of organisms and are often not distinctive (Nejedli and Tlak Gajger 2013). There are general characteristics that are common to most species (Bombonato et al. 2007). The liver parenchyma is surrounded by a dense connective tissue capsule, modelled so that it penetrates the tissue, forming septa subdividing into lobes. It is mainly composed of strands of polyhedral hepatocytes, with central nuclei distinguished by the presence of chromatin with an intensely basophilic margin, giving a dense aspect (Roberts 1978).

The pancreas of fish has both exocrine and endocrine functions. This organ is present in some species in the form of islets of exocrine pancreatic tissue scattered around the intestines or in the liver. The exocrine portion has the function of secreting digestive juices, while the endocrine portion secretes the hormones insulin and glucagon. The exocrine portion of the pancreas also functions to form glandular cells or glandular acini, with the tissue consisting of scattered serous acini (Youson et al. 2006; Nejedli and Tlak Gajger 2013). During ontogenesis, exocrine pancreatic tissue, named hepatopancreas, develops around the portal vein and gradually invades the liver along the branches of the portal vein (Alboughobish and Khaksari Mahabadi 2005; Sayrafi et al. 2011; Mokhtar 2017).

Hepatopancreas of fish plays an important role in intermediate metabolic breakdown of xenobiotics (Fasulo et al. 2010). Its functions are associated with the detoxification and biotransformation process (Van der Osst et al. 2003), being one of the organs most affected by contaminants in the water. In teleosts, the kidney is a mixed organ, composed of hematopoietic, reticuloendothelial, endocrine, and excretory elements. Usually, it is divided into two portions, cephalic or head, and exocrine or trunk. In some fish species, no division between these portions is observed, although their morphology and functions are distinct, being basically composed of hematopoietic elements and, later, of mesonephric origin, composed by excretory elements (Takashima and Hibiya 1995; Fernandes et al. 2019). Osmotic regulation of water and salts is the main function of the kidney in fish, unlike mammals, whose main function is the excretion of nitrogenous waste. In the case of fish, nitrogenous wastes are mainly excreted through the gills (Ip and Chew 2018). The nephron is the functional unit of the kidney, which has the role of filtering blood; absorbing water, hormones, and nutrients; and excreting other substances in the renal lumen, forming the urine in the glomerular filtrate (Feist 2009). The fish nephron varies considerably between marine, euryhaline, and freshwater species, reflecting functional differences. However, they have a similar basic cellular architecture (Feist

2009). One of the first organs to be affected by contaminants is the kidney (Thophon et al. 2003). This organ is important to maintain a stable internal environment in relation to the hydroelectrolytic balance (water and salt) and to the excretion and metabolism of xenobiotics.

Both hepatopancreas and kidney present early warning signs of fish health conditions (Sorour 2001) with the degree of morphological lesions being associated with changes in environmental conditions, thus indicating the degree of environmental pollution (Thophon et al. 2003; Camargo and Martinez 2007; Flores-Lopes and Thomaz 2011). Identifying warning signs through changes in the hepatopancreas and kidneys tissues is ecologically and economically relevant, as well as faster than other traditional methods because they have the potential to be used as biomarkers (Sousa et al. 2013; Lakra et al. 2021).

Two Sciaenidae fish, *Menticirrhus americanus* (Linnaeus 1758) and *Micropogonias furnieri* (Desmarest 1823), are widely distributed in the adjacent continental shelf and in semi-enclosed estuarine systems of southeastern Brazilian coast (Figueiredo and Menezes 2000). Because they have the capacity to adapt in different environmental conditions, they present high eco-physiological plasticity and tolerance to a wide range of water physicochemical conditions, being potentially suitable for environmental monitoring purposes. In the southeastern Brazilian coast, the Sepetiba Bay is a semi-enclosed system affected by several anthropogenic activities, whereas the Paraty Bay presents better environmental conditions (Assis de Brito et al. 2020). Sepetiba Bay has suffered the effects of human activities mainly in the last 40 years (Pizzochero et al. 2019), with damage to the water quality (Wasserman et al. 2001; Copeland et al. 2003) and, consequently, to the fish diversity (Araújo et al. 2017). Paraty Bay is one of the Brazil's best-preserved coastal areas, been considered a reference (least impacted) environmental protection area. The 1988 Federal Constitution has protected this bay as a Union possession, with a specific legislation to safeguarding the biota. Nowadays, this bay is granted with the status of the World Heritage Site by the UNESCO (United Nations Educational, Scientific and Cultural Organization). The hypothesis tested in this study is that there are more serious and irreversible histological changes in the hepatopancreas and kidney of individuals of *M. americanus* and *M. furnieri* from Sepetiba Bay compared to those from Paraty Bay. Thus, we evaluated histological changes in the hepatopancreas and kidney of these fish species to assess their potential to be used as biomarkers of environmental impacts, and to determine whether these two fish species are suitable to be used as "sentinel" in biomonitoring programs.

Materials and methods

Study area

Sepetiba Bay (22°54′-23°04′S, 43°34′-44°10′W) has an area of approximately 450 km^2 and is located in the State of Rio de Janeiro, southeastern Brazil, approximately 50 km west of the municipality of Rio de Janeiro. The bay has a wide variety of habitats (e.g., mangroves, sandbanks, small estuaries, rock formations, and sandy beaches) and its drainage area is home to a population of approximately two million people. In this area, there are also more than 400 industries installed, including metallurgical, petrochemical, and pyrometallurgy, a submarine shipyard, in addition to a large export and import terminal, the Port of Sepetiba (Molisani et al. 2004; INEA 2009). Water depths are about 20 m in the main access channel to the port, but most bay area has depth of less than 10 m. The continental drainage of an area with great anthropogenic influence contributes to the waters being rich in organic nutrients and the substrate being predominantly muddy (Araújo et al. 2017). Sepetiba Bay plays an important role for fish and other aquatic organisms that use the area as nursery and growth areas. Industries located in the Sepetiba surroundings discharge into the bay large loads of wastewaters (Lacerda et al. 1982; Molisani et al. 2004). Paraty Bay (23°04′-23°18′S, 44°30′-44°36′W) is a small bay that is part of the Ilha Grande Bay, southeastern Rio de Janeiro (Fig. 1). Its area covers 65,258 ha, and the perimeter accounts for approximately 350 km^2 (Creed and De Paula 2007). Although Paraty Bay faces potential deposition of atmospheric contaminants from large urban and industrial centers such as São Paulo and Rio de Janeiro, it is still considered an unpolluted area and has a relatively well-preserved ecosystem (Molisani et al. 2004; Wanick et al. 2011). The aquatic environment in the region does not seem to suffer from the

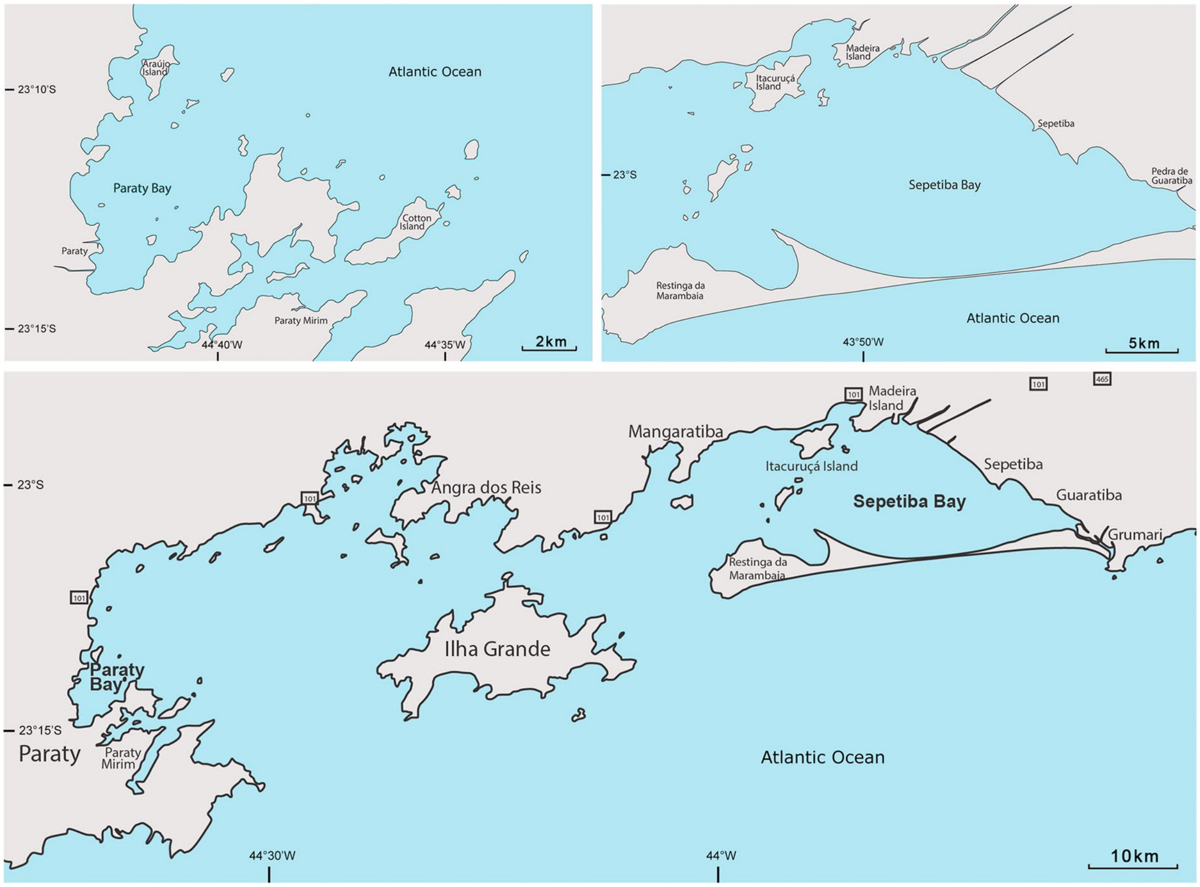

Fig. 1 Study area, showing the Sepetiba Bay and Paraty Bay bays in southeastern Brazilian coast

environmental hazard, despite the occasional incidence of contamination sources. In this study, the Bay of Paraty is taken as a reference area due to the low content of heavy metals such as Ni, Cu, Cr, Mn, Zn, and Hg (Lacerda et al. 1982; Cardoso et al. 2001; Freret-Meurer et al. 2010; Wanick et al. 2011), which is an indication that this area does not have evident effects of pollution by anthropogenic activities.

Data collection

Twenty adult fish from the Sepetiba Bay (10 individuals of *M. americanus* and 10 M. *furnieri*) and twenty-one adult fish from the Paraty Bay (10 M. *americanus* and 11 M. *furnieri*) were collected in 2015 and 2016. The collected fish were immediately identified, anesthetized with benzocaine hydrochloride (50 mg L^{-1}), as recommended by Resolution 1000 of the Federal Council of Veterinary Medicine of 2012, and euthanized. The fish was euthanized through hypothermia, measured to total length (cm), and weighed (g). All fish were immersed in formaldehyde solution after euthanasia to lessen post-mortem changes. The ICMBio license approved the current research for fish collection under process number 10707.

The size (TL, total length) of *M. americanus* ranged from 17.2 to 23.4 cm TL, and the body weight ranged from 48.5 to 99.3 g, whereas the size of *M. furnieri* ranged from 20.1 to 30.2 cm TL and the body weight ranged from 128.5 to 251.5 g. *Micropogonias furnieri* presented wider body size range than *M. americanus*, a comparatively small-sized species, but all individuals were adults. Sex was not considered in this study. The fish were dissected and the hepatopancreas and kidneys were excised. After removal, these organs were fixed in 10% neutral buffered formalin for 24 h.

Histopathological analyses

Initially, a previous classification of the histopathological alterations of each organ was performed using scores from 0 to 3 (0, no alteration; 1, mild alteration; 2, moderate alteration; and 3, severe alteration) following the protocol of Hose et al. (1996). These alterations have been modified (Poleksic and Mitrovic-Tutundzic 1994), for the following characteristics: (1) mild alteration, no damage is detected in the tissues of the organs in a way that prevents the restructuring and the return of the normal functions of the tissues if the environmental conditions improve; changes are restricted to small parts of the organ; (2) moderate alteration, more severe changes with influence on tissues associated with organ function; they are repairable injuries, but if large areas of tissue are affected or maintained in conditions of chronic pollution, they can become serious alterations; they can occur in virtually any organ; (3) serious alteration, the recovery of the organ structure is not possible, even with the improvement of the water quality or the end of the exposure to pollutants.

Histopathological changes in the hepatopancreas and kidneys were also evaluated semi-quantitatively using the histopathological alteration index (HAI). The HAI was calculated for each specimen using the following equation: $HAI = (1 \times SI) + (10 \times SII) + (100 \times SIII)$, where I, II, and III correspond to the number of alteration stages 1, 2, and 3 and S represents the sum of changes for each particular stage.

The interpretation of the HAI values followed the following order according to the range of variation of this index: (1) 0 to 10, indicate normal functioning of the organ; (2) 11 to 20, indicate minor alterations; (3) 21 to 50, indicate moderate alterations; (4) 51 to 100, indicate serious injuries; and (5) > 100, indicate irreparable organ damage (Poleksic and Mitrovic-Tutundzic 1994). Lesions were classified into three progressive stages, based on tissue repair capacity: (I) tissue reconstruction is possible (structure and function); (II) more severe alterations (effects on tissue functions); (III) tissue restoration is no longer possible. Examples of lesion cases were photographed using a photomicroscope. The percentage of each anomaly observed in the organ was calculated by dividing the number of altered fish by the total number of examined fish.

Alterations of hepatopancreas and kidneys

Tissues were fixed in 10% neutral buffered formalin for 24 h, dehydrated through a graduated series of increasing concentrations of ethanol, bleached in xylene, and embedded in paraffin. Sections of 4-μm thickness were cut, mounted on glass slides, and stained with hematoxylin and eosin for histological description of the organ. Sections of hepatopancreas were also stained with Gomori trichrome for differentiation of connective tissue, muscle, and collagen fibers. PAS (periodic acid Schiff) method was used for identification of the kidney proximal tubule with brush border. Tissue observation was performed under an Olympus B × 41 light microscope (Tokyo, Japan) connected to a Nikon Coolpix 4300 digital camera.

Results

Histological analysis of hepatopancreas

The pancreatic exocrine tissue in normal hepatopancreas in the studied species is located in the liver. The intrahepatic exocrine pancreas is organized in clusters of columnar cells, organized in acini (Fig. 2A), and presents melanomacrophage aggregates that can be pathological (Fig. 2B). It is located around the branches of the portal vein (Fig. 2C), and is separated from the liver parenchyma through a thin layer of connective tissue, composed mainly of collagen fibers.

Histological changes in hepatopancreas

The alterations in the hepatopancreas tissues (Fig. 2, Table 1) were classified into slight (stage I), moderate (stage II), and severe changes (stage III). The following slight changes were found: nuclear hypertrophy; irregular shaped nucleus; nucleus in lateral position; cellular hypertrophy; cytoplasmic vacuolization; eosinophilic granules; and melanomacrophage aggregates. The moderate changes observed in this tissue were nuclear vacuolization; cytoplasmic degeneration; blood congestion; and bile stagnation. Severe change found was necrosis.

The histological changes in *M. americanus* in the Sepetiba Bay (Table 1) showed high frequencies

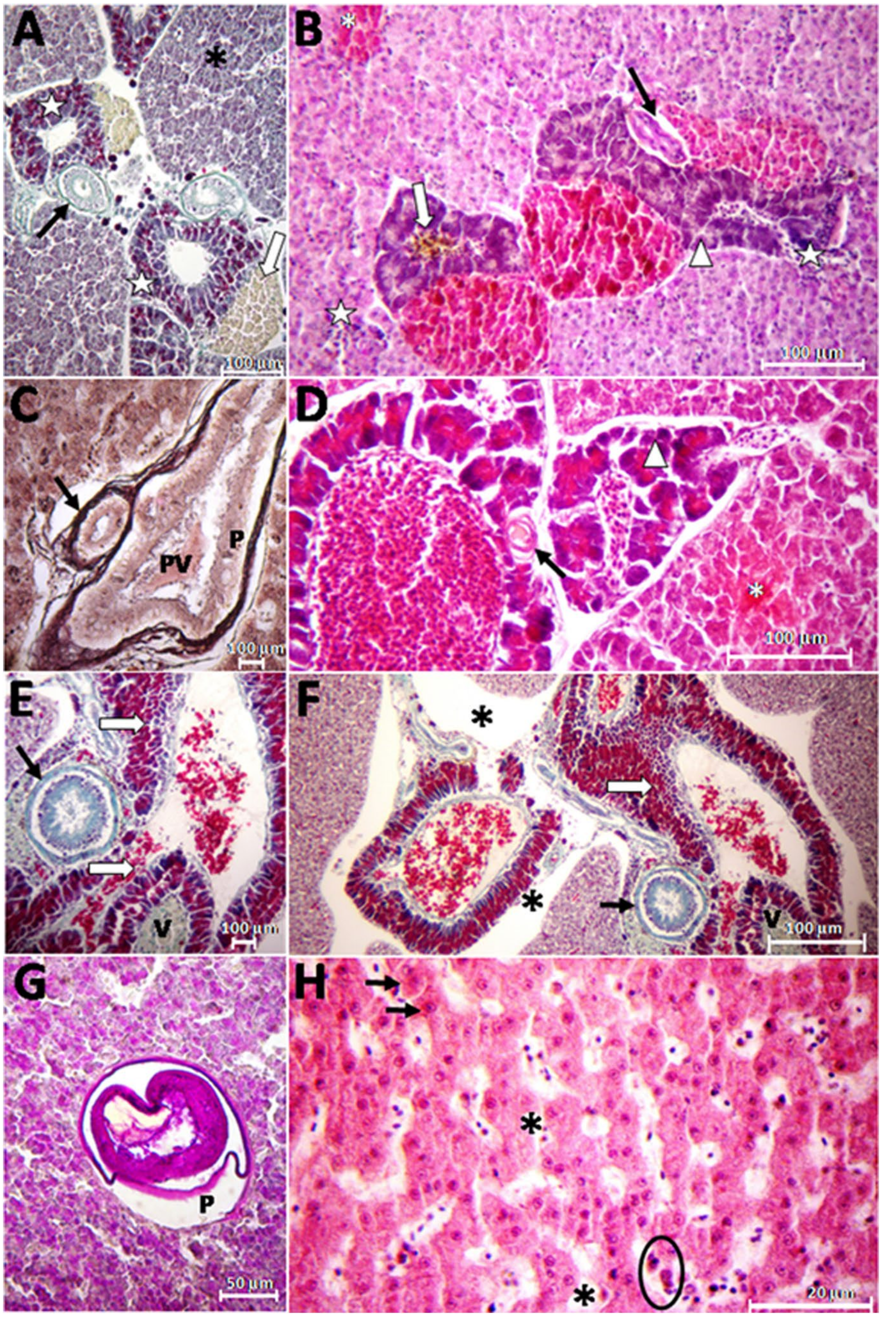

Fig. 2 Photomicrographs of the hepatopancreas. **a** Normal hepatopancreas showing hepatic parenchyma (asterisk), clusters of pancreatic acini (star), bile duct (arrow), and melanomacrophage aggregates (white arrow). Stain: Gomori trichrome, *Menticirrhus americanus.* **b** Congestion (white asterisk), parasite (arrow), melanomacrophage aggregates (white arrow), leukocyte infiltration (star), and patchy degeneration (triangle). Stain: HE, *Micropogonias furnieri*. **c** Portal vein (letters PV) with pancreatic tissue (letter P) and bile duct (arrow). Stain: Gomori method for reticulum, *Micropogonias furnieri*. **d** Patchy degeneration (triangle), congestion (white asterisk) and parasite (arrow). Stain: HE, *Menticirrhus americanus*. **e** Intravascular hemolysis in hepatoportal blood vessels (white arrow), vein (letter V), and bile duct (arrow). Stain: Gomori trichrome, *Micropogonias furnieri*. **f** Damage of hepatopancreas characterized by loss of contact between hepatocyte and pancreatic acini (asterisk), intravascular hemolysis in hepatoportal blood vessels (white arrow), vein (letter V), and bile duct (arrow). Stain: Gomori trichrome, *Micropogonias furnieri*. **g** Parasite (letter P). Stain: HE. **h** Biliar stagnation (arrow), cytoplasmic vacuolization (asterisk), and nuclear hypertrophy (circle). Stain: HE, *Menticirrhus americanus*

Table 1 Classification and occurrence of histological changes in hepatopancreas in *Menticirrhus americanus* and *Micropogonias furnieri* from Sepetiba and Paraty bays. Stage I: do not alter the normal functioning of the tissues; stage II: more severe and impair the normal functioning of the tissues; stage III: very severe and cause irreparable damage. Values of frequency of occurrence in %; the number of altered individuals in brackets

Stage	Histological changes	Sepetiba Bay		Paraty Bay	
		M. americanus	*M. furnieri*	*M. americanus*	*M. furnieri*
I	Nuclear hypertrophy	100% (10)	100% (10)	100% (10)	100% (11)
	Irregular shaped nucleus	100% (10)	100% (10)	100% (10)	100% (11)
	Nucleus in lateral position	100% (10)	100% (10)	100% (10)	100% (11)
	Cellular hypertrophy	100% (10)	90% (9)	100% (10)	90.9% (10)
	Cytoplasmic vacuolization	70% (7)	80% (8)	100% (10)	63.6% (7)
	Eosinophilic granules	60% (6)	80% (8)	40% (4)	27.3% (3)
	Melanomacrophage aggregates	80% (8)	70% (7)	40% (4)	36.7% (4)
II	Nuclear vacuolization	90% (9)	100% (10)	50% (5)	63.6% (7)
	Cytoplasmic degeneration	100% (10)	100% (10)	30% (3)	27.3% (3)
	Blood congestion	90% (9)	80% (8)	40% (4)	36.7% (4)
	Bile stagnation	50% (5)	50% (5)	70% (7)	36.7% (4)
III	Necrosis	70% (7)	70% (7)	30% (3)	27.3% (3)
	Total number of examined individuals	10	10	10	11

(50–100%) of slight alterations (stage I); these include nuclear hypertrophy (Fig. 2H), irregular shaped nucleus, nucleus in lateral position and cellular hypertrophy, cytoplasmic vacuolization (Fig. 2H), eosinophilic granules, and melanomacrophage aggregates (Fig. 2B). Moderate alterations (stage II) presented comparatively high frequencies (50–100%) of nuclear vacuolization, cytoplasmic degeneration, and blood congestion and bile stagnation (Fig. 2H). Necrosis was the only severe alteration (stage III) found and presented high frequency (50–100%). In the Paraty Bay (Table 1), *M. americanus* presented high frequencies (50–100%) of slight alterations (stage I)—nuclear hypertrophy, irregular shaped nucleus, nucleus in lateral position, cellular hypertrophy, and cytoplasmic vacuolization—and intermediate frequencies (30–50%): eosinophilic granules and melanomacrophage aggregates (Fig. 2B). Moderate alterations (stage II) also presented intermediate frequencies (30–50%) from cytoplasmic degeneration and blood congestion and high frequency (50–100%) for nuclear vacuolization and bile stagnation. Necrosis, in severe alteration (stage III), was found and presented intermediate frequency (30–50%).

The histological changes in *M. furnieri* in the Sepetiba Bay (Table 1) presented high frequencies (50–100%) of slight alterations (stage I): nuclear hypertrophy, irregular shaped nucleus and nucleus in lateral position, cellular hypertrophy, cytoplasmic vacuolization, eosinophilic granules, and melanomacrophage aggregates. Moderate alterations (stage II) presented high frequencies (50–100%): nuclear vacuolization, cytoplasmic degeneration, blood congestion, and bile stagnation. Necrosis was the only severe alteration (stage III) found and showed high frequency (50–100%). In the Paraty Bay (Table 1), *M. furnieri* presented high frequencies (50–100%) of slight alterations (stage I)—nuclear hypertrophy, irregular shaped nucleus and nucleus in lateral position, cellular hypertrophy, and cytoplasmic vacuolization—and intermediated frequency (30–50%): melanomacrophage aggregates; and eosinophilic granules presented low frequency (below 30%). Moderate alterations (stage II) also presented high frequency (50–100%) for nuclear vacuolization; intermediate frequency (30–50%) for blood congestion and bile stagnation; and low frequency (below 30%) for cytoplasmic degeneration. Necrosis was found and presented low frequency (below 30%).

No notable differences in histological changes were observed between the two fish species examined. However, the hepatopancreas of fish collected in Sepetiba Bay presented HAI above 100, indicating serious damage to these organs. On the other hand, the hepatopancreas of fish from the Bay of Paraty presented HAI values between 20 and 100, indicating moderate damage, which can reach a serious condition.

Histological analysis of kidneys

Normal kidney of fish is shown in Fig. 3A. The nephron is formed by four distinct regions: glomerulus, proximal tube, distal tube, and collecting tubule. Despite being part of the excretory system, it is composed of hematopoietic tissue and melanomacrophage aggregates present that can be pathological (Fig. 3B). The proximal tubules are covered with a glycocalyx covering (brush border) (Fig. 3A), the distal tubules have no brush edges, and the collecting tubules consist of columnar epithelia with basal nuclei without a brush border.

Histological changes in kidneys

The alterations in the kidney tissues (Fig. 3, Table 2) were classified into slight (stage I), moderate (stage II), and severe changes (stage III). The following slight changes were found: dilation of glomerular capillaries and glomerular hypertrophy (corpuscle); nuclear hypertrophy; cellular hypertrophy; cytoplasmic vacuolization; dilation of lumen; tubular regeneration; and melanomacrophage aggregates (tubules). The moderate changes found in this tissue were Bowman's space increase, blood in Bowman's space, tubular degeneration (cytoplasmic), and increase of the tubular lumen caliber. Severe changes observed were necrosis.

The histological changes in *M. americanus* in the Sepetiba Bay (Table 2) showed slight alterations (stage I) with high frequencies (50–100%): dilation of glomerular capillaries, glomerular hypertrophy, nuclear hypertrophy, cellular hypertrophy, cytoplasmic vacuolization, dilation of lumen and tubular regeneration, and melanomacrophage aggregates. The moderate alterations (stage II) presented high frequencies (50–100%): Bowman's space increase, blood in Bowman's space, tubular degeneration (cytoplasmic), and increase of the tubular lumen caliber. Necrosis (stage III) presented high frequency. In the Paraty Bay (Table 2). *M. americanus* also showed slight alterations (stage I) with high frequencies (50–100%): dilation of glomerular capillaries and glomerular hypertrophy, nuclear hypertrophy, cellular hypertrophy, cytoplasmic vacuolization, dilation of lumen and tubular regeneration, and melanomacrophage aggregates. Moderate alterations presented high frequencies (50–100%) with Bowman's space increase, tubular degeneration (cytoplasmic), and increase of the tubular lumen caliber, and intermediate frequency (30–50%) in blood in Bowman's space. Necrosis (stage III) presented intermediated frequency (30–50%).

The histological changes in *M. furnieri* in the Sepetiba Bay (Table 2) showed slight alterations (stage I) with high frequencies (50–100%): dilation of glomerular capillaries, glomerular hypertrophy, nuclear hypertrophy, cellular hypertrophy and cytoplasmic vacuolization, dilation of lumen and tubular regeneration, and melanomacrophage aggregates. The moderate alterations (stage II) presented high frequencies (50–100%): Bowman's space increase, blood in Bowman's space, tubular degeneration (cytoplasmic), and increase of the tubular lumen caliber. Necrosis (stage III) presented high frequency. In the Paraty Bay (Table 2), *M. furnieri* also showed slight alterations (stage I) with high frequencies (50–100%): dilation of glomerular capillaries, glomerular hypertrophy, nuclear hypertrophy and cellular hypertrophy, cytoplasmic vacuolization, dilation of lumen and tubular regeneration, and melanomacrophage aggregates. Moderate alterations presented high frequencies (30–50%) with Bowman's space increase, tubular degeneration (cytoplasmic), and increase of the tubular lumen caliber, and low frequency (below 30%) in blood in Bowman's space. Necrosis (stage III) presented intermediated frequency (30–50%).

Similar histologic changes were observed between the two examined fish species. However, the fish collected in Sepetiba Bay showed kidneys with HAI above 100, indicating irreversible damage to the organs. The kidneys of fish collected in the Bay of Paraty showed HAI values between 20 and 50, indicating moderate damage to the organs.

Discussion

We found no difference in histological changes between *M. americanus* and *M. furnieri*, suggesting that these two species are equally sensitive to environmental alterations. The probable exposure of these

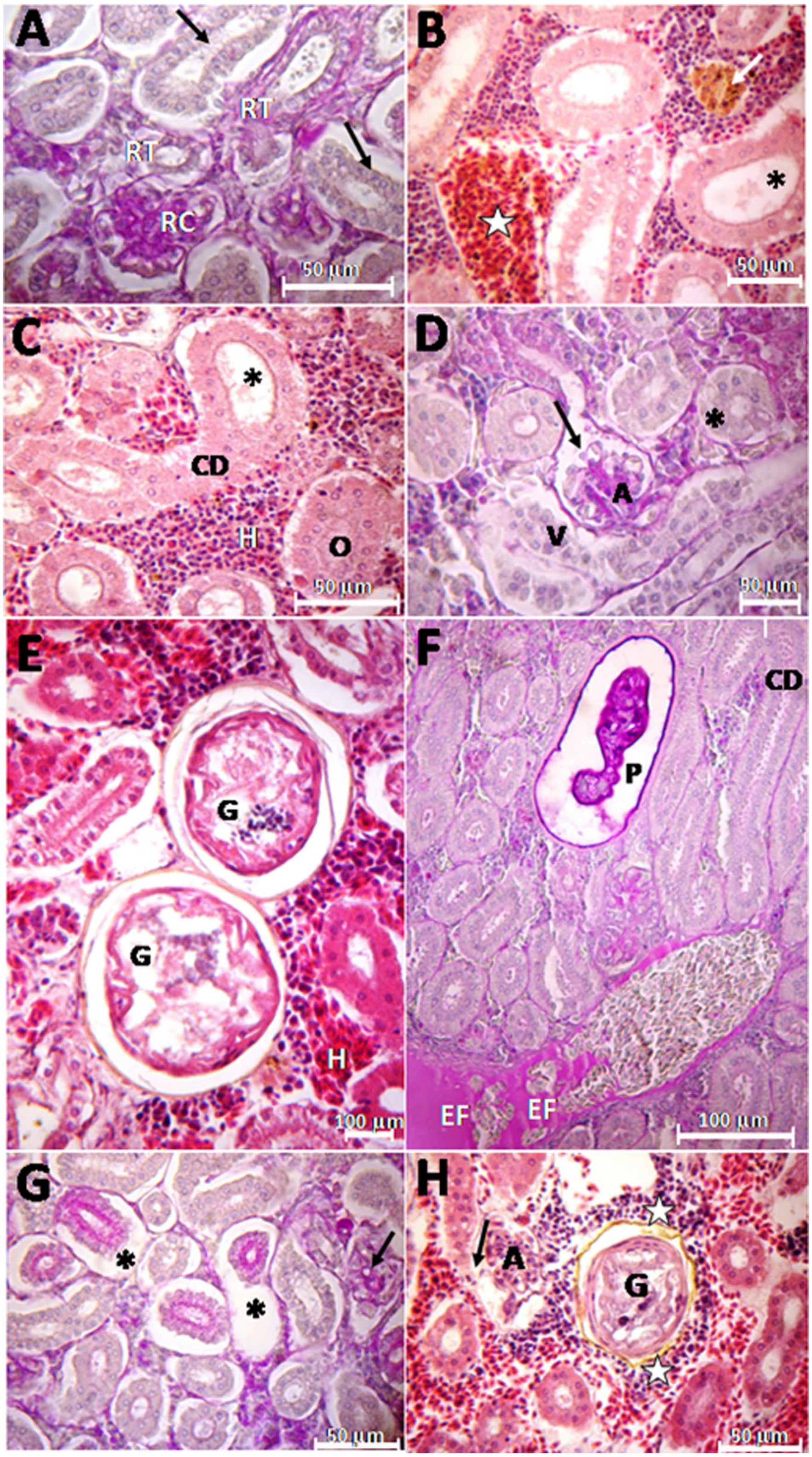

Fig. 3 Photomicrographs of the kidney. **a** Normal renal corpuscle showing the glomerulus and the Bowman's space well defined (RC) and renal tubules (RT). Note the brush border of the proximal tubule (arrow). Stain: HE, *Micropogonias furnieri*. **b** Dilation in some of renal tubules (asterisk), melanomacrophage aggregates (white arrow), and melanomacrophage aggregates with hemorrhage (star). Stain: PAS, *Menticirrhus americanus*. **c** Occlusion of tubular lumen (letter O), dilation in some of renal tubules (asterisk), hemorrhage (letter H), and cellular degeneration (letters CD). Stain: PAS, *Menticirrhus americanus*. **d** Atrophy of glomerular tuft (letter A), dilation of Bowman's space (arrow), vacuolar degeneration tubular (letter V), and cell with hypertrophied nucleus (asterisk). Stain: PAS, *Micropogonias furnieri*. **e** Hemorrhage (letter H) and granuloma (letter G) with central necrosis in the kidney tissue. Stain: HE, *Menticirrhus americanus*. **f** Parasite (letter P), cellular degeneration (letters CD), glomerular expansion (asterisk), and oedematous fluid (letters EF). Stain: PAS, *Micropogonias furnieri*. **g** Narrowing of tubular lumen (asterisk) and glomerular expansion (white arrow). Stain: HE, *Micropogonias furnieri*. **h** Granuloma (letter G) with compact and thick layer of collagen with internal necrotic content surrounded by epithelioid cells, atrophy of glomerular tuft (letter A), dilation of Bowman's space (arrow), and inflammatory infiltration (star). Stain: HE, *Menticirrhus americanus*

Table 2 Classification and occurrence of histological changes in kidneys of *Menticirrhus americanus* and *Micropogonias furnieri* from Sepetiba and Paraty bays. Stage I: do not alter the normal functioning of the tissue; stage II: more severe and impair the normal functioning of the tissue; stage III: very severe and cause irreparable damage. Values of occurrence in %; the number of altered individuals in brackets

Stage	Histological changes	Sepetiba Bay		Paraty Bay	
		M. americanus	*M. furnieri*	*M. americanus*	*M. furnieri*
I	Corpuscle				
	Dilation of glomerular capillaries	100 (9)	100 (10)	80 (8)	90.9 (10)
	Glomerular hypertrophy	100 (9)	100 (10)	80 (8)	81.8 (9)
	Tubules				
	Nuclear hypertrophy	100 (9)	100 (10)	90 (9)	100 (11)
	Cellular hypertrophy	100 (9)	100 (10)	100 (10)	100 (11)
	Cytoplasmic vacuolization	100 (9)	100 (10)	100 (10)	81.8 (9)
	Dilation of lumen	100 (9)	90 (9)	100 (10)	100 (11)
	Tubular regeneration	100 (9)	100 (10)	100 (10)	100 (11)
	Melanomacrophage aggregates	88.9 (8)	100 (10)	90 (9)	81.8 (9)
II	Bowman's space increase	100 (9)	100 (10)	80 (8)	81.8 (9)
	Blood in Bowman's space	88.9 (8)	90 (9)	40 (4)	27.3 (3)
	Tubular degeneration (cytoplasmic)	100 (9)	90 (9)	60 (6)	72.7 (8)
	Increase of the tubular lumen caliber	100 (9)	100 (10)	70 (7)	63.6 (7)
III	Necrosis	88.9 (8)	100 (10)	40 (4)	45.5 (5)
	Total number of examined individuals	9	10	10	11

fishes to a series of anthropogenic pollutants induced a negatively impact in their tissue. Since histological changes in the liver and kidney have been used as an indicator of environmental quality (Fernandes et al. 2008; Assis de Brito et al. 2020), this study provided important evidences of the poor environmental quality of these two bays, although with slight differences in environmental quality between the bays.

The histopathologic alteration index (HAI) was successfully applied to evaluate histological changes in fishes caused by pollution. The comparatively higher values of HAI for *M. americanus* and *M. furnieri* in the Sepetiba Bay indicate contamination to this coastal system resulting from exposure to pollution in this more impacted bay. This was confirmed by the severity of histological alterations observed in fishes from the Sepetiba Bay. Overall, comparatively lower HAI values were observed in Paraty Bay, with indication of better fish health because of its farthest distance from the pollution sources. Although fish is part of the nekton with the ability to move between different areas, our findings suggest that such movements are limited and that these species of the family Sciaenidae have restricted distribution in different habitats. These results were expected and corroborate other studies that suggest contamination of Sepetiba Bay by non-specific xenobiotics (Assis de Brito et al. 2020), and metals resulting from industrial centers nearby the shoreline and that are the main contaminants of Sepetiba Bay (Morales et al. 2019).

Small alterations were found in the hepatopancreas of the two species studied in both bays. Nuclear hypertrophy was characterized by an increase in cell size as reported by Hadi and Alwan (2012). It is possible that this hypertrophy is associated with contaminated sediments, since interstitial hepatocytes when exposed to contaminated sediments showed hydropic swelling (Hinton and Laurén 1990). Both species studied presented hepatopancreas with an irregularly shaped nucleus, a nucleus in a lateral position, and cytoplasmic vacuolization. This type of alteration was associated with the occurrence of fish in places contaminated by organophosphate pesticides (Fanta et al. 2003). The increase in cytoplasmic vacuolization is indicative of the degenerative process, suggesting metabolic damage, and possibly associated with exposure to contaminated water (Pacheco and Santos 2002). This alteration is possibly a cellular defense mechanism against substances harmful to hepatocytes, with the function of collecting the harmful elements and preventing them from interfering with the biological activities of these cells (Mollendroff 1973).

Corroborating our findings, histological and structural studies carried out in the sea stickleback *Spinachia spinachia* (Linnaeus 1758), in areas with low pollution levels, revealed the absence of melanomacrophage aggregates (Steinel and Bolnick 2017). In addition, the presence of heavy metals within the melanomacrophage aggregates was found in *Platichthys flesus* (Linnaeus 1758), collected in areas of greater environmental impact (Pulsford et al. 2009; Steinel and Bolnick 2017). The presence of melanomacrophages in hepatopancreas can be a strong evidence that this organ has suffered structural and metabolic damage due to exposure to polluted water (Camargo and Martinez 2007).

Moderate alterations such as nuclear vacuolization, cytoplasmic degeneration, and blood congestion were found more frequently in both species in Sepetiba Bay. Fish exposed to contamination by metals such as copper (Paris-Palacios et al. 2000) and mercury (Ribeiro et al. 2002) and by polychlorinated biphenyls (Chang et al. 2001) have shown such changes, indicating signs of tissue degeneration. In total, 50% of *M. americanus* and *M. furnieri* collected in the Sepetiba Bay presented bile stagnation, alteration characterized by the presence of bile in the form of yellow–brown granules in the cytoplasm of hepatocytes (Pacheco and Santos 2002), indicating that bile is not being released from the hepatopancreas. Accumulation of bile evidences metabolic problems (Camargo and Martinez 2007) and indicates possible damage to hepatic metabolism (Fanta et al. 2003).

Similar to hepatopancreas, the kidney reacts to exposition to anthropogenic pollution. Most common alterations found in the kidney of *M. americanus* and *M. furnieri* exposed to water affected by anthropogenic activities (Sepetiba Bay) were moderate and severe changes in corpuscle and tubular degenerative. Kidneys of fishes from the Sepetiba Bay presented a higher frequency (50–100%) of melanomacrophage aggregates when compared with those from the Paraty Bay. Melanomacrophage aggregates were observed in hepatopancreas and hematopoietic tissue of the kidney, as described in the literature (Roberts 1978). These structures contain different pigments such as melanin, hemosiderin, lipofuscin, and lipogenic pigment that can assist in the host's defense mechanisms (Roberts 1978). Melanin plays an important role in the production of bactericidal compounds, especially hydrogen peroxide (Wolke et al. 1985). It can also absorb or neutralize free radicals, cations, and other toxic agents, derived from the degradation of phagocytosed cellular material (Agius and Agbede 1984; Zuasti et al. 1998).

Occlusion of the renal tubules was observed in this study, which may be related to the accumulation of certain materials in the lumen and also as a consequence of the swelling of the cell epithelium (Takashima and Hibiya 1995). As a result of this alteration, damage to the flow of the filtrate may occur and the processes of reabsorption and secretion in the tubule may be delayed (Rand and Petrocelli 1985; Hinton et al. 1992). Occlusion of the renal tubules in fishes may be caused by increases in doses of lead nitrate and days of exposure (Brraich and Kaur 2017).

Histological changes in the Bowman's capsule, such as increase in the space of the Bowman's capsule, and the resulting formation of edema were found in both species in Sepetiba Bay. These alterations occur in the kidneys because they are the main site of blood production in fish, and xenobiotics in the blood can cause histological changes in the Bowman's capsule. The increase in the space of the Bowman's capsule, and consequently, to the formation of edema, was reported by other studies with fish exposed to lead acetate, to herbicides and to heavy metals (Hermenean et al. 2015; Cruz et al. 2016). Hypertrophied Bowman's capsule cells and aggregated melanomacrophages in the kidney of trout *Salmo trutta* Linnaeus, 1758, and tilapia *Oreochromis mossambicus* (Peters 1852) exposed to mercuric chloride were reported by Handy and Penrice (1993). Fish exposed to organic contaminants and mixed environmental contaminants have been reported as presenting similar changes (Pacheco and Santos 2002; Veiga et al. 2002).

Indications that fish from the Sepetiba Bay were exposed to adverse conditions that resulted in serious and irreparable damages to the rivers were indicated by the presence of tubular degeneration, associated with necrosis. Tubular degeneration and changes in the corpuscle in the kidneys are related to fish that are exposed to contaminated water and with the presence of metals (Takashima and Hibiya 1995). The degeneration process can change to hyaline degeneration, reflected by the presence of large eosinophilic granules within the cells (Camargo and Martinez 2007). These granules may be formed into the cells or by the reabsorption of plasma proteins lost in the urine, suggesting damage to the corpuscle as reported by Reinert (1992). At the apex, and characterizing severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya 1995).

Necrosis corresponds to a severe and irreparable change. Signs of degeneration (nuclear degeneration and nuclear vacuolization) in addition to focal necrosis indicate that the histological changes are more severe and are associated with exposure of fish to water contaminated by metals such as copper (Paris-Palacios et al. 2000) and mercury (Ribeiro et al. 2002), as well as polychlorinated biphenyls (Chang et al. 2001). The histological results confirmed by the presence of necrosis in the hepatopancreas and kidney are relevant indication of the altered environmental conditions that the Sepetiba Bay has undergone recently.

Indications that *M. americanus* and *M. furnieri* were responding to the effects of contaminants were detected in the present study through the histological changes observed in the hepatopancreas and kidney. This also indicates that anthropogenic activities lead to contamination of the bays probably caused by non-specific xenobiotics that also negatively have been impacting the ecosystem of the two studied bays. The histological alterations found indicated that this procedure was efficient as biomarkers for environmental quality assessment, particularly in tropical bays. Histological changes are effects and/or early responses to acute exposure to chemical stressors (Bucher and Hofer 1993; Camargo and Martinez 2007; Hadi and Alwan 2012) being an efficient tool in environmental monitoring and management. Histological changes in the hepatopancreas and kidney of *Menticirrhus americanus* and *Micropogonias furnieri* were also able to distinguish the lesser altered state of the Paraty Bay compared with more critical situation of the Sepetiba Bay. Although further studies covering other tropical bays in different state of contamination are still needed, we suggest that histological changes in the hepatopancreas and kidneys can be useful biomarkers to assess environmental contamination in tropical South American bays using these two studied species.

Author contribution Thatiana Luiza Assis de Brito Carvalho: formal analysis, writing—original draft. Aparecida Alves do Nascimento: formal analysis, writing—original draft. Iracema David Gomes: writing—review and editing. Francisco Gerson Araújo: writing—review and editing.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval The authors state that the research was conducted according to ethical standards. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, including Universidade Federal Rural do Rio de Janeiro, Brazil, Animal Care Protocol (# 11874).

Competing interests The authors declare no competing interests.

Author statement I declare, as corresponding author, that I am responsible for ensuring that the descriptions are accurate and agreed by all authors.

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