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Histopathological Changes in Estuarine Catfish: A Temporal Approach in a Marine Protected Area

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HIGHLIGHTS

- Hepatic changes in catfish bioindicator were effectiveness to indicate the ecosystem health.
- Human risk to resident fish population must monitored.

Abstract: The Cananéia-Iguape Estuarine-Lagoon Complex (CIELC) is an area of low anthropogenic impact; however, it is not completely isolated from human pressure, particularly in the northern sector. Considering this scenario, this study aims to identify the health of two catfish species *Cathorops spixii* and *Genidens Genidens* taking into account the hepatic pathological analysis and the accumulation of iron in the form of hemosiderin considering a temporal approach. Biometric measurements were taken, and the condition factor was obtained. The main liver alterations found in the two catfish species were necrosis, inflammation, hemorrhage, cytoplasmatic vacuolization, and pyknosis nuclei. Necrosis (2014: 57%; 2017: 71 %; and 2018: 20%) and pyknosis nuclei (2014: 86%; 2017: 43%; and 2018: 40%) was the most frequent alteration. However, cytoplasmatic vacuolization was the only change that was statistically significant over time. Regarding hemosiderin, catfish livers mostly presented a minimum degree of accumulation, which represents healthy results. Finally, histopathological observations in *C. spixii* and *G. genidens* catfish tissues are confirmed to be an efficient tool to evaluate the health of these fish and the changes induced by environmental conditions over the years. However, the absence of significant differences on the histopathological changes over time can suggest a physiological equilibrium of these fish in the CIELC.

Keywords: Biomarker; *Cathorops spixii*; *Genidens genidens*; Biomonitoring; Cananéia.

INTRODUCTION

Estuaries are considered aquatic ecosystems in transition between freshwater and marine environments, and they are characterized by great productivity and a diversity of species, as estuaries provide a high availability of nutrients, sunlight, and protection in addition to the heterogeneity of the habitat, which allows the growth of numerous organisms. However, these environments are subject to different pressures from human activities due to the growth of industrial, agricultural, and urban activities. The CIELC is an area considered to be under low anthropogenic action, even though it is in the midst of regions of intense environmental impact. In the northern area, there are already signs of impacts related to anthropogenic activities and metal input [1, 2].

Estuarine regions are very heterogeneous environments covering a wide range of environmental variables. The Cananéia-Iguape Estuarine-Lagoon Complex (CIELC) is an area with low anthropogenic influence; however, it is not completely isolated from human pressure, particularly in the northern sector. This region is part of UNESCO's Atlantic Forest reserve and was recently included in the Ramsar list of wetlands that offer an international incentive for conservation and research initiatives (https://rsis.ramsar.org/ris/2310). The CIELC is located between areas in which there is great risk of human impact. In the northern sector, this is due to exposure to Ribeira de Iguape River valley activities, and in the southern sector, this is due to ship movement in Paranaguá Bay, which acts on the hydrological system. In the northern sector of the estuary, some impacts have already been observed at higher altitudes such as silting, deforestation, and erosion of the area due to agricultural activities related to the cultivation of tea and banana crops. The influence of the urban centers of Iguape and Ilha Comprida, located in the lower valley of the Ribeira de Iguape River, is also present in this area [1]. There is a long history of mining activities being conducted in the estuary; this has contributed to the presence of lead in the water, which occurs in concentrations above those permitted by the provisional sediment quality guidelines (ISQG) [2].

Histopathological data have been used in biomonitoring studies of the aquatic environment, and a higher incidence of tissue changes is found in fish from areas with high anthropogenic influence [3, 4, 5]. Tissue changes are the organism's response to external stressors, and these changes, which can occur in several organs, can be used to detect external stressors' effects; livers are highly represented in the analysis of histological lesions [6, 7]. Tissue changes may represent responses to chemical contaminants present in the aquatic compartment, among which polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethane (DDT), dieldrin, and chlordane normally come from industrial activities, harbors, agricultural activities, urban centers, and sewage discharges, all of which contribute to water contamination [6]. In addition to these substances, trace metals such as lead, copper, and cadmium are also promoting histopathological changes; they can bioaccumulate in the tissues of aquatic animals and induce changes in metabolic functions [7, 8].

The fish of the Ariidae family, namely *Cathorops spixii* (*C. spixii*) and *Genidens genidens* (*G. genidens*), have been used as bioindicators of contamination in several studies in recent years [2, 6, 9, 10, 11, 12]. The works involving these fish conduct analyses of biochemical biomarkers; tissue lesions in different tissues, such as gills and livers [6]; genotoxicity biomarkers [3]; and bioaccumulation of metals in the muscles [13]. The anomalies normally occur in anthropized environments close to urban centers and industrial activities, and contamination at different levels takes the form, in general, of substances from pesticides, fertilizers [6], minerals, and oil products [13]. Studies demonstrate that the use of these fish as bioindicator species has been effective in quantitative and qualitative analyses; thus, these fish are appropriate bioindicators of environmental quality [3, 4, 9, 12, 13]. *C. spixii* (Agassiz 1829) and *G. genidens* (Valenciennes 1839) show a wide distribution on the Brazilian coast in places such as estuaries and estuarine lagoons, usually occurring in bays with a biogenic bottom and low depths [14, 15]. They have a feeding habitat in the benthic zone and can be found in shallow waters with muddy or sandy bottoms [14, 16, 17, 18].

Considering the importance of the CIELC as a Ramsar site, this is, a priority area for conservation, the goal of this study is to identify and categorize the histopathological changes in livers of the bioindicators estuarine catfish *C. spixii* and *G. genidens* (Siluriformes, Ariidae) as primary and secondary responses to human influence in this aquatic system and its effect on the native biota. The pathological alterations in the hepatic tissues of the catfishes will be analyses over time. In addition, metabolic responses with respect to process of iron accumulation, as hemosiderin, will be also evaluated in the bioindicators catfishes.

MATERIAL AND METHODS

Sampling sites, fish collection, and biometric data

This study was carried out within the scope of the environmental monitoring and diagnosis research project entitled "Impact of xenobiotics on the estuarine teleost metabolism", which has been carried out since 2004 with bioindicator catfish in the CIELC [4, 8, 9, 11, 12, 19, 20, 21, 22]. 135 *C. spixii* and 50 *G. genidens* specimens were collected in August (winter period) of 2014 (*C. spixii* – n = 43; *G. genidens* – n = 22), 2017

(*C. spixii* – n = 41; *G. genidens* – n = 7), and 2018 (*C. spixii* – n = 51; *G. genidens* – n = 21) in the CIELC (Figure 1) and analyzed regarding pathological changes in the hepatic tissue of the fish. The sampling sites were properly georeferenced using a GPS device (vessel).

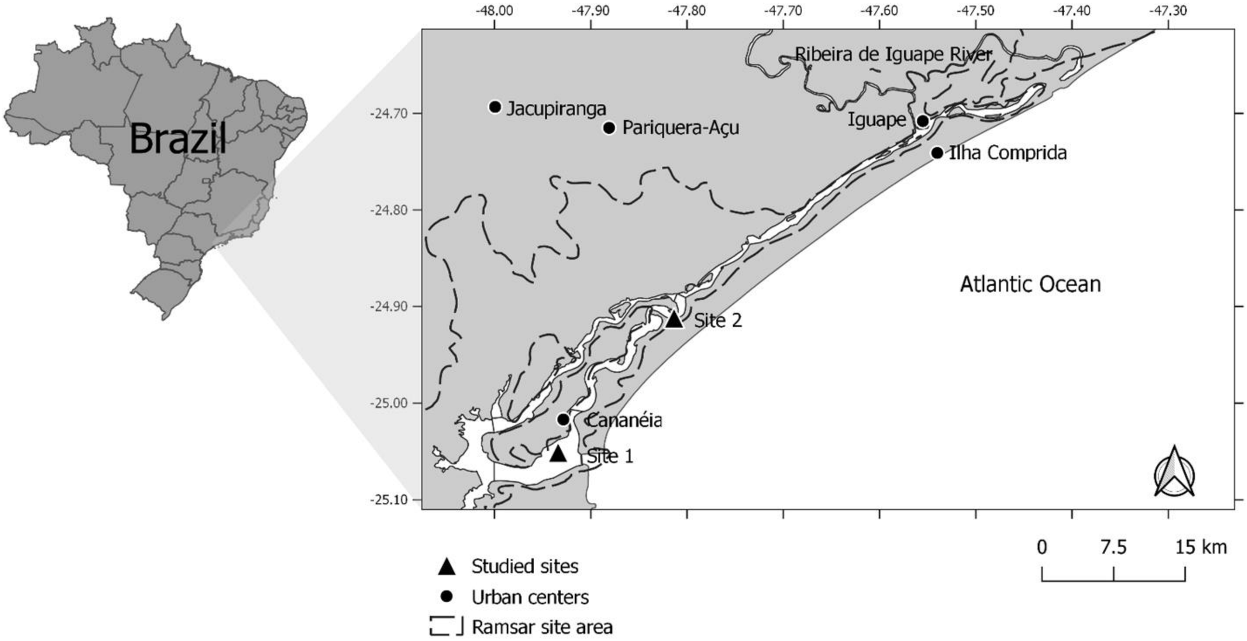

Figure 1. Map of the Cananéia-Iguape Estuarine-Lagoon Complex (CIELC), indicating the sites where the fish were collected in the northern and southern areas.

Site 1 represents an area with a greater maritime influence, while Site 2 represents the innermost area of the estuary. The salinity of the water decreases towards the interior of the complex, reaching its lowest value at the mouth of the Valo Grande channel and its highest value in Cananéia [1]. Regarding mud distribution in the CIELC, was observed points to higher granulometry in the northern region of the estuary near the urban centers of Iguape, in the Mar Pequeno, while in the Cananéia region, the sludge is of low and medium character, and thus characteristic of the sandy sediment in this region [1, 2]. There are higher levels of metals such as copper, zinc, and lead in the region around the urban center of Iguape, and low levels in the southern region of the estuary [2]. The pH in the CIELC presents a decreasing gradient towards the interior of the estuary: it is higher at Site 1, which has a greater maritime influence, and lower at Site 2, which is located further inside the estuarine complex [19]. The levels of organic matter and dissolved oxygen in the water show low variation along the estuary [23]. The predominant species of fish observed in the region is *C. spixii* followed by the species *Stellifer rastrifer*, *Notarius luniscutis*, *Chloroscombrus chrysurus*, *Isopisthus parvipinnis*, *Cynoscion leiarchus*, and *Symphurus tesselatus* [24, 25]. Regarding the Ariidae family, the main species present at the site are *Cathorops spixii*, *Netuma barba*, *Genidens genidens*, *Sciadeichthys luniscutis*, *Bagre marinus*, *Bagre bagre*, and *Notarius grandicassis* [24, 26].

The fish were collected on board the research boat *Albacora* of the Oceanographic Institute of São Paulo University using a bottom otter trawl (1.6” mesh wall, 1.2” mesh cod end, and 11 m length) in sampling periods lasting 10 minutes each, and trawling at a mean depth of 6 m at 3 mph. Catfish were separated from the other fish, and immediately taken to the laboratory so that biometric data could be taken (total length=TL, total weight=TW, and body weight=BW) and for dissection, so that the livers and gills could be extracted and fixed for histopathological procedures. Using the biometric data, the isometric condition factor (CF_{iso}) was calculated using the expression CF_{iso}=TW)/TL^3*100 [26]. In the laboratory, the catfish were identified [14, 27], considering the main characteristics of the occipital process of the cranium and dentition. Since the catfish use all estuary along their lifetime [28, 29], *G. genidens* and *C. spixii* collected in sites 1 and 2 were grouped e analyzed with respect effects on the estuarine system.

All collected and analyzed *C. spixii* and *G. genidens* specimens were authorized by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade [Sisbio processes nº 20398-1; 63087-1]) following the recommendations of the Animal Ethics Committee of the Federal University of São Paulo (CEUA-Unifesp, n°

2438280618). The voucher specimens MZUSP-48529 and MZUSP-49319 are deposited in the ichthyological collection of the Museu de Zoologia da USP.

Histological procedures

Liver samples were processed and stained for routine histology. Still in the field, immediately after fish sampling and dissection, the obtained liver sections were preserved in ALFAC fixative solution (80% ethanol, 37–40% formaldehyde, and glacial acetic acid) for 16 h and stored in 70% ethanol for future procedures in the laboratory. They were dehydrated in a graded series of ethanol baths, embedded in Paraplast-Plus resin (Sigma©), and two slides with five-micrometer-thick sections were obtained.

For general histological examinations, one slide of each fish was stained with Harris Hematoxylin-Eosin. For hemosiderin analysis, the duplicate slide was stained with Perls (Prussian blue). Subsets of slides were scored independently to check for observer bias. The slides were also processed in batches containing controls and treatments to eliminate staining artefacts. All qualitative and semi-quantitative analyses were made under an optical microscope (200x and 400x magnification) and photographs were taken using ZEN2011 software in the Zeiss-Axiophot photomicroscope.

The hepatic lesion index was calculated considering the importance and extension of the observed pathologies [6]. For the diagnosis of histopathological lesions, four grades of lesions were established: no anomaly detected (0), minimal (1), moderate (2), and marked pathological importance (3), which indicated that more than 75% of the tissue was taken over by the injury. Besides the lesion's extent, three levels of pathological importance (importance factor) were considered: minimum (1), moderate (2), and great pathological importance (3). Based on the scores, median values were calculated per sampling site following the expression:

$$I_{org} = \sum pr \sum alt\ (a \times w)$$

where I_{org} = organ lesion index; pr = reaction pattern; alt = change; a = change score; and w = importance factor.

The semi-quantitative analysis of hemosiderin in the liver tissues was conducted by viewing the degree of blue color extension in relation to total hepatic tissue area as an indication of the iron distribution within it. This varied from 0 to 3: not detected (0); minimum, up to 25% of the tissue (1); moderate, between 25 and 75% (2); and larger extension deposition, greater than 75% (3). Each tissue was analyzed for the entire length of the permanent slide.

To avoid false positive histological data and, therefore, to ensure the reliability of the analysis considering the different steps taken in the procedures to obtain the permanent slides, only samples without artifacts were used. Thus, slides with artifacts that would compromise the results were discarded.

Statistical analysis

Biometric data and the isometric condition factor (CF_{iso}) are expressed in mean values and standard deviation. The normality of the data was checked using the Shapiro-Wilk test. Since no normal distribution was observed, nonparametric analysis of variance (ANOVA) was applied. Therefore, the Kruskal-Wallis test with Dunn's multiple comparisons was used to check differences concerning the biometric data and CF_{iso} between years and catfish species.

Concerning the histopathological confidence, all observations of the permanent slides were analyzed three times, with a minimum interval of 30 days each. From these results, the error was calculated using the formula:

$$E = \left(\frac{fo - io}{io}\right) \times 100$$

where E = error calculation; io = initial observation; and fo = final observation. Considering the proposed formula, only results with an error of less than 20% were used in the analyses.

The liver and gill pathology and hemosiderin data are shown in occurrence tables for each, regarding catfish species and year. The statistical differences were tested using the Chi-square (χ^2) and Mann-Whitney test using Sigma plot 12.0 and Past 4.02 software. The confidence interval used for all tests was 95% ($p < 0.05$).

RESULTS

The 2014, 2017, and 2018 samples revealed the distinct environmental properties in the northern (Iguape) and southern (Cananéia) sections of the CIELC, with intermediate conditions present in the center of the system. The northern section presented the lowest values of salinity (0 – 15.6), while the southern section presented the highest values of salinity (22.5 – 30.6); this is due to freshwater input from the Ribeira de Iguape River and the more expressive seawater movement resulting from the tides, respectively. There were no significant differences between the TL and TW measures in individual *C. spixii* catfish from 2014 and 2017. The smallest and lightest individuals were sampled in 2014 (TL = 17 ± 4 cm; TW = 53 ± 47 g) and 2017 (TL = 18 ± 4 cm; TW = 68 ± 71 g), while the largest and heaviest individuals were sampled in 2018 (TL = 28 ± 8 cm; TW = 259 ± 181 g), showing a statistically significant difference between the other years ($p < 0.001$). Regarding the CF of the *C. spixii* individuals, 2017 was the only year with a significant difference ($p < 0.001$); that is, a higher value than the other years ($CF_{2014} = 0.86 \pm 0.11$; $CF_{2017} = 1.01 \pm 0.24$; $CF_{2018} = 0.92 \pm 0.16$) (Table 1). With respect to *G. genidens*, the TL of these individuals was significantly different in all the years ($p < 0.001$), with the smallest individuals found in 2017 (TL = 13 ± 1 cm), followed by individuals from 2014 (TL = 17 ± 2 cm). The largest individuals were found in 2018 (TL = 23 ± 4 cm). Regarding weight, there was no significant difference between the years 2014 (TW = 37 ± 13 g) and 2017 (TW = 20 ± 6 g), while individuals with the highest weights (TW= 110 ± 62 g) were caught in 2018, showing a significant difference between the years ($p < 0.001$). Regarding the CF_{iso} of *G. genidens*, there was only a statistically significant difference between the years 2014 and 2017 ($p < 0.001$); that is, a greater value in 2017 ($CF_{2014} = 0.76 \pm 0.05$; $CF_{2017} = 0.87 \pm 0.08$; $CF_{2018} = 0.80 \pm 0.09$) (Table 1). Regarding the areas, no statistically significant difference was observed for both *C. spixii* and *G. genidens* ($p>0.05$).

Table 1. Biometric data (mean ± standard deviation) of the two catfish species (*Cathorops spixii* and *Genidens genidens*) in the Cananéia-Iguape Estuarine-Lagoon Complex (CIELC).

Species	**Year**	**TL (cm)**	**TW (g)**	**CF_{iso}**
C. spixii	2014	17 ± 4^{a}	54 ± 47^{a}	0.86 ± 0.11^{b}
	2017	18 ± 4^{a}	68 ± 71^{a}	1.01 ± 0.24^{c}
	2018	28 ± 8^{b}	259 ± 18^{b}	0.92 ± 0.16^{bc}
G. genidens	2014	17 ± 2^{a}	37 ± 13^{a}	0.76 ± 0.05^{a}
	2017	13 ± 1^{b}	20 ± 6^{a}	0.87 ± 0.08^{b}
	2018	23 ± 4^{c}	110 ± 6^{b}	0.80 ± 0.09^{ab}
Species	**Sector**	**TL (cm)**	**TW (g)**	**CF_{iso}**
C. spixii	Northern	17 ± 6^{a}	65 ± 83^{a}	0.84 ± 0.12^{b}
	Southern	23 ± 8^{ab}	58 ± 165^{ab}	0.96 ± 0.19^{bc}
G. genidens	Northern	19 ± 5^{a}	65 ± 56^{a}	0.79 ± 0.08^{a}

(TL) Total length; (TW) total weight; (CF_{iso}) isometric condition factor. Different letters indicate statistically significant differences with respect to the same biological parameter (ANOVA, Kruskal-Wallis, $p < 0.05$). *Considering the sampling periods, no *G. genidens* were found in the southern sector.

With respect to histological assay and validation, the error of the observations was obtained, as this study is a qualitative and semi-quantitative analysis. The mean error between the analyses performed for the liver and gill tissues was 18% and 19%, respectively. Regarding the hemosiderin analyses, the error between the analyses was 15%. This value was lower than that proposed in the methodology (20%) to ensure that the subjective nature of the analyses did not impact the data obtained.

The main histopathological changes observed in liver of the ariids are shown in Figure 2. The most frequent pathological changes found in the livers of *C. spixii* (n = 93) and *G. genidens* (n = 26) were necrosis (*C. spixii*: 57%; *G. genidens*: 54%), inflammation (*C. spixii*: 12%; *G. genidens*: 31%), hemorrhage (*C. spixii*: 8%; *G. genidens*: 4%), cytoplasmatic vacuolization (*C. spixii*: 18%; *G. genidens*: 15%), and pyknosis nuclei (*C. spixii*: 3%; *G. genidens*: 65%). The data on the occurrence of the main liver changes observed in *C. spixii* and *G. genidens* can be seen in Table 2.

For the three years in which this species was collected, necrosis had the highest occurrence (2014: 67%; 2017: 53%; and 2018: 54%). There was a statistically significant difference in the presence of hemorrhage between the three years ($\chi^2 = 6.645$; $p = 0.036$); the greatest values were in fish collected in 2014 (19%). The change in the cytoplasmatic vacuolization type was also statistically significant ($\chi^2 = 14.783$; $p = 0.001$); this increased over the years and reached its highest value in 2018 (42%). Changes such as necrosis, inflammation, and the presence of pyknosis nuclei did not show statistically significant differences between the years of collection. The data on the occurrence of the main liver changes observed in *G. genidens* can be seen in Table 2. Regarding this species, for the three years of collection, the most frequent changes were necrosis (2014: 57%; 2017: 71 %; and 2018: 20%) and pyknosis nuclei (2014: 86%; 2017: 43%; and 2018: 40%). Changes in the type of hemorrhage were only detected in 2018 (20%), and there was no statistically significant difference between the three years. The change in inflammation was not statistically significant between the years (2014: 36%; 2017: 43%; and 2018: 0%). Finally, cytoplasmatic vacuolization was the only change that was statistically significant between the three years of collection (χ^2=9,649; p=0.008); the highest values were recorded in fish from 2018 (60%).

Table 2. Occurrence (%) of the main liver changes observed in *Cathorops spixii* and *Genidens genidens* in the Cananéia-Iguape Estuarine-Lagoon Complex *(*CIELC*)*.

Species	**Year**	**Necrosis**	**Inflammation**	**Hemorrhage**	**Vacuolization**	**Pyknosis nuclei**
C. spixii	2014	67^{a}	15^{b}	19^{b}	4^{d}	4^{d}
	2017	53^{ab}	8^{c}	3^{c}	13^{c}	5^{d}
	2018	54^{ab}	15^{b}	4^{c}	42^{b}	0
G. genidens	2014	57^{a}	36^{a}	0	7^{d}	86^{a}
	2017	71^{a}	43^{a}	0	0	43^{c}
	2018	20^{c}	0	20^{a}	60^{a}	40^{c}
Species	**Sector**	**Necrosis**	**Inflammation**	**Hemorrhage**	**Vacuolization**	**Pyknosis nuclei**
C. spixii	Northern	50^{ab}	20^{b}	25^{ab}	0	0
	Southern	63^{a}	22^{b}	30^{a}	0	3^{d}
G. genidens	Northern	54^{ab}	50^{a}	23^{a}	0	62^{b}

To the same pathology, different letters indicate statistical differences (Chi-square, p <0.05). *Considering the sampling periods, no *G. genidens* were found in the southern sector.

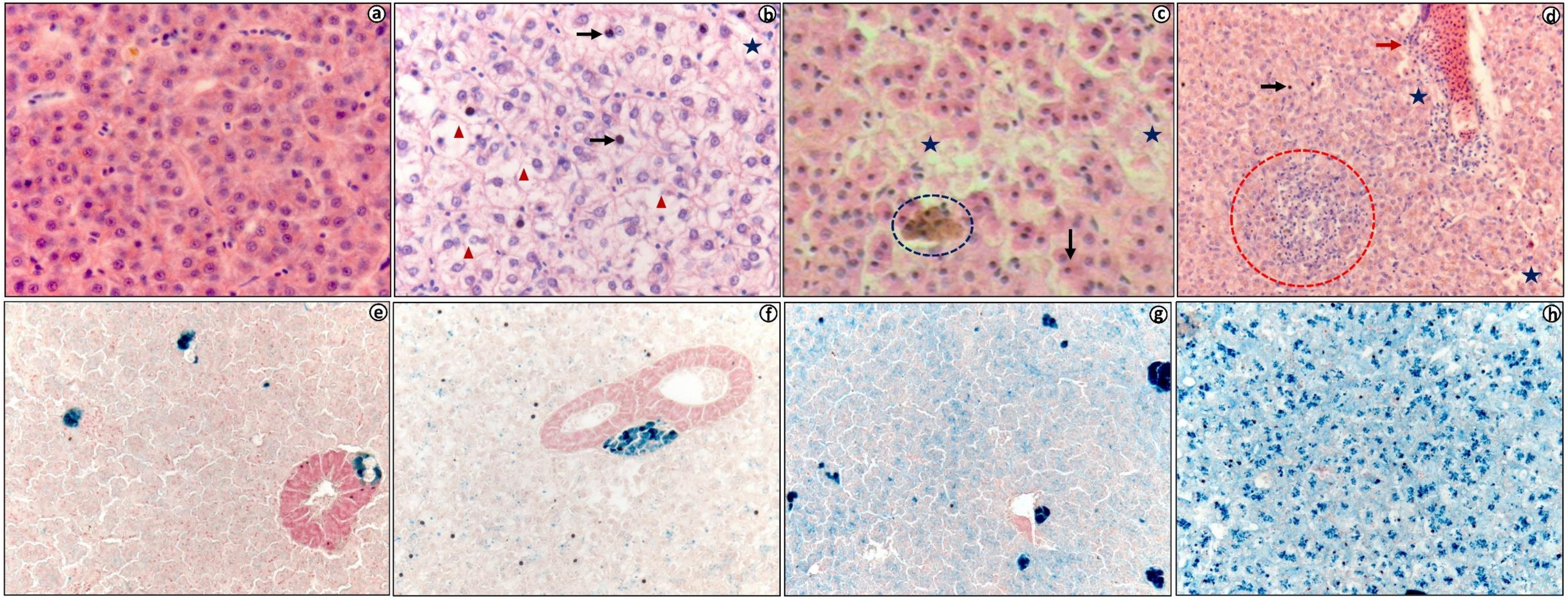

Figure 2. Cross section of the liver tissue of *Cathorops spixii* and *Genidens genidens* collected in the CIELC. Normal liver area (a) and hepatic histopathological changes (b-d), besides hemosiderin grades (e: undetected; f: minimum; g: moderate, h: large). vacuolization (▲); pyknosis nuclei (➞); necrotic area (★); melanomacrophage (◌);inflammatory cell infiltrate (◌); and blood vessel with hemorrhagic (➞). 200x magnification.

Based on the identification of the most significant changes in the liver tissue of *C. spixii* and *G. genidens* catfish, the injury index was calculated according to Bernet and coauthors (1999) [6]. In general, the liver injury index for *C. spixii* remained the same among the sample years (I_{Liver} 2014: n = 27, Mdn = 3, $Q_{1/4}$ = 0, $Q_{3/4}$ = 4; I_{Liver} 2017: n = 40, Mdn = 3, $Q_{1/4}$ = 0, $Q_{3/4}$ = 3.7; I_{Liver} 2018: n = 26, Mdn = 3, $Q_{1/4}$ = 0.7, $Q_{3/4}$ = 4), with a median of 3 and no statistically significant temporal differences (Kruskal-Wallis H = 1.683; p = 0.431). In turn, for *G. genidens* catfish, higher injury index values were observed for fish collected in 2014 (I_{Liver} 2014: n = 14, $\dot{X}$ = 71 ± 3.79), with a reduction for the years of 2017 and 2018 (I_{Liver} 2017: n = 7, $\dot{X}$= 4.14 ± 0.9; I_{Liver} 2018: n = 5, $\dot{X}$ = 3.60 ± 4.50). The liver injury index for the species *G. genidens* for 2014 was the only one that showed a statistically significant difference in relation to the other years of collection (ANOVA p = 0.033; Duncan's method p < 0.05) (Figure 3).

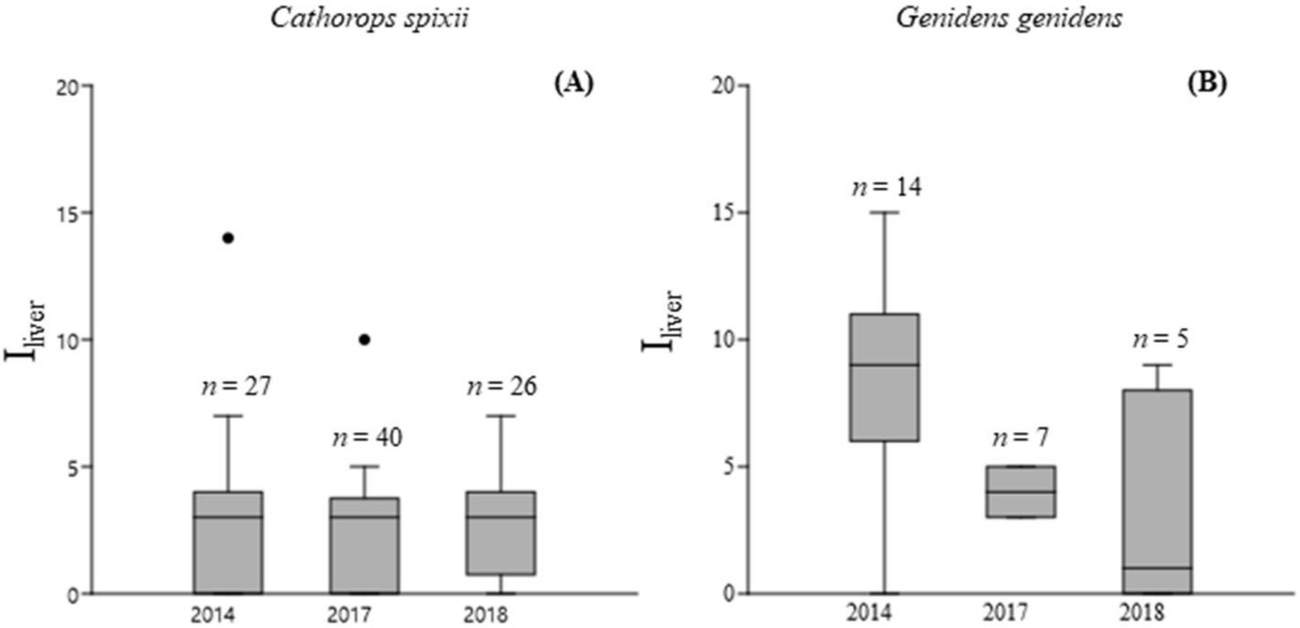

Figure 3. Boxplot indicating the values of the liver injury index in *C. spixii* (A) and *G. genidens* (B). The data are presented in boxes with limits that indicate the first and third quartiles; the inner line marks the median value; the mustache strands above and below indicate the upper and lower limits; outliers are shown as black dots; n = sample number.

Regarding the liver injury index among the fish species collected in the CIELC, without considering the temporal variation, the species *C. spixii* (n = 93, Mdn = 3, $Q_{1/4}$ = 0, $Q_{3/4}$ = 4) showed lower values compared to the species *G. genidens* (n = 26, Mdn = 5.5, $Q_{1/4}$ = 3.7, $Q_{3/4}$ = 9). There were statistically significant differences in relation to the rate of liver injury between catfish species (Mann-Whitney T= 2246; p < 0.001). Despite the low sample number, *G. genidens* from the northern area of the CIELC shown more hepatic damage than *C. spixii* from both northern and southern sector (Figure 4). However, statistically significant differences were no found (p>0.05).

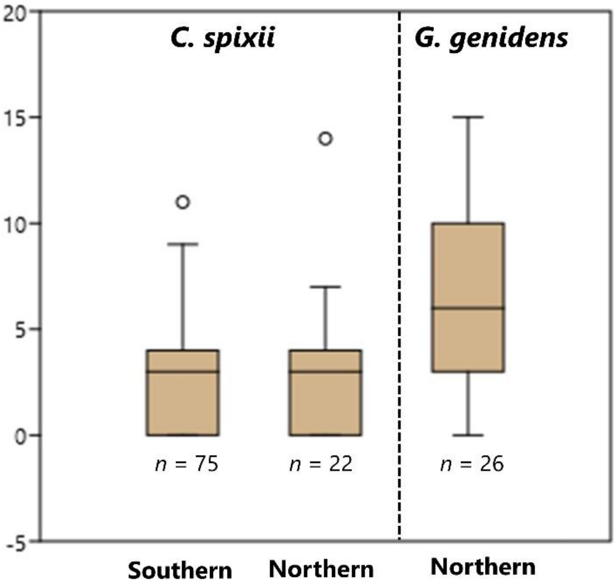

Figure 4. Boxplot of the liver injury index in *C. spixii* and *G. genidens* collected in the northern and southern areas of the CIELC. The data are presented in boxes with limits that indicate the first and third quartiles; the inner line marks the median value; the mustache strands above and below indicate the upper and lower limits; outliers are shown as black dots.

A semi-quantitative analysis of the hemosiderin accumulation in the liver tissues of *C. spixii* and *G. genidens* fish was performed using the samples submitted to Perls staining. The hemosiderin observed in the livers of *C. spixii* and *G. genidens* indicates the four levels of hemosiderin analyzed in the tissue; these range from 0 (undetected) to grade 3 (large extent, with the presence of hemosiderin in more than 75% of the tissue).

Regarding the hemosiderin analyses (Table 3), *C. spixii* from 2017 showed more minimum (43%) and moderate (40%) hemosiderosis. However. C. fish collected in 2018 had less hemosiderosis (minimum grade = 64%). The same profile was observed to G. genidens, since catfish caught in both 2017 and 2018 had a major occurrence of minimum hemosiderosis (2017 = 57%, and 2018 = 50%). No statistically significant differences between the years were found (*C. spixii* - Mann-Whitney T = 1264; *p* = 0.162; *G. genidens* - Mann-Whitney T = 51.50; *p* = 0.181).

Table 3. Occurrence (%) of hemosiderin grade in the liver tissue of *Cathorops spixii* and *Genidens genidens* catfish species in the Cananéia-Iguape Estuarine-Lagoon Complex (CIELC).

	year	**0 (undetected)**	**1 (minimum)**	**2 (moderate)**	**3 (large)**
C. spixii	2017	5	43	40	13
	2018	3	64	25	8
G. genidens	2017	29	57	0	14
	2018	0	50	33	17

DISCUSSION

Biomonitoring studies of the aquatic environment are useful for assessing environmental quality; such studies implement strategies that use biological parameters to obtain environmental information. The CF is a practical quantitative measure of fish welfare that reveals recent feeding conditions; the results suggest that in 2017 environmental feeding conditions were better utilized by the catfish species [26]. In the years 2014 and 2017, *C. spixii* fish did not show differences in their biometric parameters; however, when analyzing the CF, it was noted that in the year 2017 the value was higher. In 2018, although *C. spixii* fish obtained higher biometric values compared to previous years, the CF was lower, which indicates the lower availability of resources or less use of the environment by these fish. The CF for *G. genidens* also increased in 2017, but the biometric values of these fish decreased in that year and increased in 2018, when they reached the highest value. For both species of fish, the year 2017 promoted greater well-being (CF), indicating greater use of environmental resources by these fish compared to other years. The values referring to the CF of all the years in the present study were higher than that found by other authors studying fish of polluted estuarine region [30]. It is possible that the presence of contaminants at the site may have interfered with the biometric factors; however, these CF values may be due to differences between the species examined in their study.

The investigation of histological changes is an effective tool that allows access to the health status of fish, and it serves as an advance warning of the possible environmental risks these fish are subject to; thus, histological changes can predict long-term adverse effects, allowing preventive measures to be taken before the effects become irreversible [31]. Necrosis is a serious change taking the form of an irreversible lesion that can be caused by several abiotic factors in addition to the presence of chemical, pesticide, and metal contaminants in the water [5]. The data from the present study suggests that the high prevalence of this lesion (60%) in *C. spixii* and *G. genidens* fish is like that found in places with great environmental impacts [4, 30, 32]. The occurrence of necrosis in *C. spixii* fish in the present study did not vary between years, fluctuating at rates of around 60% in the analyzed fish. Azevedo and coauthors [4] obtained similar results for the occurrence of necrosis as the results of the present work: the authors used *C. spixii* fish to assess the influence of anthropogenic activities in Santos (SP), Brazil, a region in which port, industrial, and tourism activities take place; it is also home to urban centers that contribute to the entry of contaminants into the site. The data suggesting the occurrence of necrosis in the fish in the present study could be explained by exposure to contaminants in the CIELC aquatic compartment; for example, the levels of metals such as lead - which occurs in concentrations of approximately 80 mg Kg^{-1} in the northernmost regions - in the sediment of the estuary [2]. However, the distribution of trace metals in sediments is not homogeneous: the distribution is greater in the northern sector of the CIELC due to the historical presence of mining in the highlands of the Ribeira de Iguape River valley, but it does not reach toxic levels [33].

Inflammation, besides representing a bodily defense mechanism, can also be associated with necrosis, since phagocytes remove dead cells from tissue. This may explain the occurrence of inflammation in *G. genidens* fish, whose values for inflammation were slightly below those for the occurrence of necrosis. However, the same is not the case with *C. spixii* fish, whose inflammation prevalence values were far below those for necrosis. Hemorrhage, on the other hand, is a lesion that is usually associated with necrosis, as the extravasation of red blood cells can occur near blood vessels. The highest occurrence of hemorrhage found for *C. spixii* fish was in 2014 (19%); however, the values found for both species in this study were low compared to those in a study by Katsumiti and coauthors [3], in which the authors collected *C. spixii* catfish after the accident involving the Vicuña oil tanker in Paranaguá Bay (PR), Brazil, in 2004. This suggests that the high rates of hemorrhage in their study were the result of the area being contaminated with pollutants after the accident.

There were high values for the occurrence of cytoplasmic vacuolization in both species in 2018 (*C. spixii*: 42%; *G.genidens*: 60%). These values are like those found by other authors that investigated the lesions in the livers of *Labrisomus philippii* fish collected in the Bay of São Jorge, Chile, a place characterized by high levels of trace metals and metalloids (copper, zinc, cadmium, and arsenic) from port activities and sewage discharges [32]. However, similar values for the occurrence of cytoplasmic vacuolization were found in the livers of *C. spixii* in the control group of the study carried out by Katsumiti and coauthors [3] in Paranaguá Bay (PR), Brazil. The CIELC is an area characterized by significant differences in salinity: the northern regions are subject to great fluvial influence - with salinity between 0 and 5 ppm close to Site 2, while there are regions with great maritime influence - with salinities reaching values of up to 35 ppm [1]. This great variability in salinity can induce physiological stress in fish subject to these variations, causing imbalances that may be related to the formation of cytoplasmic vacuoles in the liver [34]. Thus, the higher occurrence of vacuolization in the livers of the fish in 2018 may be associated with the greater availability of contaminants in the water in this particular year as well as with variations in homeostatic balance resulting from variations in salinity. For *C. spixii* fish, the prevalence of this change increases over the years of collection.

The occurrence of pycnotic nuclei was considerably higher in *G. genidens* than in *C. spixii*. The occurrence value of pyknosis found for *C. spixii* fish was lower than found when studying the same fish collected in Santos (SP), Brazil, a location with great anthropogenic interference [4]. This anomaly may be associated with an increase in the metabolic activity of fish cells upon exposure to stressors; however, it can also be observed in the liver tissue of females during the reproductive period. This last explanation is discarded since the fish were collected outside the reproductive period, which occurs between spring and autumn [18].

The lesion index is configured as a quantitative approach to the histopathological changes observed qualitatively; this allows for an understanding to be gained of the severity of the changes regarding their pathological importance and dimensions within the analyzed tissue. The values found for the injury index in *C. spixii* fish did not vary between the years of collection. These results were lower than those found in a study carried out in Paranaguá Bay (PR), Brazil, in which they used the same species of fish to assess the anthropogenic impact on this region [35], which is characterized mainly by port and industrial activities and where urban waste represents a great source of water contamination. This value is also like the value found in *C. spixii* fish in the Santos-São Vicente Estuary - more specifically, in Santos Bay - which is a location less impacted by industrial activities but with intense marine hydrodynamic influence [4]. This suggests that the CIELC fish were under more severe stress in 2014 because of anthropogenic pressures or natural environmental stressors such as marine hydrodynamics. In subsequent years, the liver injury index values for *G. genidens* fish were lower and similar to the levels found in *C. spixii* fish.

Without considering the temporal analysis, the lesion indexes of the two species show higher values for *G. genidens* compared to *C. spixii*. In the northern sector of the CIELC, local sediments contain trace metals such as zinc, copper, and lead due to local anthropogenic influences and the presence of urban centers [2]. Thus, the higher rate of injury found in *G. genidens* fish can be explained by the restriction of this species to regions further north of the estuary, which have higher levels of contaminants in the water [2]. However, this result should be interpreted carefully, because due to the low sample size of the *G. genidens* species (n = 26), this result may not reflect the true condition of the population in the CIELC.

Concerning the hemosiderin found in the livers of *C. spixii* and *G. genidens*, the minimum values for iron distribution were found in 2017 and 2018; these values represent healthy results because hemosiderin, under normal conditions, is found in small amounts in this organ. However, moderate occurrences were also found in *C. spixii* fish in two years of collection (2017: 40%; 2018: 25%) and in *G. genidens* fish in 2018 (33%), indicating that some fish showed a greater accumulation of hemosiderin in their livers, with no difference between the years of collection. The accumulation of hemosiderin in the livers of fish may be related to the occurrence of vacuolization in *C. spixii* fish in 2017 (13%) and 2018 (42%) and in *G. genidens* fish in 2018 (60%).

Histological changes in fish livers have been found in studies showing a strong relationship between the presence of contaminants and the occurrence of pathologies [5, 36]. Therefore, the occurrence of serious injuries such as necrosis - as found in catfish livers in the present study—may be related to the presence of contaminants, xenobiotic metabolism, and the presence of trace metals such as zinc, copper, and lead in the CIELC [4]. Both vacuolization and pycnotic nuclei can be the result of an increase in metabolic expenditure resulting from an accentuated hydrodynamic process in the estuary, which is subject to a semi-diurnal tide regime; thus, this increased stress may be due to natural environmental stressors such as variations in salinity.

Catfishes from the CIELC show evidence of histopathological damage in their livers, and the examination of this damage is an efficient tool by which to reveal the health of these animals. However, no significant differences were observed concerning the temporal approach. The reversible changes observed in the livers (i.e., vacuolization) of both catfishes' species may be related to anthropogenic disturbances of the estuary, such as salinity inputs or metals contents mainly in the norther area [2, 19, 33]. However, we reinforce the importance of chemical analysis in catfishes from CIELC to corroborate it. Concerning hemosiderin, since it was found at mostly minimal and moderate levels, its presence may be related to the occurrence of some reversible lesions in the liver tissue.

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REFERENCES

1. Barcellos RL, Camargo PB, Galvão A, Weber RR. Sedimentary organic matter in cores of the Cananéia-Iguape lagoonal-estuarine system, São Paulo State, Brazil. J. Coast. Res. 2009 Jan; 56: 1335-9.
2. Tramonte KM, Figueira RCL, Lima Ferreira PA, Ribeiro AP, Batista MF, Mahiques MM. Environmental availability of potentially toxic elements in estuarine sediments of the Cananéia-Iguape coastal system, Southeastern Brazil. Mar. Pollut. Bull. 2016 Feb; 103(1–2): 260–9.
3. Katsumiti A, Domingos FXV, Azevedo M, Silva MD, Damian RC, Almeida MIM, et al. An assessment of acute biomarker responses in the demersal catfish *Cathorops spixii* after the Vicuña Oil Spill in a harbour estuarine area in Southern Brazil. Environ. Monit. Ass. 2009 May; 152(1–4):209–22.
4. Azevedo JS, Braga ES, Silva de Assis HC, Oliveira Ribeiro CA. Biochemical changes in the liver and gill of *Cathorops spixii* collected seasonally in two Brazilian estuaries under varying influences of anthropogenic activities. Ecotoxicol. Environ. Saf. Oct; 2013 96:20–30.

5. Norregaard RD, Dang M, Bach L, Geertz-Hansen O, Gustavson K, Aastrup P, et al. Comparison of heavy metals, parasites and histopathology in sculpins (*Myoxocephalus* spp.) from two sites at a lead-zinc mine in North East Greenland. Environ. Res. Jan; 165: 306–16.
6. Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T. Histopathology in fish: Proposal for a protocol to assess aquatic pollution. J. Fish Dis. 1999 Dec; 22(1): 25–34.
7. Yancheva V, Velcheva I, Stoyanova S, Georgieva E. Histological biomarkers in fish as a tool in ecological risk assessment and monitoring programs: A review. Appl. Ecol. Environ. Res. 2016 Jan; 14(1): 47–75.
8. Azevedo JS, Sarkis JES, Hortellani MA, Ladle RJ. Are Catfish (Ariidae) effective bioindicators for Pb, Cd, Hg, Cu and Zn? Water Air Soil Pollut. 2012 May; 223(7): 3911–22.
9. Azevedo JS, Fernandez WS, Farias LA, Fávaro DTI, Braga ES. Use of *Cathorops spixii* as bioindicator of pollution of trace metals in the Santos Bay, Brazil. Ecotoxicol. 2009 Jul; 18(5):577–86.
10. Braga ES, Azevedo JS, Kuniyoshi L, Fávaro DIT. Zn, Co, Cr, As, and genotoxic effects in the ichthyofauna species from polluted and non-polluted/protected estuaries of the São Paulo Coast, Brazil. An. Acad. Bras. Cienc. 2019 Mar;91(4):1–18.
11. Pecoraro GD, Hortellani MA, Hagiwara YS, Braga ES, Sarkis JE, Azevedo JS. Bioaccumulation of Total Mercury (THg) in Catfish (Siluriformes, Ariidae) with Different Sexual Maturity from Cananéia-Iguape Estuary, SP, Brazil. Bull. Environ. Contam. Toxicol. 2019 Nov;102(2):175–9.
12. Amaral TF, Miyasaki FH, Braga ES, Azevedo JS. Temporal and spatial toxicogenetic damage in estuarine catfish *Cathorops spixii* from a marine protected area with evidence of anthropogenic influences. Sci. Total Environ. 2021 Dec; 799:149409.
13. Angeli JLF, Trevizani TH, Ribeiro A, Machado EC, Figueira RCL Market B, et al. Arsenic and other trace elements in two catfish species from Paranaguá Estuarine Complex, Paraná, Brazil. Environ. Monit. Assess. 2013 Apr; 185(10):333–42.
14. Figueiredo JL, Menezes NA. [Manual of marine fish from southeastern Brazil. II. Teleostei (1)]. São Paulo: Museu de Zoologia da Universidade de São Paulo; 1978.
15. Schimidt TCS, Martins IA, Reigada ALD, Dias JF. [Taxocenosis of marine catfish (Siluriformes, Ariidae) from the São Vicente estuarine region, SP, Brazil]. Biota Neotrop. 2008 Dec; 8(4):73–81.
16. Fávaro LF, Frehse FA, Oliveira RN, Schwarz Júnior R. [Reproduction of the Madamango sea catfish, *Cathorops spixii* (Agassiz) (Siluriformes, Ariidae), from Pinheiros Bay, an estuarine region off the coast of Paraná, Brazil]. Rer. Bras. Zool. 2005 Dec; 22(4): 1022–9.
17. Wakabara Y, Flynn MN, Tararam AS. Ingestion and selection of suprabenthic crustaceans by small-sized fishes in a lower saltmarsh system. Rev. Bras. Oceanogr. 1996 Oct; 44(2): 89–103.
18. Gomes ID, Araújo FG, Azevedo MCC, Pessanha ALM. [Reproductive biology of marine catfish Genidens genidens (Valenciennes) and Cathorops spixii (Agassiz) (Siluriformes, Ariidae), in Sepetiba Bay, Rio de Janeiro, Brasil]. Rev. Bras. Zool. 1999 Jun; 16(suppl 2): 171–80.
19. Azevedo JS, Braga ES, Fávaro DT, Perretti AR, Rezende CE, Souza CMM (2011) Total mercury in sediments and in Brazilian Ariidae catfish from two estuaries under different anthropogenic influence. Mar. Pollut. Bull. 2011 Dec; 62(12): 2724–31.
20. Morais IS, Azevedo JS. [The use of microanalysis in catfish otolith bioindicators in environmental biomonitoring studies]. Biota Amaz. 2017; 7(2): 65-77. https://doi.org/10.18561/2179-5746/biotaamazonia.v7n2p65-77
21. Azevedo JS, Vaz-dos-Santos AM, Perin S, Braga ES, Rossi-Wongtschowski CLDB. *Cathorops spixii* (Agassiz 1829) at the Cananeia-Iguape Estuarine system. In: Vaz-Dos-Santos AM, Rossi-Wongtschowski CLDB. (eds.). Growth in fisheries resources from the Southwestern Atlantic, São Paulo: Instituto Oceanográfico – USP; 2019. 68-70.
22. Vaz dos Santos AM, Azevedo JS, Perin S, Braga ES, Rossi-Wongtschowski CLDB. *Cathorops spixii* (Agassiz 1829) at the Santos-São Vicente Estuarine System. In: Vaz-Dos-Santos AM, Rossi-Wongtschowski CLDB. (eds.). Growth in fisheries resources from the Southwestern Atlantic, São Paulo: Instituto Oceanográfico – USP; 2019. 60-64.
23. Aguiar VMC, Braga ES. Transport of salt, phosphorus and suspended particulate material in the Cananéia Lagoon-estuarine System, São Paulo, Brazil. 4th International Symposium of Environmental Geochemistry of Tropical Countries; 2004.
24. Contente RF. [Ecological and multidecadal patterns of the ichthyofauna of the Cananéia-Iguape estuary]. [Doctor’s thesis]. São Paulo: Universidade de São Paulo, Instituto Oceanográfico; 2013. 146f. https://www.teses.usp.br/teses/disponiveis/21/21131/tde-19052014-181026/publico/TESE_Riguel_Feltrin_Contente.pdf
25. Mishima M, Tanji S. [Environmental factors related to the distribution and abundance of catfish (Osteichthyes, Ariidae) in the Cananéia Estuarine Lagoon Complex (25ºs; 48ºw)]. Bol. Inst. Pesca. 1983; 10:17-27.
26. Vazzoler AEAM. [Reproductive biology of teleost fish: theory and practice]. Maringá: EDUEM; 1996.
27. Marceniuk AP. [Identification key for marine catfish species (Siluriformes, Ariidae) from the Brazilian coast]. Bol. Inst. Pesca, 2005 Oct; 31(2): 89-101.
28. Mishima M, Tanji S. [Food niche of marine catfish (Teleostei, Ariidae) in the Cananéia Lagoon Estuarine Complex (25ºs, 48ºw)]. Bol. Inst. Pesca 1982; 9:131-40.

29. Rios EP. [Role of the estuary in the life cycle of the dominant species of the ichthyofauna of the Cananeia-Iguape Estuarine-Lagoon Complex]. [Doctor’s thesis]. São Paulo: Universidade de São Paulo, Instituto Oceanográfico; 2001. 128f.
30. Oliveira Ribeiro CA, Katsumiti A, França P, Maschio J, Zandoná E, Cestari MM, et al. Biomarkers responses in fish (*Atherinella brasiliensis*) of paranaguá bay, southern Brazil, for assessment of pollutant effects. Braz. J. Oceanogr. 2013 Mar; 61:1–11.
31. Van Der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ. Toxicol. Pharmacol. 2003 Feb; 13(2): 57–149.
32. Montenegro D, González MT. Evaluation of somatic indexes, hematology and liver histopathology of the fish Labrisomus philippii from San Jorge Bay, northern Chile, as associated with environmental stress. Rev. Biol. Mar. Oceanogr. 2012 Apr; 47(1): 99–107.
33. Chiozzini VG. [Study of abundance and provenance and elemental distribution patterns in surface sediments and sedimentary profiles in the Cananeia-Iguape estuarine-lagoon complex, São Paulo-Brazil, with emphasis on rare earth elements and their environmental significance]. [Doctor’s thesis]. São Paulo, Universidade de São Paulo, Instituto Oceanográfico; 2017. 414f. doi: 10.11606/T.21.2018.tde-19032018-153950
34. Sterzelecki F, Rodrigues E, Fanta E, Ribeiro C. The effect of salinity on osmoregulation and development of the juvenile fat snook, *Centropomus parallelus* (POEY). Braz. J. Biol. 2013 Aug; 73(3): 609–15.
35. Domingos FXV, Assis HCS, Silva MD, Damian RC, Almeida AIM, Cestari MM, Randi MAF, Oliveira Ribeiro CA (2009) Anthropic Impact Evaluation of Two Brazilian Estuaries Trough Biomarkers in Fish. J. Braz. Soc. Ecotoxicol. 2009; 4(1–3): 21–30. doi: 10.5132/jbse.2009.01.004
36. Saleh YS, Marie MAS. 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. 2016 May; 106(1–2): 308–22.

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