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Alterations in Morphometric and Organosomatic Indices and Histopathological Analyses Indicative of Environmental Contamination in Mullet, *Mugil liza*, from Southeastern Brazil

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Abstract Mullet (*Mugil liza*) were sampled in five different areas along the Guanabara Bay, southeastern Brazil, classified as non-contaminated, moderately contaminated and contaminated. Morphometric (Fulton condition factor, relative condition factor and weight to length scaling coefficient) and organosomatic (hepatosomatic index) indices of environmental stress were analysed. Fish from the differentially contaminated areas show statistically different Fulton and relative condition factors and hepatosomatic indices, but not the weight to length scaling coefficient. The Kn and the FCF followed the same trend, with fish from São Gonçalo (1.07 ± 0.04 and 0.89 ± 0.03), Itaipu (0.84 ± 0.01 and 0.86 ± 0.01) and the Rodrigo de Freitas Lagoon (1.03 ± 0.01 and 0.87 ± 0.20) showing higher FCFs than fish from Magé (0.96 ± 0.01 and 0.81 ± 0.01). Fish from Itaipu showed significantly higher HSI values than the other sampling sites (1.68 ± 0.07), with fish from Olaria and Ipiranga showing the lowest (1.56 ± 0.12 and 1.60 ± 0.07, respectively).

Keywords Stress indices · Mullet · Histopathology · Environmental contamination

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Introduction

Fish are useful biomarkers regarding environmental contamination (Espino 2000). Several studies (Laflamme et al. 2000; Norris et al. 2000; van der Oost et al. 2003) indicate that fish exposed to environmental contamination show changes in morphological parameters that determine and describe environmental interference, i.e. the Hepatosomatic Index (HSI), that identifies liver disorders and hepatic stress and is a measure of fish energy reserves; the Fulton condition factor (FCF), that evaluates general fish health, the recently introduced weight to length scaling coefficient (SC), that describes the growth trajectory of a fish population, and the relative condition factor (Kn), that also evaluates fish condition by allowing comparisons between fish with different mean sizes, by using the SC instead of a fixed exponent value.

Coastal and estuarine areas are constantly under threat of chronic release many anthropogenic pollutants (Halpern et al. 2008). The Guanabara Bay is an estuary of approximately 400 km^2 in Rio de Janeiro, south-eastern Brazil. It is an important fishing area, but receives daily environmental

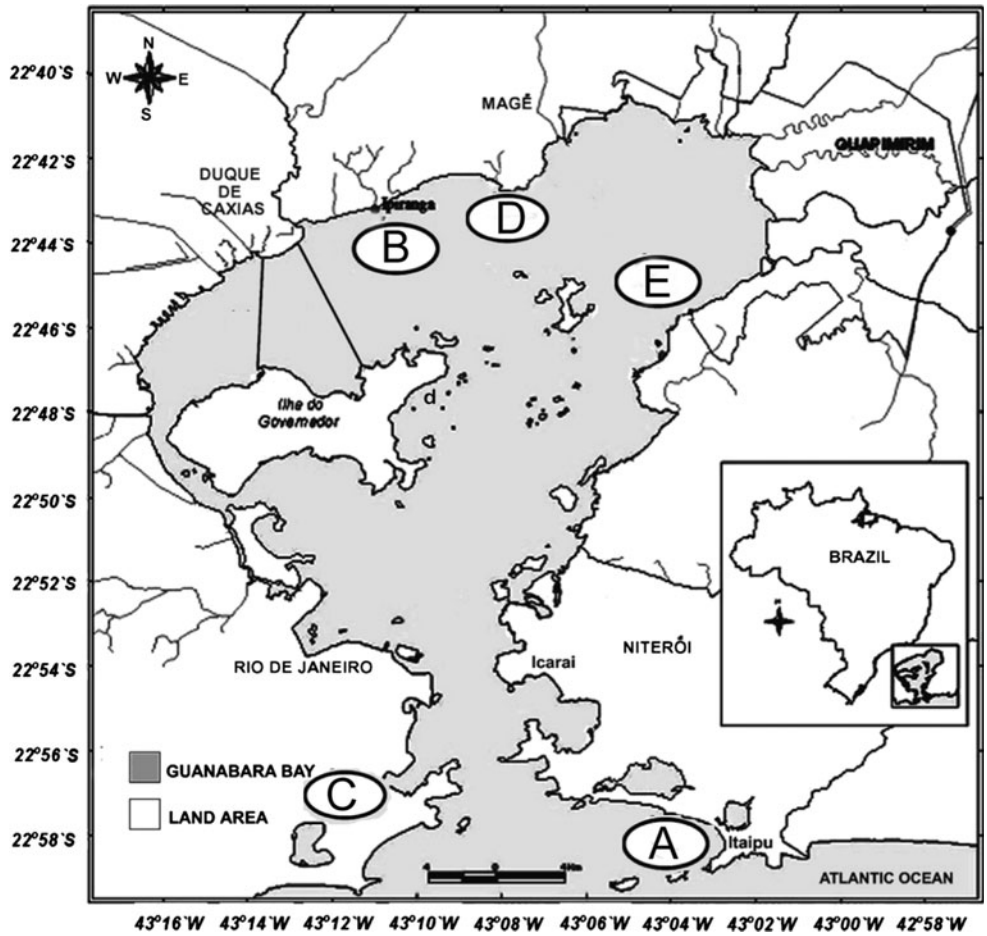

Fig. 1 Guanabara bay map, representing the five fish sampling areas: Itaipu (*a*), Ipiranga (*b*) Rodrigo de Freitas Lagoon (*c*), Olaria (*d*) and São Gonçalo (*e*) [Modified from (Neves et al. 2007)]

impacts, i.e. sewage and non-treated industrial effluents (Neves et al. 2007). Its drainage basin is an impacted area, suffering the effects of, among others, organic matter, oils, and metals. Areas close to open waters, however, show no history of contamination. This paper discusses environmental stress data obtained over 5 years in Mullet (*Mugil liza*), a commercially important fish species, from different sites from the Guanabara Bay, validated by liver histopathological analyses. As a detritivorous species these fish are more vulnerable than other species to pollutants, since many compounds are usually present in high concentrations adsorbed to sediment surfaces present throughout the water column (Mansour and Sidky 2002) making them ideal biomonitors for environmental contamination.

Materials and Methods

A total of 278 Mullet were sampled (August 2005–February 2010) by trawl-nets, from different areas of the Guanabara Bay, with different environmental contamination histories. These sites, from less to more contaminated, are: (1) Itaipu, a non-contaminated site with no history of environmental contamination, located at the mouth of the bay, where contamination by environmental pollutants is lower, due to the location near open waters. This site has been used as a reference site in other monitoring studies (Soares-Gomes, et al. 2010); (2) São Goncalo, which is also close to open seas (Fig. 1), where a lesser degree of contamination is present; (3) Ipiranga and (4) Olaria, extremely contaminated sites located at the far end of the bay. These sites are located near the municipality of Duque de Caxias, where there are two oil pipelines, exposing the area to chronic pollution to heavy metals and organic compounds (Francioni et al. 2005; Neves et al. 2007); (5) the Rodrigo de Freitas Lagoon, environmentally impacted for several years but now recovering. This area has been shown to be contaminated by metals and organic compounds (Loureiro et al. 2009). However, the Rio de Janeiro municipality has taken measures in the last decade to environmentally recover this site, with dredging of contaminated sediment and new sea connections, allowing water renewal and consequent contaminant dilution at this site, reducing contaminant levels (INEA 2009).

Fish were euthanized, sexed, measured (±1.0 mm), weighed and immediately processed. Livers were excised from each of the fish and weighed.

Indicators of fish condition based on ratios of morphometric indices are broadly used in the literature (Couture and Rajotte 2003).

The HSI and FCF were calculated according to (Maddock and Burton 1998):

(a) HSI = 100 (W_L/W_T); liver weight, W_L (g), is compared to total fish weight, W_T (g);
(b) FCF = 100 (W_T/L_T^3); total fish weight, (W_T (g) is compared to total fish length, L_T (cm).

The Kn was calculated according to Le Cren (1951): Kn = W/W′; where the weight of each individual (W) is compared to its expected weight (W′), which is estimated by a length-weight regression ($W = a\, L_T{}^b$). The exponent b is the calculated value of SC, a descriptor of the growth trajectory of a fish population, calculated as the slope of W_T to L_T relationship of a sample on a double logarithmic plot. The SC has been reported as season-insensitive and has been extensively recommended as a biomonitoring tool (Couture and Rajotte 2003; Eastwood and Couture 2002).

Livers were fixed in a buffered formalin solution and embedded in Paraplast Plus resin. Sections (5 µm thick) were stained in haematoxylin and eosin and examined by light microscopy for evidence of cytotoxic damage.

Hypothesis tests were used to verify differences between the means and the correspondence analysis and ANOVA tests were conducted to verify if differences were significant. Model assumptions (normality/homogeneity of residual variance) were tested by Anderson–Darling and Bartlett tests. A two sample *t* test with an assumption of variance equality was also used. The significance level was $\alpha = 0.05$.

Results and Discussion

From the 278 specimens collected, 216 individuals (78.00 %) could be sexed; 69 females (32.00 %) and 147 males (68.00 %). Fish weight and length are shown in Table 1.

We verified that the only site that approached the expected 50/50 sex ratio between males and females was the reference area of Itaipu, (58.23 % males and 41.77 % females). The others site consisted of consistently more males than females during the sampling campaigns: São Gonçalo presented 61.54 % male specimens and 38.46 % females, the Rodrigo de Freitas Lagoon presented 86.64 % males and 13.16 % females, and Ipiranga 68.06 % males and 31.94 % females. Thus, this metric does not seem to be impacted by the degree of contamination at the different sampling sites, except for the reference site, which did, in fact, approach the expected ratio.

An ANOVA test was conducted considering FCF and sex, and a significant difference between their means was observed. Data regression analyses were conducted considering W_T as the dependent variable and L_T as the predictor variable, for both sexes. The estimated coefficient with their respective estimative standard errors and the determination coefficient (R^2) of the statistical model for males (1) and females (2) were obtained, where SC is the slope:

$$\log \hat{W}_T = \underset{(0.080)}{-1.890} + \underset{(0.049)}{2.880} \log L_T, \quad R^2 = 96.10\ \% \tag{1}$$

$$\log \hat{W}_T = \underset{(0.123)}{-1.732} + \underset{(0.073)}{2.806} \log L_T, \quad R^2 = 95.70\ \% \tag{2}$$

Table 2 shows the data regression analyses data and the SC for both sexes. After obtaining the SC, the Kn was calculated. Table 3 shows the Kn, FCF and SC for each sex and grouped. Outliers were excluded from the analysis by the Dixon's Q test.

Figures 2 and 3 illustrate these results, showing the Kn and FCF means for each sampling site (95 % confidence interval).

At Olaria only 1 female was collected, so the SC, Kn and FCF were not calculated for this sex at this site. The lowest values for these indices were observed at Itaipu, for both sexes and when males and females were grouped. When this data was grouped, values above three were observed at São Gonçalo. Significant differences between the Kn and FCF means for both sexes at each site were

Table 1 Weight (g) and length (cm) by sampling site for each sex and for both

Site	W_T (g)			L_T (cm)		
Sex	♂	♀	Both sexes	♂	♀	Both sexes
Itaipu	914.70 ± 41.30	1046.80 ± 62.80	969.80 ± 36.10	47.16 ± 0.89	48.84 ± 1.17	47.86 ± 0.71
Ipiranga	626.00 ± 38.60	989.00 ± 99.60	753.40 ± 45.20	42.47 ± 0.88	47.92 ± 1.85	44.21 ± 0.88
R.F.Lagoon	556.50 ± 27.50	616.10 ± 62.40	592.20 ± 30.50	40.32 ± 0.80	40.86 ± 1.23	40.39 ± 0.70
Olaria	442.10 ± 73.30	–	449.10 ± 65.00	37.43 ± 1.57	–	37.22 ± 1.40
São Gonçalo	863.50 ± 15.80	1792.00 ± 209.0	1409.00 ± 140.00	52.01 ± 1.79	55.96 ± 2.43	53.53 ± 1.49

Data for n = 1 was not included (shown as –)

Table 2 Data concerning the data regression analyses and the SC at each sampling site

Sex	Site	n	Equation	SC	R^2	p^a
Males	Itaipu	46	$\hat{W}_T = 0.029L_T^{2.675}$	2.675 ± 0.089	95.30	0.174
	Ipiranga	49	$\hat{W}_T = 0.010L_T^{2.923}$	2.923 ± 0.082	96.50	0.156
	R.F.Lagoon	33	$\hat{W}_T = 0.015L_T^{2.841}$	2.841 ± 0.120	94.70	0.266
	Olaria	8	$\hat{W}_T = 0.003L_T^{3.258}$	3.258 ± 0.150	98.70	0.736
	São Gonçalo	8	$\hat{W}_T = 0.010L_T^{2.935}$	2.935 ± 0.368	91.40	0.582
Females	Itaipu	33	$\hat{W}_T = 0.032L_T^{2.665}$	2.665 ± 0.122	93.90	0.478
	Ipiranga	23	$\hat{W}_T = 0.017L_T^{2.815}$	2.815 ± 0.067	98.90	0.254
	R.F.Lagoon	5	$\hat{W}_T = 0.006L_T^{2.842}$	2.842 ± 0.120	96.76	0.265
	São Gonçalo	5	$\hat{W}_T = 0.033L_T^{2.700}$	2.700 ± 0.183	98.60	0.070

[a] p values for the normality residue test. $p > 0.05$ implies a normal data distribution

Table 3 Kn, SC and FCF for males, females and grouped (–, n = 1)

Site	Kn			SC		FCF		
Sex	♂	♀	Both sexes	♂	♀	♂	♀	Both sexes
Itaipu	0.83 ± 0.01	0.87 ± 0.01	0.84 ± 0.01	2.675 ± 0.089	2.665 ± 0.122	0.85 ± 0.01	0.87 ± 0.17	0.86 ± 0.01
Ipiranga	0.97 ± 0.01	0.99 ± 0.06	0.96 ± 0.01	2.923 ± 0.082	2.815 ± 0.067	0.79 ± 0.01	0.83 ± 0.01	0.81 ± 0.01
R.F.Lagoon	1.05 ± 0.01	1.05 ± 0.04	1.03 ± 0.01	2.841 ± 0.120	2.842 ± 0.120	0.87 ± 0.01	0.89 ± 0.01	0.87 ± 0.20
Olaria	0.96 ± 0.02	–	0.98 ± 0.04	3.258 ± 0.150	–	0.80 ± 0.01	–	0.84 ± 0.04
São Gonçalo	1.01 ± 0.03	1.22 ± 0.04	1.07 ± 0.04	2.935 ± 0.368	2.700 ± 0.183	0.81 ± 0.02	1.00 ±0.02	0.89 ± 0.03

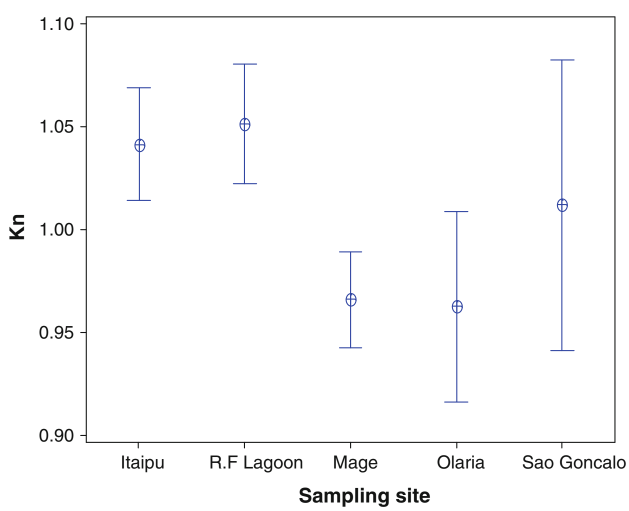

Fig. 2 Kn means by sampling site

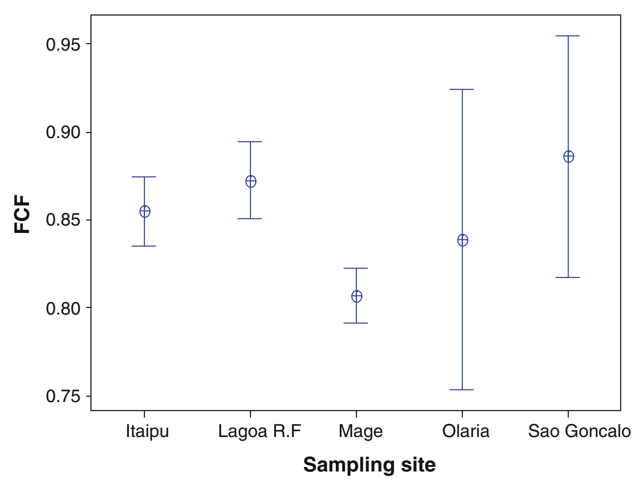

Fig. 3 FCF means by sampling site

found. The Kn showed the same trend for both sexes: fish from São Gonçalo, Itaipu and the Rodrigo de Freitas Lagoon show higher Kn values than those from Magé. An FCF closer to 1 indicates fish in better health condition. This index showed the same trend as the Kn, with fish from São Gonçalo presenting the theoretical best score for this index. For both indices (FCF and Kn) males Olaria showed one of the lowest values.

Significant differences between the HSI for both sexes by sampling site were also observed. The HSI at Itaipu and Magé showed similar means when separated by sex. However, when grouped, Itaipu showed significantly higher values than all other sampling sites. Fish from São Gonçalo showed the highest HSI. The HSI for females could not be calculated at the Rodrigo de Freitas Lagoon and Olaria (Table 4).

Table 4 HSI for males, females and grouped

Site	HSI		
Sex	♂	♀	Both sexes
Itaipu	1.59 ± 0.32	1.79 ± 0.55	1.68 ± 0.07
Ipiranga	1.59 ± 0.39	1.62 ± 0.42	1.60 ± 0.07
R.F.Lagoon	1.66 ± 0.45	NA	1.66 ± 0.02
Olaria	1.46 ± 0.25	NA	1.56 ± 0.12
São Gonçalo	2.09 ± 0.34	2.96 ± 0.26	2.52 ± 0.18

NA non available

Potential toxicants in sediments are the primary form of contamination in coastal and estuarine systems. Since Mullet are detritivorous and ingest large quantities of sediment foraging throughout the water column, they are more vulnerable to sediment-related pollution than other species (Mansour and Sidky 2002), and thus ideal biomonitors regarding environmental contamination.

Regarding the indicators of fish condition based on ratios of morphometric indices, in this study SC < 3 were found in several of the differentially contaminated sampling sites, and the lowest value was observed at the reference site. These values, however, did not differ statistically, contrasting with other studies, in which SC < 3 is noted as indicative of environmental stress (Couture and Rajotte 2003). However, those authors note that SC < 3 may also be found in populations from clean environments where other pressures (i.e. over population, disturbed food webs) can alter the growth trajectory of the populations. Thus, more studies must be conducted regarding this index in Mullet. Because of this, the SC was not used alone as an indicator of environmental exposure.

Fish body condition is routinely employed as indicative of fish condition since it is a simple alternative to tissue analysis (Sutton, et al. 2000) with low or declined condition factors interpreted as a depletion of energy reserves (Jenkins 2004). An FCF = 1 indicates excellent fish health, while FCF < 1 indicates worse health condition. The FCF in this study distinguished between contaminated and non-contaminated Mullet: Magé fish (contaminated) showed the lowest FCF values while, São Gonçalo fish, nearer open waters, showed the highest values. Olaria males also had low values, indicating that this population is also in worse condition, comparable to Magé.

Some studies indicate that the Kn may be preferable over the FCF, since it takes into account the fact that, if a fish tightly follows the growth trajectory described by the SC of its population, and if this SC is higher than 3, then its FCF will increase as it grows. As the Kn uses the SC as the exponent b instead of the fixed value of 3, it would remain unchanged and therefore more realistic (Couture and Rajotte 2003). The Kn thus allows for comparisons between fish with different mean sizes, indicating whether the fish are in better ($K > 1$) or worse ($K < 1$) condition than an average individual with the same length. The mean Kn observed in the present study fish from the most contaminated sites (Olaria—males only and Magé—both sexes) showed significantly lower values than São Gonçalo, Itaipu and the Rodrigo de Freitas Lagoon. Therefore, this study also supports the combined use of the FCF and Kn as condition indicators for Mullet.

The HSI is a measurement of fish energy reserves (Nikolsky 1963; Querol et al. 2002), and is a useful indicator of aquatic contamination (Maddock and Burton 1998). In the present study, female HSI were higher than male values, as expected, and significantly lower values were found at the contaminated sites, the same trend as Kn and FCF.

Other evidence for environmental stress was also analysed: macro and microscopic analyses in fish from the cleanest and most contaminated areas (Itaipu and Magé, respectively, were conducted (n = 14). Several abnormalities were repeatedly observed in Magé livers, like nodules, tumours, granular appearance, yellowish tinge and visible ectoparasites. A dark red liver colour in fish is considered normal, while light brown and yellowish are indicative of fatty livers (Adams, et al. 1993). More than 50 % of the Magé fish presented abnormal liver color. Microscopical Magé liver sections showed melanomacrophage centers, leucocitary infiltrations and endoparasites, while Itaipu fish showed normal fish histopathology, with the exception of one Itaipu fish that showed only slight indications of melanomacrophage agglomerations, with no evidence of citotoxic damage (Fig. 2). These abnormalities have been observed in fish from contaminated areas (Miranda et al. 2008; Silva et al. 2009). Macroscopical gill abnormalities were also repeatedly present in Magé fish, such as shortened gill arches, visible ectoparasites and frayed/pale gills. These abnormalities validating the results of the index analyses showing differences in fish condition from Magé and Itaipu. Ectoparasites attached to fish scales throughout the body and frayed fins were also visible in some fish from Magé (Fig. 4).

The three morphometric indices (FCF, Kn and HSI) reflected the different environmental contamination status of the 5 sampling sites, proving to be useful indicators of environmental contamination for Mullet. The SC, however, did not reflect this. These results were readily validated by histopathological liver analyses. Thus, this study indicates Mullet are useful indicators of environmental contamination and the FCF, Kn and HIS are good indicators of Mullet health and should be further investigated.

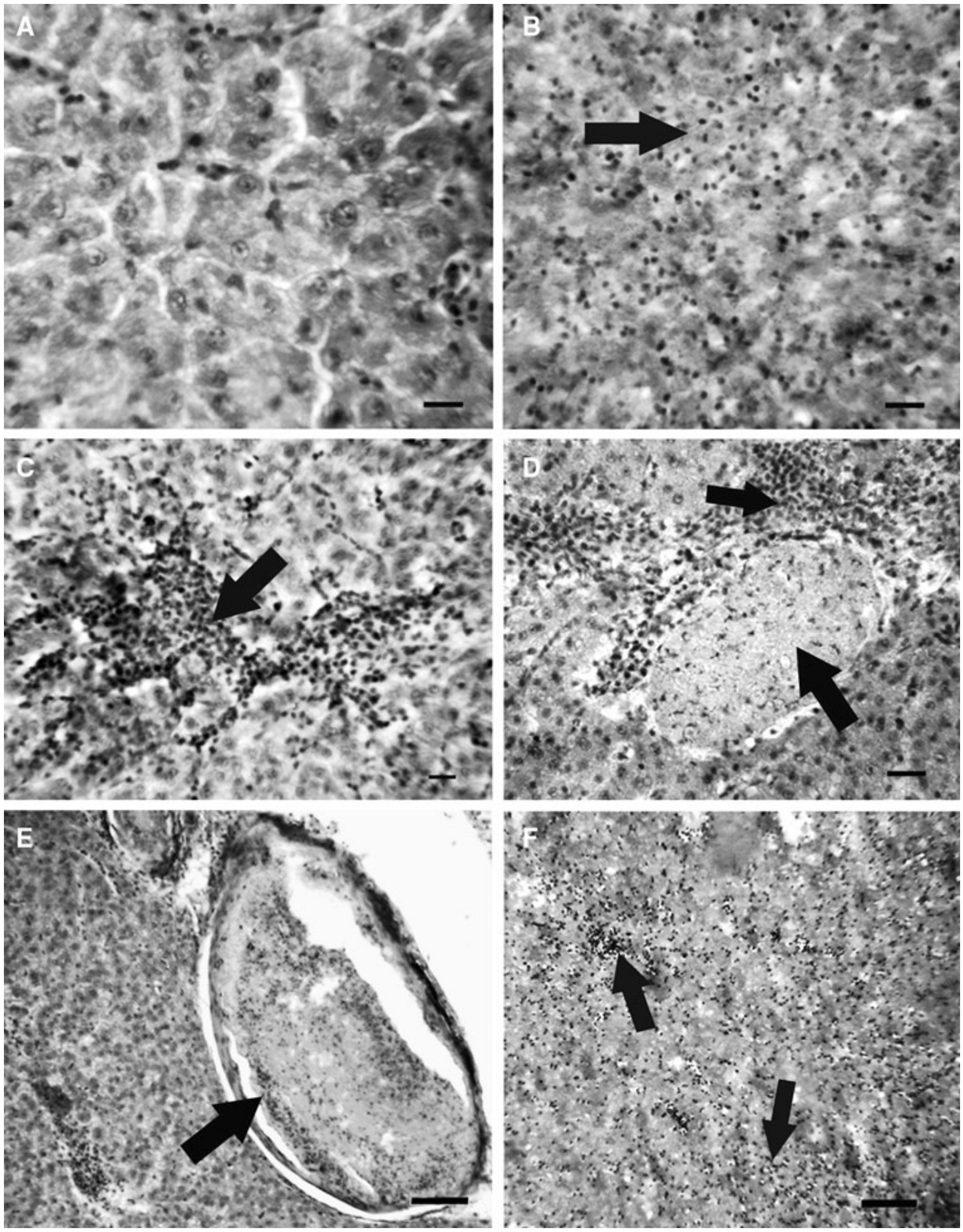

Fig. 4 Liver cross sections of *M. liza* from different studied sites located at Guanabara Bay. **a** Liver parenchyma without apparent damages or alterations (Itaipu). **b** and **f** Alterated tissue showing high bacterial infestation (*arrows*) (Magé–Praia de Ipiranga*)*. **c** Inflammatory response showing Leucocyte infiltration (*arrow*) (Magé–Praia de Ipiranga). **d** The *small arrow* shows both inflammatory response and melanomacrophage centers and the *bigger arrow* indicates the occurrence of preneoplasic focus (Magé–Praia de Ipiranga*)*. **b** Ectoparasites are observed (*arrow*) (Magé–Praia de Ipiranga*)*. *Scale bar* = 20 μm

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