



ECOTOX - BRASIL

Ecotoxicol. Environ. Contam., v. 8, n. 2, 2013, 09-15
doi: 10.5132/eec.2013.02.002

EEC

Fish histopathology as biomarker to evaluate water quality

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(Received May 01, 2012; Accept July 04, 2013)

Abstract

Aquatic environments contaminated by industrial effluents and urban sewage present as common characteristic the release of xenobiotics complex mixture that potentially affects important aspects related with physiological aspects in fish. In the present work, fish specimens, *Astyanax aff. fasciatus* and *Oreochromis niloticus*, were used to evaluate the anthropogenic impact in two lakes (Lake Park and Cedeteg) situated in Guarapuava City, southern of Brazil. The occurrence of morphological changes in gills and liver associated with somatic indexes (Hepatosomatic - HSI and Condition Factor - CF) were used as biomarkers, and two reference sites were used. According to the results, individuals from both studied sites presented morphological damages in gills (aneurysms, hyperplasia, lamellar fusion and neoplasia) and liver (necrosis and leukocyte infiltration). The lesion indexes for liver and gills studied in both lakes were significantly impacted when compared with reference sites, but individuals from Lake Park were more affected. The HSI in both species was not different, but the CF in *A. fasciatus* decreased in both studied sites. These results suggest that the morphological findings found in the present study were developed due to the urban sewage release, reinforcing the importance of histopathological investigation in biomonitoring programs to evaluate the water quality and environmental assessment.

Keywords: biomonitoring, histopathology, urban lakes, freshwater fish.

INTRODUCTION

In the last few decades, the increasing of environmental degradation has contributed for the distribution of toxic chemicals to the environment and consequently serious effects in wild aquatic organisms' health can be noticed. Untreated sewage effluents consist of complex mixtures of toxicants that increase significantly the impact on aquatic ecosystems, decreasing the species diversity observed in different regions of the world (Schmitt *et al.*, 1999; Amisah & Cowx, 2000). The tolerance of wild organisms to toxicants in domestic effluents may vary among species and their integrative effects may lead to reproductive failure or reduction of fish species number (Wendelaar Bonga, 1997; Minier *et al.*, 2006).

According to Oga (2003) and Adams (2002), the response to chemical stress can be used as biomarkers or sentinels of environmental conditions. Biomarkers are early responses or measurable biological event due to exposure to pollutants after acute or chronic exposure and the morphological findings has been largely considered in biomonitoring studies (Lagadic *et al.*, 2000; Winkaler *et al.*, 2001; Oliveira Ribeiro *et al.*, 2005; Miranda *et al.*, 2008). According to Winkaler *et al.* (2001) and Ayas *et al.* (2007), histopathological events are considered fast and efficient for detection of acute and chronic adverse effects in fish; and may express the health condition of exposed individuals (Myers & Fournier 2002; Oliveira Ribeiro *et al.*, 2005; Miranda *et al.*, 2008). Field investigations are important to interpret assessment risks and understand biological effects of chemicals under natural conditions, representing also a real

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health state (Mallatt 1985; Lagadic *et al.*, 2000; Winkaler *et al.*, 2001; Alberto *et al.*, 2003).

Fish exposure to chemical contaminants induce lesions in different target organs, especially in liver (Oliveira Ribeiro *et al.*, 2005; Rabbito *et al.*, 2005; Mela *et al.*, 2007; Miranda *et al.*, 2008) and gills (Winkaler *et al.*, 2001; Tkatcheva *et al.*, 2004; Oliveira Ribeiro *et al.*, 2005; Benli *et al.*, 2008). According to Ayas *et al.* (2007) and Lemes & Braccini (2004), liver is an important target organ related to important metabolic and detoxification mechanisms. In addition, gills are in permanent contact with water and represent an important target organ to pollutants dissolved in water. This organ is essential on gas exchanges and osmotic regulation. Morphological changes in gills can represent adaptive strategies for conservation of some physiological functions or endpoints to evaluate acute or chronic exposure to chemical present in water and sediment (Winkaler *et al.*, 2001; Tkatcheva, *et al.*, 2004). Also, physiological evaluation through somatic indexes as condition factor (CF) and hepatosomatic index (HSI) are indicatives of metabolic fish condition that increase the information about water quality, as described by Schibatta (2005).

Astyanax aff. fasciatus is an abundant and widely distributed species across Central and South America (Araujo 1997), while *Oreochromis niloticus* tilapia is one of the most important freshwater finfish species in aquaculture around the world (Shaw & Handy 2006). This study investigated the occurrence of morphological changes in liver and gills of fish tropical species through morphological damages in target organs and somatic indexes parameters.

MATERIAL AND METHODS

Study areas and sampling

The present study considered two lakes (Municipal Lake Park and University of the Midwest - Lake Cedeteg) located at Guarapuava City - Paraná State, Southern of Brazil. Both studied sites are impacted by domestic sewage effluents. The reference site for *A. fasciatus* was the Segredo Hydroelectric power plant and for *O. niloticus*, the aquaculture farm at Araucaria City, both sites also located at Paraná State (Fig. 1). Twenty five adult male and female fish of each species *A. fasciatus* and *O. niloticus* were collected from the studied and reference sites and used in the present study.

Fish analysis

The specimens were anesthetized with ether benzocaine (200ppm) before measure the weight (total body and liver) and length (total body). After anesthesia the specimens were killed by medullar section and liver and gills were sampled for hepatic somatic index and histological procedures.

The Condition Factor (CF) was estimated using the formula $TL = TW/TL^b$, where TW is the total weight (g) and TL is the total length (cm), b is the angular coefficient weight-

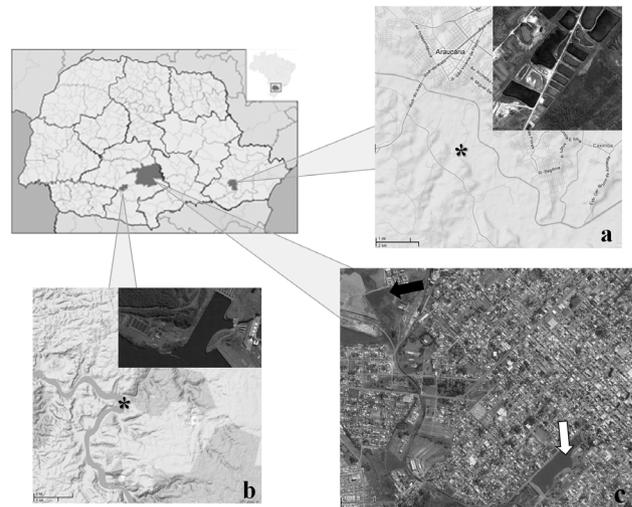


Fig. 1 - Map of the studied sites. (A) Reference site to *Oreochromis niloticus* in Araucaria City (star); (B) Reference site of *Astyanax aff. fasciatus* in Segredo Hydroelectric power plant (star) and the studied sites: (C) Municipal Lake Park (white arrow) and Lake Cedeteg (black arrow) both in Guarapuava City.

length relation obtained from linear regression between total weight and total length. The calculation of hepatic somatic index (HSI) was estimated by the formula $HSI = LW/TW \times 100$ where LW represents the liver weight (g) and TW is the total weight (g).

Histopathological procedures

Gills and liver samples were preserved in ALFAC fixative solution (ethanol 80%, formaldehyde and glacial acetic acid) for 16 h, dehydrated in a graded series of ethanol baths and embedded in Paraplast Plus resin (Sigma®). The sliced sections (5 µm) were stained in hematoxylin and eosin, observed under light microscope Leica and described according to Hybia (1982).

Results were expressed as a prevalence of fishes with histopathological lesions and gill and liver lesion index determined according to Bernet *et al.* (1999). Briefly, this index is based on the sum of the score rankings and importance factors for each considered lesion in the tissue. The score ranking was based on the percentage of lesions. The importance factor was determined for each lesion depending on the effects considered as minimal (1), moderate (2) and irreversible (3).

Statistical Analysis

All data were compared using one-way analysis of variance (ANOVA) followed by Tukey's test. All the tests were regarded statistically significant when $p < 0.05$.

RESULTS

The histopathological analysis showed severe lesions in liver and gills of fishes from both Municipal Park and Cedeteg Lakes when compared with individuals from reference sites.

Morphological Aspects

The gills structure of *A. fasciatus* and *O. niloticus* were similar to that described to other freshwater teleost species, presenting primary and secondary lamellae (Fig. 2a and 3a) (Mallat, 1985, Wilson & Lauren, 2002).

Circulatory lesions as aneurisms were found in the majority of individuals from both studied sites, but increasing in individuals from Lake Park for both studied species (Table 1 and 2). This lesion represents a disruption of pillar cells and consequent erythrocytes bleeding (Fig. 2b and 3b). A proliferation of epithelial cells in primary lamellae induces the lamellae fusion and consequently decreasing the surface area for gas exchange. This cellular hyperplasia on epithelial

tissue was observed also in the majority of individuals from both studied areas and absent in individuals from reference site (Tables 1 and 2). Those findings represent a more chronic exposure while aneurisms are more related with acute exposure (Fig. 2d,e and 3c,d). Those cells presented enlarged nuclei suggesting a high nuclear activity or cell metabolism (Fig. 2e and 3c). The occurrence of differentiated cellular regions that characterize a neoplastic event between two secondary lamellae (Fig. 2c) was found only in *A. fasciatus* mainly from Lake Park (Table 1). This kind of lesion is related with chronic exposure. Ectoparasites (no characterized) were observed in gills from both studied species (Fig. 2f and 3e).

According to the lesion index determinated to gills, the Park and Cedeteg Lakes were more impacted ($p < 0.05$) than the reference sites, but individuals of *A. fasciatus* are more affected when compared with *O. niloticus* in both studied sites and when compared with individuals from the same species from Lake Cedeteg (Fig. 6A).

The liver morphology of both considered species *A. fasciatus* and *O. niloticus* also showed similar structure to that described to other freshwater fish species (De Andrade Brito *et al.*, 2012, Silva *et al.*, 2012). The hepatocytes were

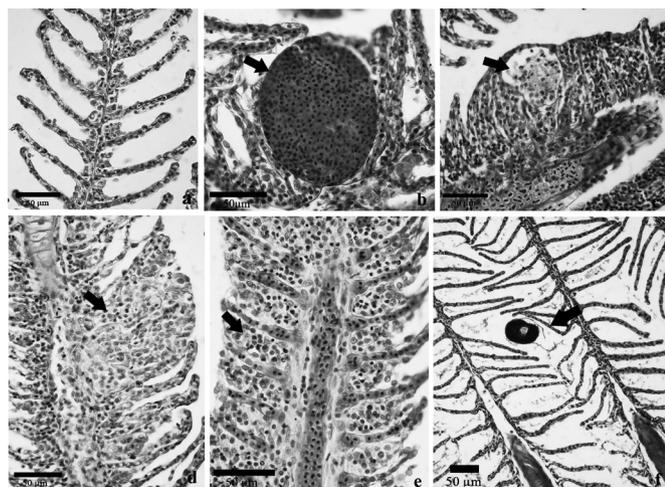


Fig. 2 - Cross sections of gills of *Astyanax aff. fasciatus*. (A) General view of the gills (reference site); (B) The arrow shows aneurisms in secondary lamellae; (C) Neoplasia in secondary lamellae (arrows); (D) and (E) Hyperplasia of epithelial cells on secondary lamellae showing the fusion between lamellae; (F) The arrow shows the presence of ectoparasite among secondary lamellae. Hematoxylin/Eosin stain.

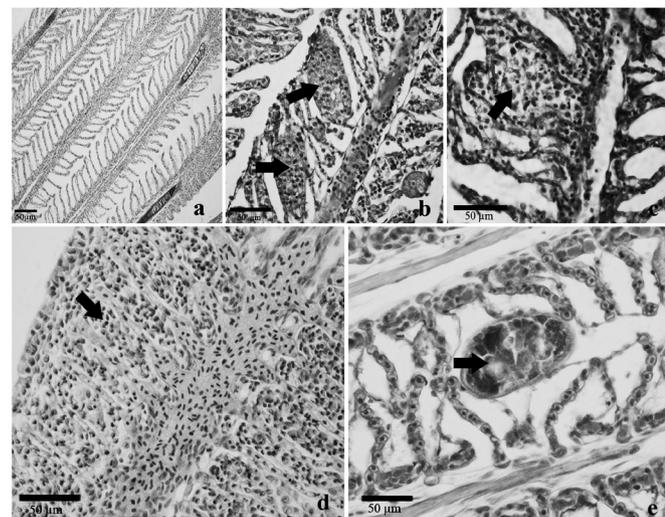


Fig. 3 - Cross sections of gills of *Oreochromis niloticus*. (A) General view of the gills (reference site); (B) Aneurism in secondary lamellae (arrow); (C) Fusion among secondary lamellae (arrow); (D) Hyperplasia of epithelial cells on secondary lamellae (arrow); (E) Parasite among secondary lamellae (arrow). Hematoxylin/Eosin stain.

Table 1 - Prevalence of histopathological findings (%) in gills and livers of *Astyanax aff. fasciatus* from Reference, Lake Cedeteg and Lake Park.

Lesion	Reference	Lake Cedeteg	Lake Park
<i>Gills</i>			
Hyperplasia	0	40	60
Lamellar fusion	0	28	56
Aneurysm	20	36	56
Neoplasm	0	04	24
Ectoparasites	0	0	08
<i>Liver</i>			
Necrosis	0	32	76
Leukocyte infiltration	20	16	60
Parasites	0	08	44

Table 2 - Prevalence of histopathological findings (%) in gills and livers of *Oreochromis niloticus* from References, Lake Cedeteg and Lake Park.

Lesion	Reference	Lake Cedeteg	Lake Park
<i>Gills</i>			
Hyperplasia	04	20	28
Lamellar Fusion	0	28	52
Aneurysm	16	44	64
Ectoparasites	0	0	04
<i>Liver</i>			
Necrosis	0	28	72
Leukocyte infiltration	08	20	56
Parasites	0	0	0

easily identified as large and polyhedral cells with rounded nuclei forming cords around capillary sinusoids that radiate from a central vein. The bile ducts presented variable size and were easily identified due to cubical or cylindrical epithelium usually associated with veins (Fig. 4a and 5a).

Two main lesions were found in liver of both species, the presence of leukocytes infiltration and necrosis areas. The presence of leukocytes infiltration was found in all groups including the reference, but the incidence in groups from the studied sites was higher mainly in individuals from Lake Park (Table 1 and 2). The leukocytes traverse the hepatic vessels featuring a leukocyte infiltration (Fig. 4b and 5b). This event was in general associated with the occurrence of necrosis (cell death), in individuals from studied sites but not in fish from reference sites (Tables 1 and 2). The hepatic lesions in

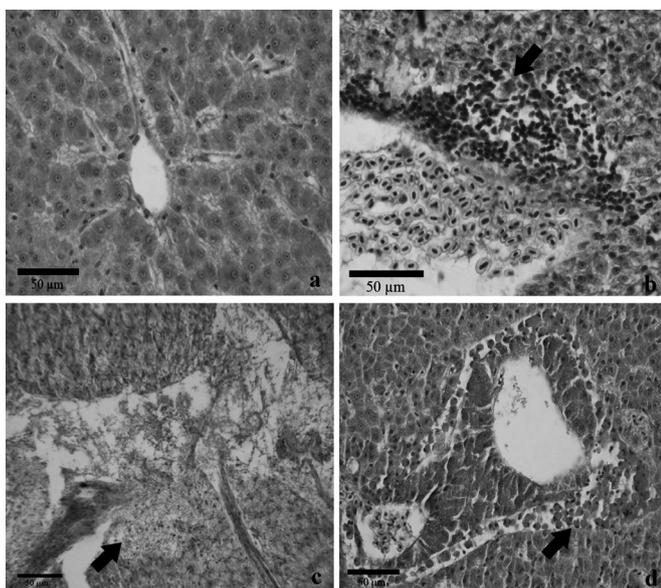


Fig. 4 - Cross section of liver of *Astyanax aff. fasciatus*. (A) General aspect of liver (reference site). (B) The arrow shows a leukocyte infiltration; (C) Necrosis area; (D) Incidence of parasites around vessels (arrow). Hematoxylin/Eosin stain.

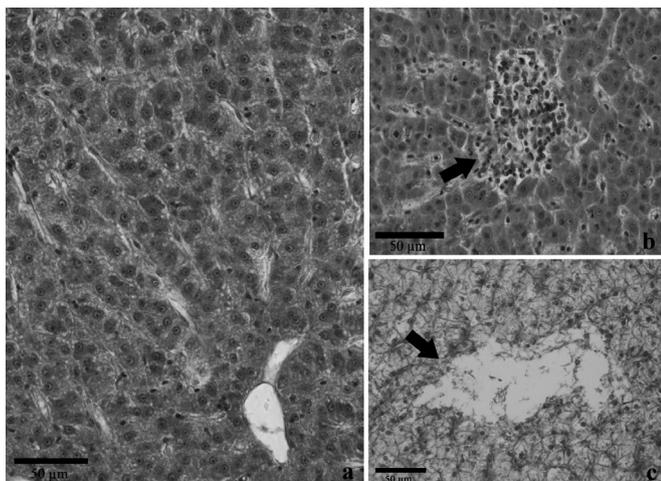


Fig. 5 - Cross section of liver of *Oreochromis niloticus*. (A) General aspect of liver (reference site); (B) Leukocyte infiltration (arrow); (C) Necrosis area (arrow). Hematoxylin/Eosin stain.

individuals of Lake Park had a higher incidence in both species (Tables 1 and 2 and Fig. 4c and 5c). The high occurrence of parasites in liver may represent an immunological system failure, observed by the presence of intra-hepatic parasites genus *Calyptospora* (Protozoa: Apicomplexa) around the liver vessels (Fig. 4d).

The lesion index in the liver corroborate the results found in the gills, which for both species studied, the urban lakes are more impacted than the reference sites, and also the Lake Park is more affected than the Lake Cedeteg. Considering the lesion index to liver, it was observed that, differently that observe to gills, there were no difference between the same species in both sites studied (Fig. 6B).

The HSI for *A. fasciatus* and *O. niloticus* was not different between the studied sites (Fig. 6C and E), but the condition factor (CF) in *A. fasciatus* showed a decrease in both sites studied, as compared to the reference sites (Fig. 6D), while *O. niloticus* showed no difference (Fig. 6F).

DISCUSSION

The structure of liver and gills described to *Astyanax aff. fasciatus* and *Oreochromis niloticus* were also reported to other teleost species by Mallat (1985), Machado (1999) and Lemes & Braccini (2004), favoring the comparisons with other studies where the chronic exposure to contaminants

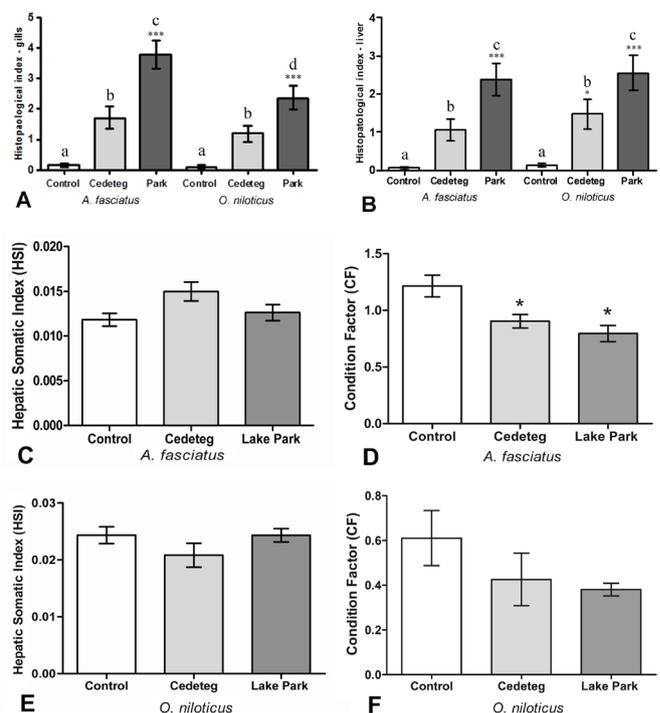


Fig. 6 - Histopathological lesions index to gill (A) and liver (B) to *A. fasciatus* and *O. niloticus* from control (reference), Lake Cedeteg and Urban Lake Park groups. Hepatic somatic index of *A. fasciatus* (C) and *O. niloticus* (E), and Condition factor of *A. fasciatus* (D) and *O. niloticus* (F) from control (reference), Lake Cedeteg and Lake Park sites; Difference are indicated by * (p<0.05) and *** (p<0.001). The index difference between the species are indicated by letters (p<0.05).

were investigated. According to Winkaler *et al.* (2001) and Tkatcheva *et al.* (2004), some morphological changes in gills and liver may represent adaptive strategies to maintaining physiological functions, but the histopathological lesions as described in the present study indicated that fishes were affected by the exposure to pollutants presented in water and sediment. The presence of surfactants, phenols, PAHs (polycyclic aromatic hydrocarbons), metals and other urban pollutants certainly were the reason to those damages, as also described by Tkatcheva *et al.* (2004). The reduction of oxygen may be also the source of physiological disturbances that can sometimes reflect in morphological damages (Fernandes & Rantin, 1994; Alberto *et al.*, 2003).

The branchial findings described in fish chronically exposed to urban sewage in the present study are similar to those observed by Alberto *et al.* (2003) and Monteiro *et al.* (2009) respectively in *Astyanax altiparanae* and *A. fasciatus*. The exposure of gills to different contaminants may be marked by the occurrence of lamellar fusion, tissue hyperplasia as neoplastic events, cellular hypertrophy and aneurysms (Tkatcheva *et al.*, 2004 and Oliveira Ribeiro *et al.*, 2000 and 2005).

Hyperplasia and lamellar fusion in gills found in individuals from Lake Park and Lake Cedeteg are considered as moderate severity according to the classification of Bernet *et al.* (1999). These types of lesion may occur as a tissue response to the presence of pollutants diluted in water, increasing the distance between the blood capillary and lamellae surface, reducing in theory the pollutants uptake. However this cell proliferation also leads to respiratory dysfunction, affecting gas exchange due to the decreasing of gill surface and also leading to disturbances in animal osmoregulation (Mallat, 1985). According to Valdez Domingos *et al.* (2009) and De Andrade Brito *et al.* (2012), the occurrence of secondary lamellar fusion is frequently found in fishes exposed to urban sewage.

The presence of pre-neoplastic foci or tumors in gills was described by Ali & Sreekrishnan (2001) and Shailaja & D'Silva (2003) as a cause of chronic exposure to pollutants as PAHs or organochloride compounds. Those findings were also reported by Oliveira Ribeiro *et al.* (2005) in liver and spleen of *Anguilla anguilla* chronically exposed to the organic pollutants. Pre-neoplastic foci and tumors mean a non reversible lesion and chronic exposure to highly toxic pollutants in small concentrations, not easily detectable. According to the results both studied sites are chronically impacted by pollutants that may potentially induce the development of neoplastic effects.

The application of the lesion index of Bernet, associated with image analyses allows a more accurate evaluation of morphological effects caused by pollutants. After the establishment of the index is easier to compare the level of injuries between the target organs on the same individual, between two or more different species and also among groups of the same species from different locations studied. According to the lesions and alterations described to gills of *O. niloticus* from Lake Park, this site can be considered the most impacted by pollutants dissolved in water. According to the same parameter

Lake Cedeteg showed a higher impact level than the reference sites, where was not found evidences of impact.

The hepatic necrosis are described by Bernet *et al.* (1999) as irreversible injury to tissue. Those findings were observed in *A. fasciatus* and *O. niloticus* from both sites studied but absent in individuals from the reference sites. Necrosis was also reported in liver of Brazilian tropical and subtropical fish species exposed to multiple contaminants in different impacted regions (Miranda *et al.* 2008; Valdez Domingos *et al.* 2009). According to Gonzales *et al.* (1993), necrosis found in the fish liver are usually related to contaminants found in water or sediment. Khan *et al.* (1994) and Ayas *et al.* (2007) described that those lesions in liver of fish is one of the most important histopathological responses due to exposure to contaminants. The principle of hepatic necrosis results from the presence of chemicals within cells causing disturbs on biochemical process as enzyme inhibition, failure on protein synthesis, carbohydrate metabolism, reactive oxidative species production, damages in cell membrane and failure of ATP synthesis (Manahan 1994; Rabitto *et al.*, 2005; Mela *et al.*, 2007).

Leukocyte infiltration is another morphological disturbance found in liver of individuals exposed to pollutants and represents a first line of immunological response due to cell death, and presents a moderate severity according to the classifications proposed by Bernet *et al.* (1999). This finding was observed in the majority of individuals from Lake Park, corroborating the chronic exposure of gills to pollutants described, and also signaling the presence of infections agents (Wedemwyer *et al.* 1990). According to Bernet *et al.* (1999), leukocyte infiltration is often associated also with other changes but in general, the main functions are related to neutralize and destroy the source aggressor, cleaning the tissue, removing the aggressor agent and dead cells, and induce the recovery of damaged tissue.

As described to gills, the lesion index calculated to liver from both studied lakes showed that these sites are more impacted than reference ones for both considered species. According to the liver lesion index the reference sites are not impacted by pollutants and reinforce the theory that animals found in the studied sites are affected by subchronic, chronic and trophic exposure to pollutants.

The occurrence of ecto and endoparasites is common in gills and liver of fish but depending on the incidence may be considered as a potent immunological biomarker (Oliveira Ribeiro *et al.*, 2005). Although significative parasite infestations have not been found in gills of individuals from the current work, the occurrence of high infestation of endoparasites as *Calyptospora* sp in liver of *Astyanax aff. fasciatus*, may represent disturb of the immunological defense system, as described by Casal *et al.* (2007). The higher incidence found in individuals from the Lake Park corroborate the hypothesis that this lake is more affected by human activities.

Condition factor (CF) is an indicative of the health status and may be used to assess field contamination. Alterations in

growth rates are induced by contaminants and the explanation is the reallocation of energy to detoxification mechanisms, depleting reserves that were originally destined for growth (Nicholson & Lan 2005; Valdez Domingos *et al.*, 2009). Animals collected in the field often display low CF values at sites contaminated with PAH's, PCB's (Poli Chloride Biphenyls), TBT (Tributyltin), metals and urban sewage (Alberto *et al.*, 2003; Nicholson & Lan 2005;). The low CF values of *A. fasciatus* in Lake Cedeteg and Lake Park indicates the worst condition of fish in the sites studied. This condition was not observed to *O. niloticus* in the same sites studied suggesting that this species is physiologically more adapted to the impacted condition present in the urban lakes. These data showed that introduced species as *O. niloticus* present advantages on native species concerns to tolerance to chemical stress.

Hepatic somatic index HSI is also an indicative of the metabolic condition in fish. The increase of HSI levels is normally associated with enhanced detoxification activities in response to the presence of toxic compounds (Pereira & Kuch, 2005). In contrast, decreases in the HSI level means that fish populations are under chemical stress (Kopecka *et al.*, 2006). Despite of all lesions observed in liver of both considered species in the present study, no statistical differences were found. But according to De la Torre *et al.* (2005), individuals exposed to contaminants may also show lower or similar index rates among groups, meaning that this parameter must consider also other findings.

CONCLUSION

The data in the current work showed that according to the morphological analysis the Lake Park is more impacted than Lake Cedeteg. This is supported by the severity of lesions found differently in both considered species. Also the results conclude that both studied sites are impacted by different pollutants and a further monitoring and assessment program in these lakes is strongly suggested.

In addition the results reinforce the high potential of histopathological lesions to reveal chronic and acute exposure of fish to pollutants, increasing the credibility of the diagnosis in impacted studies of water quality of aquatic ecosystems.

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