

Gill and Liver Histopathology in *Goodea atripinnis* Jordan, Related to Oxidative Stress in Yuriria Lake, Mexico

Histopatología en las Branquias e Hígado de *Goodea atripinnis* Jordan,
Relacionada con el Estrés Oxidativo en la Laguna Yuriria, México

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SUMMARY: In aquatic ecosystems, the complex mixture of pollutants may mediate the formation of free radicals and cause oxidative damage to the biota. Yuriria Lake (a Ramsar site in Central Mexico) receives input of wastewater from its tributaries, agricultural runoff, and municipal discharge. We studied the lipid peroxidation (LPO), antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and histopathology of gill and liver of the native fish *Goodea atripinnis* in Yuriria Lake. Results were compared to a control group of fish cultivated in the laboratory. LPO, SOD, and CAT showed no significant differences compared to controls, but GPx showed greater and significant differences in both tissues. Three class sizes were identified; organisms of classes I and II had slight vasocongestion in the liver as compared to controls. Hepatocytes of class III showed cytoplasmic vacuolization, cellular disorganization, and the liver showed marked fibrosis compared to controls. Gills of controls and classes I and II showed no damage in gill filaments. Tissue damage in class III included hypertrophy, loss of the typical morphology, and edema in the gill filaments. The longer exposure of older organisms to Yuriria Lake conditions may have resulted in their poorer health condition.

KEY WORDS: Mexican wetlands; Oxidative stress; Histopathology; Fish health condition; Liver; Gill.

INTRODUCTION

Wetlands are important sites of transition between land and water; these environments are considered among the most productive ecosystems in the world (Environmental Protection Agency, 2001). As in other regions of the world, in Mexico, wetlands serve several functions in the environment, such as storage and filtration of water and as habitat for wildlife (Environmental Protection Agency). These environments are critical stopover and wintering grounds for many of North America's waterfowl and other migratory birds (Wilson & Ryan, 1997) and are of vital importance for fulfilling requirements in the life cycle of migratory and resident Nearctic and Neotropical waterfowl. Unfortunately, they also act as receptors of pollutants over time, and such inputs could have serious consequences for the aquatic biota, outcomes that may not be apparent until they emerge at the population or ecosystem level (Linde-Arias *et al.*, 2008). In Mexico, these ecosystems have undergone processes of transformation for various purposes, but unfortunately a lack of knowledge about their fragility and their inadequate management are among the problems that threaten their conservation (Comisión Nacional del Agua, 2010). One of these consequences is oxidative

stress on the biota, which is defined as a disturbance caused by an imbalance between the production of free radicals or pro-oxidant agents and the antioxidant capacity of an organism. Several pollutants when entering water ecosystems become or mediate reactive oxygen species (ROS; O₂⁻, OH, H₂O₂), which are highly reactive molecules that interact with critical macromolecules such as DNA, proteins, and lipids, leading to physiological disruption. In healthy cells, ROS are metabolized by specific antioxidant enzymes, among the more important of which are catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) (Prior, 2004; Rendón von Osten, 2005; Ochoa & Gonzalez, 2008; Aras *et al.*, 2009). The activity of these enzymes coupled with the damage exerted by lipid peroxidation (LPO) can be assessed and quantified through biomarkers that represent responses to or alterations by exposure to pollutants at several organizational levels: biochemical, physiological, morphological, and histopathological (Rendón von Osten). Evaluation at different levels can provide a comprehensive assessment of the effects of xenobiotics on wildlife, giving an overview of an ecosystem's health (Cazenave *et al.*, 2009).

Because of their relatively high mobility, fish are generally considered the most viable organisms for assessing the effect of pollution in aquatic systems (van der Oost *et al.*, 2003). They also can generate a stress response that involves all levels of organization, from the cellular to the individual and population levels (chronic exposure) (Iwama, *et al.*, 2004). Assessing several biomarkers in an organism yields a set of responses that could complicate the global interpretation. The integrated biomarker response (IBR) method helps resolve this issue, allowing visualization of the interaction among assessed biomarkers, facilitating comparisons between groups or time periods, and aiding evaluation of the global responses of a biomarker assessed in a plot. This study examined oxidative stress in the liver and gills of the native fish *Goodea atripinnis* Jordan, in Yuriria Lake, evaluating oxidative stress with a set of

biomarkers: of LPO, antioxidant enzymes, and histopathological markers.

MATERIAL AND METHOD

Study site. Lake Yuriria, in the state of Guanajuato, is located in the Lerma River basin in the Central Plateau, one of the most polluted regions of Mexico due to the input of domestic sewage, industrial effluents, municipal waste and agricultural runoff (Mestre, 1997). Geographically is located between 101° 12' 03" and 101° 03' 45" west longitude and 20° 17' 22" and 20° 12' 55" north latitude with an elevation of 1766 meters, the lake is in a semi-arid climate (Fig. 1). This system receives agricultural runoff, domestic discharges, municipi-



Fig. 1 Geographic location of Yuriria Lake.

pal sewage and industrial effluents from its two main tributaries: the Lerma River and the La Cinta channel. Among the pollutants that have been detected in water and sediments of the lake are some metals (Cd, Cr, Cu, Fe, Ni, Pb, Zn, Al, Hg), polycyclic aromatic hydrocarbons (benzene, toluene and xylene) and organophosphorus pesticides (López-López *et al.*, 2011).

Water analyses. To evaluate the water chemical characteristics of Yuriria Lake, surface water samples were taken with a van Dorn-type bottle (Wildco Supply Co. Saginaw, Mich.) sampler. Water temperature ($^{\circ}\text{C}$), dissolved oxygen (mgL^{-1}), total dissolved solids (TDS) in gL^{-1} , were measured *in situ* with a Quanta sonde (Hydrolab).

Biochemical oxygen demand (BOD_5) mgL^{-1} , chloride (mgL^{-1}), and fecal and total coliform counts by most probable number method ($\text{MPN } 100 \text{ mL}^{-1}$) were analyzed according to APHA (2005). The concentrations of nitrite (mgL^{-1}), nitrate (mgL^{-1}), ammonia (mgL^{-1}), orthophosphate (mgL^{-1}), hardness (mgL^{-1}), total suspended solids (mgL^{-1}), and color (Pt-Co Units) were analyzed with a Hach DRL 2500 spectrophotometer.

Collection of specimens. The studied species, *Goodea atripinnis*, is a viviparous fish native to the Central Plateau. A total of 136 organisms of *G. atripinnis* were collected, 30 of them were dissected *in situ* to remove the liver and gills that were stored in liquid nitrogen, the remainder organisms were sacrificed and fixed in a 10% formaldehyde solution with phosphate buffer for histological and population analysis.

Biochemical assays. Two groups for biochemical assays were analyzed, the first was with organisms obtained from Yuriria Lake (field group) and the second group was with fish cultivated under controlled conditions in laboratory, free from contaminants exposure (control group). Each group was composed of 30 organisms. Livers of both groups were homogenized while gill archs were removed and were homogenized with a homogenizer Daigger electric model 398.

The level of LPO was estimated in liver and gill homogenates using the method of Buege & Aust (1978), the results are expressed in nmol MDA mg^{-1} protein. The activity of SOD was assessed by the method of Sun *et al.* (1988), expressed as units (U) mg^{-1} protein. Activity of CAT was determined by measuring the consumption of H_2O_2 as indicated by the technique Cohen *et al.* (1970), whose activity was calculated with the constant first-order rate of decomposition of H_2O_2 .

The method of Lawrence & Burk (2012) was used to determine GPx activity using cumene hydroperoxide as

substrate. Its activity is expressed as $\text{nmol NADPH min}^{-1} \text{mg}^{-1}$ protein. Protein content was determined as indicated by the technique of Bradford (1976). All the assays were performed in duplicate.

Histological analyses. A group of 36 organisms were used for histological study, removing the liver and gills. The liver and gills were removed and fixed in 10% formalin for processing by paraffin embedding and thin sections ($7 \mu\text{m}$ thick) were obtained using a microtome Leica®. The latter were stained using hematoxylin & eosin and Masson's Trichrome techniques to obtain micrographs using a photomicroscope Leica ATC 2000 to subsequent analysis of results.

Population analysis. A total of 70 organisms were used to obtain the size classes of *G. atripinnis*, each was weighed (g) with an electronic balance Ohaus® brand Scout II model (with 0.01g accuracy) and standard length (mm) was obtained using a vernier Mitutoyo® digital brand model CD-8 "CS. A frequency histogram was performed by class size and cohorts were detected by the presence of groups with normal distribution, the method of Bhattacharya was used to validation of each group.

Statistical analysis. Biomarker data are presented as the mean value and comparisons between control and field groups were obtained by applying an ANOVA test.

The integrated biomarker response was assessed following the method described by Beliaeff & Burgeot (2002), the mean of each biomarker (x) and tissue ($Y = (x - m)/s$) was assessed, where m is the overall mean of the biomarkers and s is the standard deviation. The minimum absolute value (min) of the Y values of the biomarkers from both groups (field and control) was added to the Y values to standardize the biomarker data. Each standardized biomarker was plotted as a vector in star plots; the IBR was defined by the area enclosed in the polygon formed by joining the extreme of each vector. The IBR value was estimated using the procedure proposed by Beliaeff & Burgeot.

RESULTS

Water quality. Yuriria Lake water was well oxygenated but with high BOD_5 (Table I) and a high content of total dissolved solids. The nutrients N and P were high in the lake, as was particularly evident by the presence of nitrites and ammonia. The lake had alkaline and medium-hard waters and was remarkable for high values for total and fecal coliform (Table I).

Table I. Yuriria Lake water quality parameters.

Parameter	Mean
Dissolved oxygen (mg L ⁻¹)	9.34
BOD ₅ (mg L ⁻¹)	13.89
NO ₃ (mg L ⁻¹)	3.95
NH ₃ (mg L ⁻¹)	1.75
NO ₂ (mg L ⁻¹)	0.0087
Orthophosphates (mg L ⁻¹)	0.58
Hardness (CaCO ₃ mg L ⁻¹)	57.53
Color (Pt-Co Units)	72.86
Total coliform MPN (100 mL ⁻¹)	479
Fecal coliform MPN (100 mL ⁻¹)	86
Alkalinity (mg L ⁻¹)	210.58
Chloride (mg L ⁻¹)	44.13
Conductivity (mS cm ⁻¹)	563.93
Water temperature (°C)	17.64
Total dissolved solids (g L ⁻¹)	0.219
Total suspended solids (mg L ⁻¹)	93.63
pH	8.19

MPN= more probable number.

Population analysis. We identified three size classes for individuals of *G. atripinnis* in Yuriria Lake; Class I included organisms from >1 to 39 mm, Class II organisms from 40 to 59 mm, and Class III organisms of 60 mm or more.

Biochemical assays. In gills, no significant differences relative to controls were found in the LPO (p>0.05) or in the activity of SOD or CAT (p>0.05), but GPx activity was significantly increased compared to controls (p<0.05). In the case of the liver, neither the antioxidant enzyme activities (SOD, CAT, and GPx) nor LPO showed significant differences relative to controls (p>0.05) (Fig. 2).

It is noteworthy that LPO was higher in gills than in liver (p<0.001), while the activity of CAT was higher in liver (p<0.001) and GPx and SOD activities were similar between both tissues (p>0.05).

The IBR in gills showed a clear increase in the field group, with a value 2.11 times higher than that of the controls, mainly based on the response of SOD and GPx resulting from damage by LPO. The control group, by comparison, showed lower LPO and SOD values and increased GPx activity. (Fig.3).

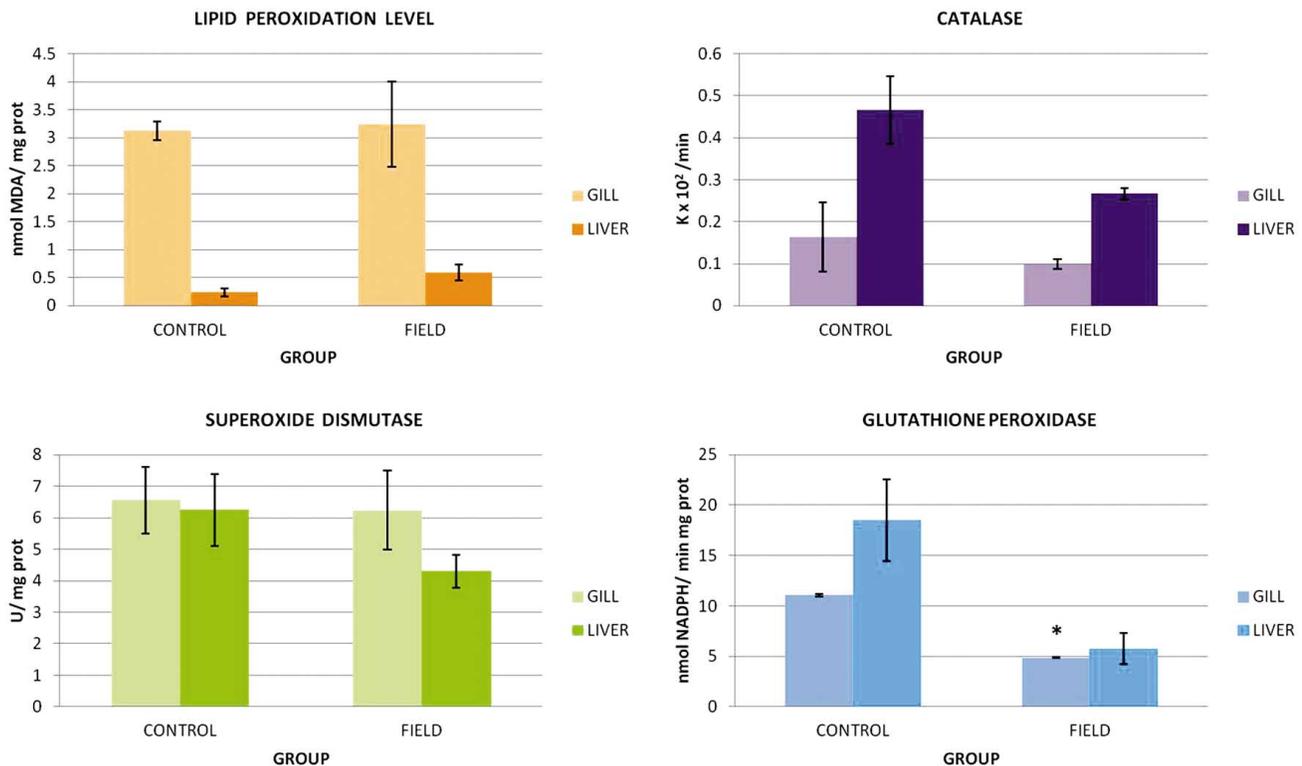


Fig. 2 Mean values of biomarkers in gill and liver of *Goodea atripinnis* for the control and field groups.*= significant difference at p<0.05.

The IBR in liver was lower than in gills in both the control and field groups, attributable to low GPx and SOD activity and a low LPO (Fig. 3). However, the IBR of liver from the field group was 5.25 times higher than that of the control.

Histological analyses. In the gills of the control group, the primary lamellae with their support cartilage were detectable, as were secondary lamellae with their typical shape (Fig. 4a). In liver, hepatocytes typically consisted of a number of strands one or two cells thick with well-defined cell membranes and round nuclei in a central position. Typically, pancreatic tissue was arranged in acini, with each acinus separated from the liver tissue by a layer of connective tissue diffusely distributed within the liver parenchyma (Fig. 4b).

Field group gills of classes I and II did not show changes relative to control; however, Class III showed various features, as follows: hypertrophy in the lamellar epithelium, manifested as epithelium thickening resulting from an increased number of cell layers; the presence of cysts or resistance parasite structures within the connective tissue in the primary lamellae, surrounded by a layer of epithelium; fusion of primary lamellae in the connective tissue and a portion of cartilage; and sphacelation (detachment of the epithelium in tissue) mainly at the apical portion (Fig. 5).

Field group livers of Class I showed the presence of bleeding in different tissue sections, mainly in areas close to blood vessels. In organisms of classes II and III, in addition to the bleeding patterns, some vacuoles were also found

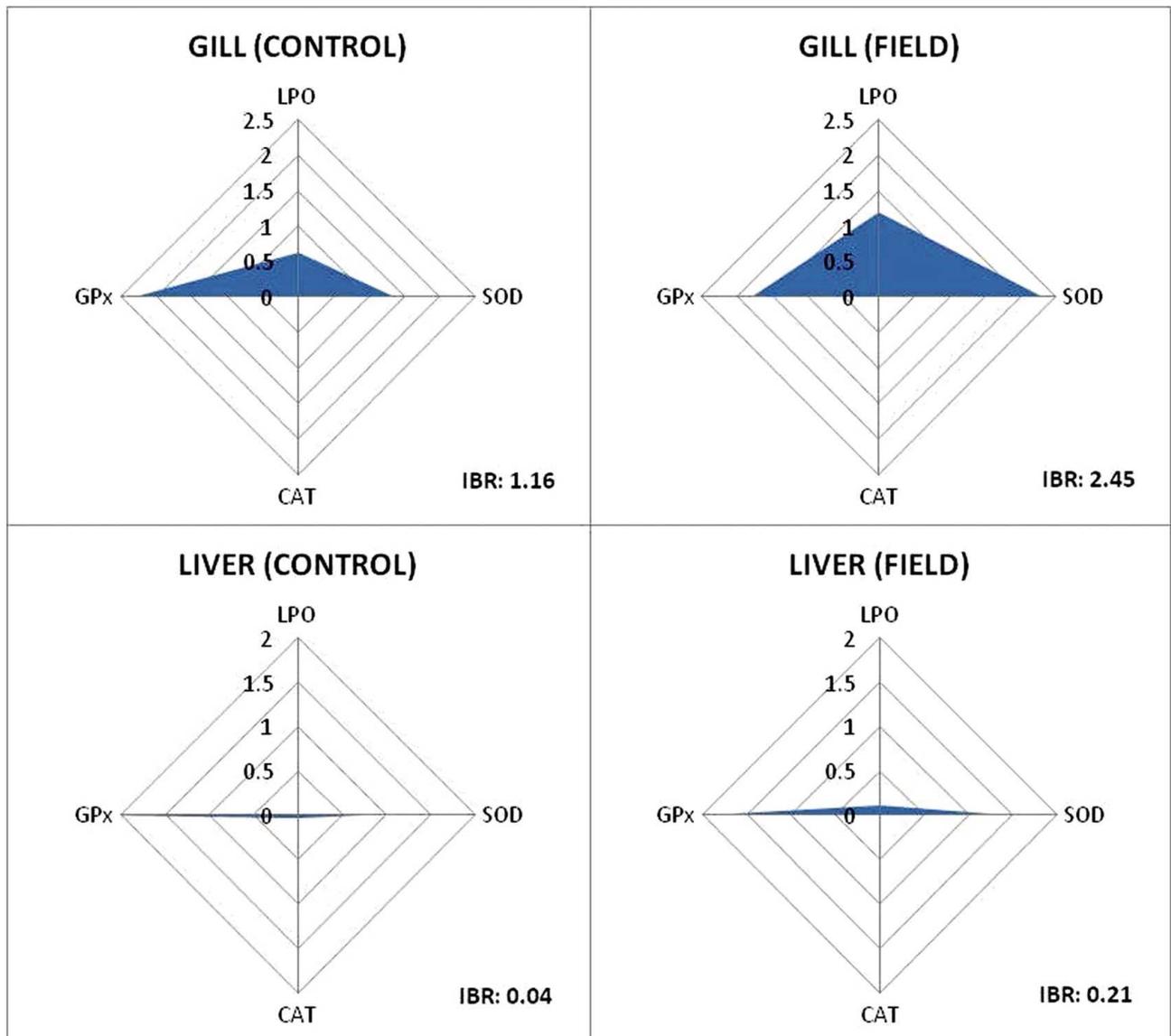


Fig. 3 Integrated biomarker response in liver and gill for *Goodea atripinnis* in the control group and field group.

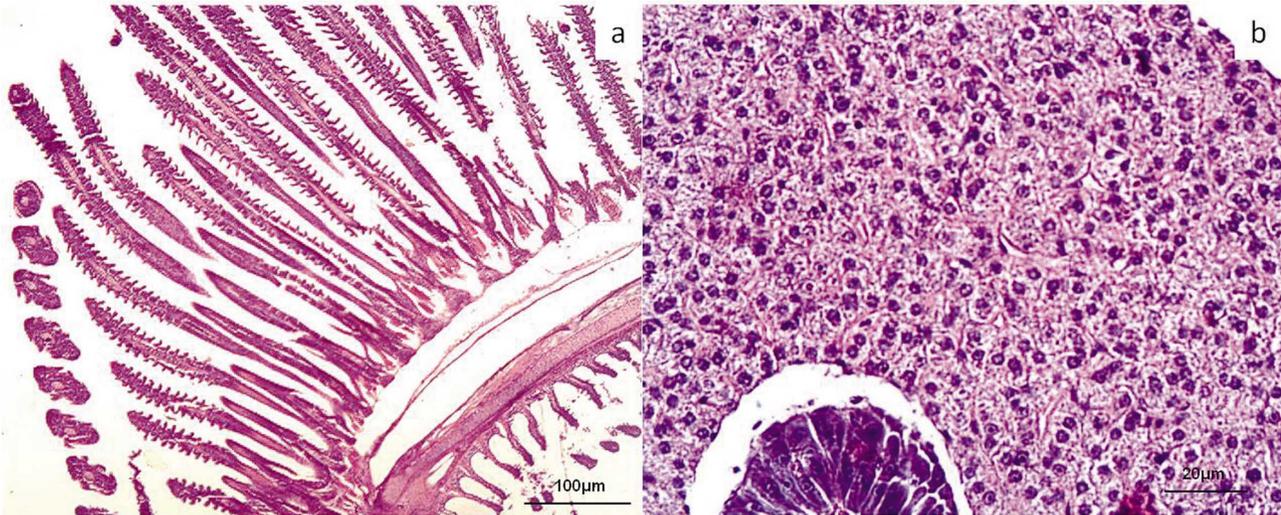


Fig. 4 Gills for control group (a) and liver for control group (b) in *Goodea atripinnis*. Hematoxylin-Eosin. Magnification 10X and 40X.

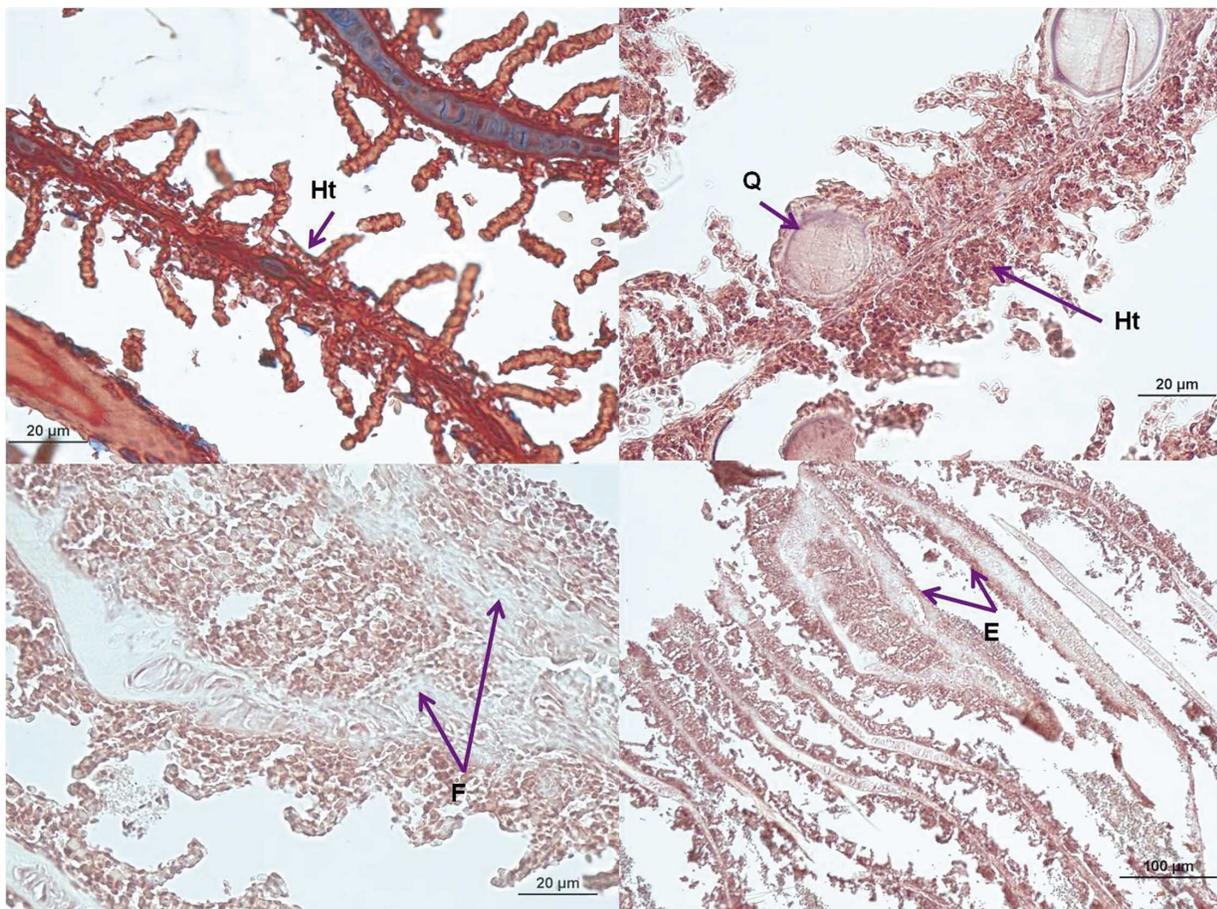


Fig. 5 Gills of *Goodea atripinnis* (field group): hypertrophy in the lamellar epithelium (HT), sphaclation (E), fusion of primary lamellae (F), and “cysts” (Q). Hematoxylin-Eosin and Masson’s trichrome, Magnification 10X and 40X.

displacing the nuclei of the hepatocytes to the periphery. Other organisms showed thickened fibers that appeared either from the blood vessels and areas with pancreatic tissue.

Both the fibers and the presence of vacuoles generated cellular disorganization by altering the typical arrangement of hepatocytes (Fig. 6).

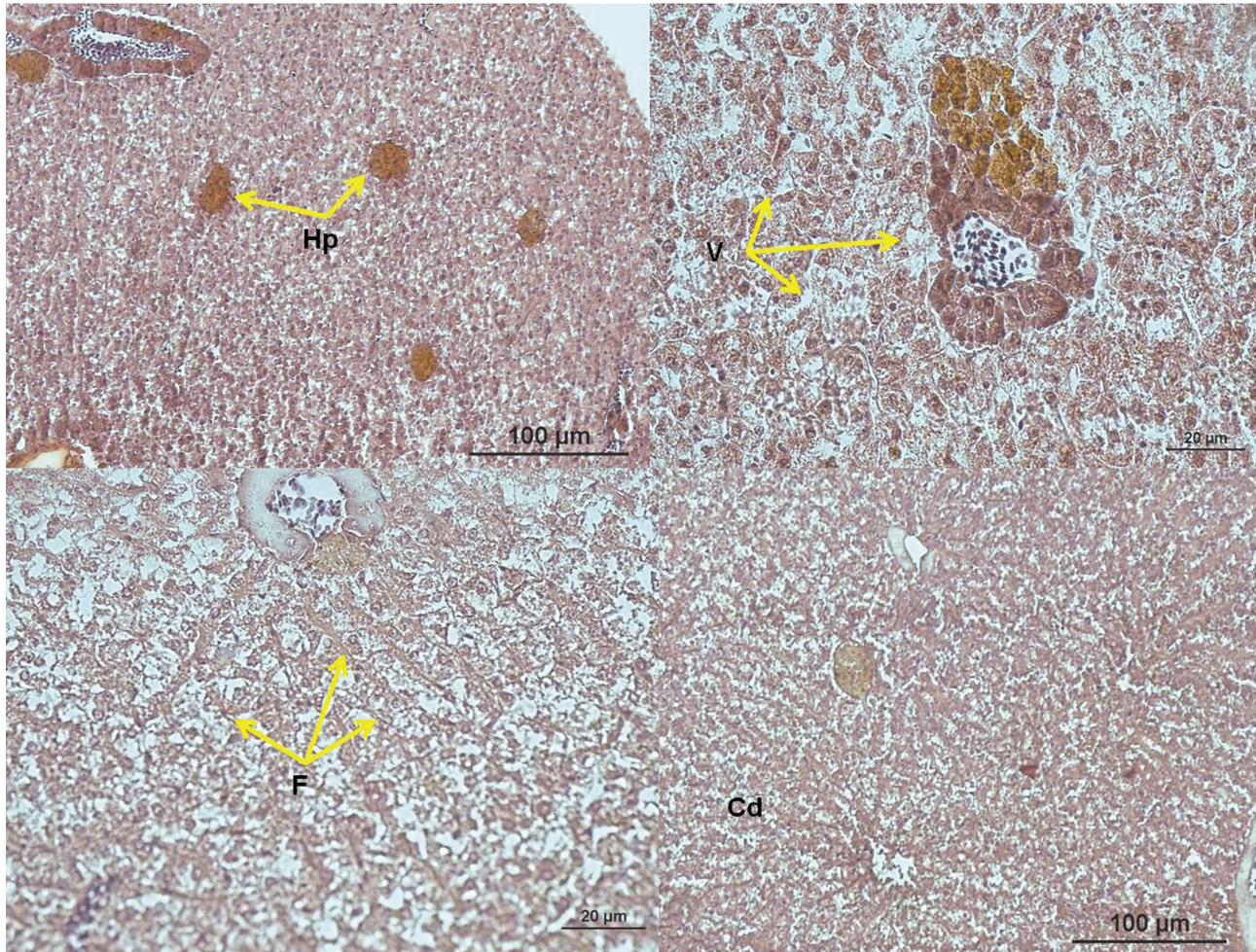


Fig. 6 Liver of *Goodea atripinnis* (field group): vacuoles (V), bleeding process (HP), fibrosis (F), and cellular disorganization (CD). Hematoxylin-Eosin. Magnification 10X and 40X.

DISCUSSION

Water quality. Yuriria Lake is located in one of the most densely populated and polluted zones of Mexico (Mestre). Major activities around the lake include agriculture, which generates the input of several agrochemicals (fertilizer and pesticides), and human settlements, some of which input untreated wastewater into the lake. Furthermore, the main problems with water quality are the inputs from the Río Lerma, which supplies most of the water to the lake; the river drains a large industrial and agricultural region of Mexico along with the input of several pollutants (Sedeño-Díaz & López-López, 2007). As a consequence, water characteristics in Yuriria Lake include evidence of a high level of deterioration. The high values for coliforms are also strong indicators of wastewater inputs.

Biomarkers. Gills. LPO has been widely used as a

biomarker of oxidative stress in environmental assessment studies with different organisms (Achuba & Osakwe, 2003; López-López *et al.*, 2006; Parvez & Raisuddin, 2006; Fernandes *et al.*, 2008; Zhu *et al.*, 2008). In this study, LPO had higher gill values relative to the LPO liver values because the former are in direct and continuous contact with the water and their pollutants; furthermore, in gills, the antioxidant enzymes are less efficient than those of the liver, which increases the vulnerability of the former to ROS (Ahmad *et al.*, 2006). These results are in concordance with those reported by Belge *et al.* (2009), Lopez-Lopez *et al.* (2006), Zhu *et al.*, and Achuba & Osakwe in studies with *Capoeta barroisi* (Lortet), *Girardinichthys viviparous* (Bustamante), *Carassius auratus* (Linnaeus), and *Clarias gariepinus* (Burchell), respectively, who found a higher level of LPO in gills compared to other tissues. However, unlike those

studies, our results indicate no significant difference relative to controls, as also was observed by Ahmad *et al.* and Parvez & Raisuddin in *Channa punctata* (Bloch) and *Anguilla anguilla* (Linnaeus), respectively; this similarity may be attributable to an adaptation of the organisms of Yuriria Lake conditions, manifesting as a similar activity of the antioxidant enzymes SOD and CAT (Achuba & Osakwe). It is noteworthy that the decrease in GPx activity may be attributed to inactivation of the same enzymes by free radicals but also has been related to water turbidity (Ahmad *et al.*).

In some studies, IBR has been used to compare the effect of different concentrations of perfluorinated compounds, as in the cases of *Cyprinus carpio* (Linnaeus) (Kim *et al.*, 2010), assessing stress in the Yangtze River (Wang *et al.*, 2010), using *Carassius auratus* as a study organism for evaluating different regions of the Bay of Cannes (Mediterranean Sea), and with mussels (Damien *et al.*, 2007); most of these studies applied IBR to compare study sites or treatments. In this work, the IBR showed an increase in SOD gill activity in the field group where the level of LPO was also increased, while GPx activity decreased when compared to the control. Furthermore, the CAT activity remained practically similar to the control. This outcome suggests an activation of SOD in Yuriria Lake organisms that could be the result of exposure to xenobiotics, although its increase over the control was not statistically significant. The total IBR value in the gills of the field group (2.45) was double the value of the control group (1.16), indicating that residents of Yuriria Lake face oxidative stress that provokes responses of the antioxidant system (Gül *et al.*, 2004; Iwama *et al.*, 2004; Ferreira *et al.*, 2005; Tejada-Vera *et al.*, 2006; de la Torre *et al.*, 2007; López-López *et al.*, 2011).

Gill histopathology may be indicative of general stress from exposure to various xenobiotics, metals, organic pollutants, toxic algae, and suspended solids (Rendón von Osten). The histopathological changes in this tissue, in general, are a response to exposure to non-specific contaminants (Au, 2004). Hypertrophy observed in the gills and lamellar fusion have also been reported by Fernandes *et al.* and Rondón-Barragán *et al.* (2007) in their studies with *Liza saliens* (Risso) and *Piaractus brachipomus* (Cuvier), respectively; the authors stated that changes in the lamellar epithelium constitute a defense mechanism that increases the diffusion distance between blood and contaminants in water and that these changes affect epithelial permeability, which in turn affects osmoregulation.

Liver. The absence of significant differences in LPO and the activity of antioxidant enzymes in liver can be explained by the fact that this organ has high enzyme activity (SOD

and CAT) (Gül *et al.*); this situation may help the fish face and adapt to oxidative stress conditions where they are living (Belge *et al.*). Our results confirm those described by Ahmad *et al.* and Parvez & Raisuddin, but differ from those reported by Wilhem-Filho *et al.* (2001), López-López *et al.* (2006), Zhu *et al.*, and Achuba & Osakwe, who identified an increase in LPO in liver test organisms exposed to mixtures of pollutants and the activation of antioxidant enzymes in this tissue. On the other hand, Atli & Canli (2010), in their study with *Oreochromis niloticus* exposed to heavy metals, found that the activity of GPx decreased.

In the IBR, the vectors of standardized biomarkers showed a slight increase in the level of LPO related to the control group, and also SOD and GPx activity were increased, revealing a slight induction of these enzymes while the activity of CAT was lowest in both groups. Furthermore, the overall value of the IBR showed that the field group had a response five times greater (0.21) than that of the control group (0.04). The IBR value in contrast to the individual values of biomarkers show that fish living in Yuriria Lake are facing oxidative stress, which could be attributed to the mixture of pollutants to which these organisms are exposed (Gül *et al.*; Iwama *et al.*; Ferreira *et al.*; Tejada-Vera *et al.*; de la Torre *et al.*; López-López *et al.*, 2011).

The histopathology of the liver is a general indicator of fish health status as well as of various urban pollutants and toxic levels (Rendón von Osten). Histopathological lesions in the liver usually are not specific to a single pollutant (Au). Vacuoles found in this study are an indicator of toxic contaminant exposure (Gül *et al.*), which causes different levels of LPO (Wolf & Wolfe, 2005) and also could represent a strategy to reduce the availability of lipophilic xenobiotics (Rondon-Barragán *et al.*). Liver fibrosis is rare, but it is not unusual to see a cirrhotic reaction caused by inflammation or toxic agents (Wolf & Wolfe). Bleeding patterns have not been reported in other studies but may be associated with cell lysis caused by LPO damage (Gül *et al.*).

Our results showed that each tissue and organ displayed a different defense antioxidant status, similar to the results of Wilhem-Filho *et al.* The enzymatic changes are quick adaptive responses, while the histological changes are a slow adaptive response, depending on the level of contamination in the water (Gül *et al.*).

It has been proposed that organisms chronically exposed to polluted environments can develop adaptive mechanisms or compensation (Ferreira *et al.*), meaning that the homeostatic process begins as an integrated set of adaptive responses to adjust metabolic processes coupled with the

effects of pollutants (Iwama *et al.*; de la Torre *et al.*). However, in *G. atripinnis*, the compensation mechanisms at the biochemical level were not enough to avoid damage at the histological level. This pattern could explain the presence of more pronounced damage at the histological level compared to what was found at the biochemical level. Furthermore, due to the complexity of natural ecosystems and the factors influencing the response of individuals, it is difficult to establish precise quantitative relationships between stressors and different responses at different levels of organization (Adams *et al.*, 2000). Studies employing several biomarkers have the advantage of allowing different evaluation criteria for analyzing the action of pollutants in the same organism, providing results that are complementary (Valdez-Domingos *et al.*, 2007). Also, using statistical tools that allow display of the integrated response of the biomarkers studied provides a better understanding of organism responses to stress events. In our study the IBR was a useful tool to make evident the global response of the LPO and the antioxidant enzymes which reflect a greater response than control group and of the individual responses of each biomarker.

Yuriria Lake, due to the inputs that receive from domestic discharge, agricultural runoff, and industrial effluents, is exerting environmental pressure on fish inhabiting the lake and also is causing damage at different levels of organization (biochemical, histological, individual). The oldest organisms of *G. atripinnis* have been exposed to these stress conditions over a long period of time, which can reduce their "fitness" by reducing their lifespan and reproductive capacities. Therefore, the population of *G. atripinnis* in Yuriria Lake could be affected.

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RUIZ-PICOS, R. & LÓPEZ-LÓPEZ, E. Histopatología en las branquias e hígado en el *Goodea atripinnis* Jordan, relacionada con el estrés oxidativo en la laguna Yuriria, México. *Int. J. Morphol.*, 30(3):1139-1149, 2012.

RESUMEN: En los ecosistemas acuáticos, la mezcla compleja de contaminantes presente puede mediar la producción de radicales libres y causar daño por estrés oxidativo a la biota. La laguna de Yuriria (sitio RAMSAR en la parte central de México) recibe aguas residuales de sus afluentes, escorrentías agrícolas y descargas municipales. Se estudió la peroxidación lipídica (LPO), las enzimas antioxidantes superóxido dismutasa, (SOD), catalasa (CAT) y glutatión peroxidasa (GPx); y la histopatología de las branquias e hígado del pez nativo *Goodea atripinnis* en la Laguna de Yuriria. Los resultados obtenidos fueron comparados con un grupo control de peces cultivados en laboratorio. La LPO, SOD y CAT no mostraron diferencia significativa en comparación con los controles; pero la GPx mostró diferencias significativas para ambos tejidos. Se identificaron tres clases de talla; los organismos de la clase I y II tuvieron una ligera vasocongestión en el hígado en comparación con los controles. Los hepatocitos de la clase III mostraron vacuolización citoplásmica, desorganización celular y el hígado mostró una marcada fibrosis. Las branquias de los organismos control y de las clases I y II no mostraron daño en sus filamentos. El daño tisular en los organismos de la clase III incluyó hipertrofia, pérdida de la morfología típica y edema en los filamentos branquiales. La exposición prolongada a las condiciones de la Laguna de Yuriria pudo haber influido en el precario estado de salud de los organismos más longevos.

PALABRAS CLAVE: Humedales mexicanos; Estrés oxidativo; Histopatología; Condición de salud del Pez; Hígado; Branquias.

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