Shallow Lakes of the Mexican Central Plateau: Assessing their Health Condition with Oxidative Stress Biomarkers in Sentinel Organisms

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http://dx.doi.org/10.5772/63350

Abstract

In the Mexican Central Plateau (MCP), due to their long history of geologic instability, numerous fluvial systems that were blocked formed extensive shallow lakes. Environmental conditions of this area have favored the agricultural land use and the settlement of great industrial corridors and cities. Human activities in MCP are largely sustained by intense water use that has led to a high deterioration in the water bodies of this area. We analyze the water quality of two selected shallow lakes of the MCP: Yuriria Lake and Xochimilco Lake and early warning biomarkers of native sentinel species of each lake. Both studied lakes are influenced by the input of complex mixtures of pollutants. We assess water quality index and a set of oxidative stress biomarkers in native endemic species of each lake. Results showed that the input of xenobiotics and changes in the periods of dry and rains in the shallow lakes studied provoke a stronger response in sentinel organisms because dilution effects are minimal in a small water column. Furthermore, resuspension of sediments in shallow lakes can release pollutants to the water column that could exert damage to the health condition of the aquatic biota compromising the survival of endemic sensitive species.

Keywords: Yuriria Lake, Xochimilco Lake, lipid peroxidation, antioxidant defenses, water quality, *Ambystoma mexicanum*, *Chirostoma jordani*

1. Introduction

The Mexican highland has capture the interest of several researchers as this area is considered a biodiversity hot spot [1] and, also, is a biogeographic transition zone between the Nearctic and Neotropical zones. Furthermore, this area has a recent volcanic origin that provides singular habitats giving rise to particular evolutionary process to the development of endemic species [2].

The Mexican Plateau is a large arid-to-semiarid plateau that occupies the northern and central Mexico, ranges from the United States border in the north to the Trans-Mexican Volcanic Belt in the south, and is bounded by the Sierra Madre Occidental and Sierra Madre Oriental to the west and east, respectively. A low east-west mountain range in the state of Zacatecas divides the plateau into northern and southern sections: the Northern Plateau and the Central Plateau.

The Central Plateau (Southern Plateau and also called "Mesa de Anáhuac") is a high land lying at elevations from 1800 to 2300 m, which contains numerous valleys originally formed by ancient lakes [3]. This area was dominated by high volcanic activity; consequently, numerous fluvial systems blocked have formed extensive shallow lakes and swamps on the plateau [4]. The ancient name of this area in Nahuatl language was "Anáhuac," which means "Land on the Edge of the Water." These lakes have faced interesting evolutionary process that leads to the development of endemic species [5].

In this zone, a temperate climate, relatively abundant rainfall, and rich alluvial and volcanic soils create favorable agricultural conditions, and much of the land supports extensive farming as well as cattle grazing in some of the drier basins. Land uses are characterized by agriculture (corn, sorghum, beans, wheat, and sugarcane), industry (sugar refineries and grain mills, mining, textile, oil, chemicals, among others), and urban (the area is densely populated and encompasses major urban centers of Mexico including Mexico City) [6, 7]. This area harbors more than 11% of the total population of Mexico, approximately 11 million people, in <3% of Mexican territory, making this region one of the most densely populated in the country. The human activities and development in Central Mexico are largely sustained by intense water use that has led to a high deterioration in the water bodies of this area [8]. Although the most distinctive feature of the Mexican hydrology involves the presence of fluvial systems, the Central Plateau offers the opportunity to study the scarce but interesting lakes originated along with the Trans-Mexican Volcanic Belt.

The agriculture, industrial, and urban activities provoke the input of anthropogenic pollutants (metals, hydrocarbons, pesticides) to aquatic systems, which form complex mixtures of xenobiotics, with the potential to exert many adverse effects on receiver ecosystems [9]. The assessment of the stress effects on the health of the biota in water bodies is very difficult given that exposure to multiple stressors produces both antagonistic and synergistic effects [10]. The bioindicator approach is a validated bioassessment method that monitors responses of key aquatic organisms (sentinels) as integrators of stress effects and as sensitive response (early warning) indicators of environmental health [11]. This integrated approach involves measuring early warning biomarkers and indicators at different levels of organization, from the individual to the population or community level. When applied in field situations, both of these biological indicators can be extrapolated to enable predictions of damage at higher levels of organization that may not be reversible. Some authors [12] have designed protocols for rapid assessment of the health condition, recommending the use of sentinel species to allow monitoring of the response of a biological marker as well as population-based indicators of various environmental stressors.

Early warning biomarkers are useful tools for environmental assessment, which targets analysis of quantification at different levels of organization: molecular, biochemical, physiological, and morphological changes resulting from stress generated by environmental modifications [13]. Several stressors exert damage via oxidative stress, defined as the imbalance between the production of reactive oxygen species (ROS) and antioxidant defense, causing damage to lipids, proteins, carbohydrates, nucleic acids, as well as to the cell membrane [14].

Various xenobiotics are known to exert oxidative stress due to their redox cycling properties and their potential to produce ROS [14]. Lipid peroxidation (LPO) is one of the most commonly used biomarkers for evaluating oxidative stress; it reflects the action of ROS on lipids and is quantified by the presence of lipid metabolites, such as malondialdehyde. Aerobic organisms have developed antioxidant defenses to eliminate or prevent cell damage caused by ROS [15]; some of these defenses include various enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [16].

For this research, we study the water quality of two selected shallow lakes of the Mexican Central Plateau (MCP): Yuriria Lake (Lerma Basin) and Xochimilco Lake (basin of México), and the oxidative stress responses (lipid peroxidation and antioxidant enzymes) of native sentinel species of each lake, the silverside fish *Chirostoma jordani* (in Yuriria Lake), and the amphibian (Axolotl) *Ambystoma mexicanum* (in Xochimilco Lake).

2. Description of the studied lakes

Yuriria Lake is located in the Mexican Central Plateau in the Lerma–Chapala basin (**Figure 1A**), at 1740 m.a.s.l., with an area of 66 km^2 and a maximum depth of 3.2 m. It is an artificial lake constructed by a diversion of Lerma river, which is their main tributary, the same that carries pollutants such as agrochemicals, heavy metals, organic matter, waste from mining activities, livestock, industrial, urban and rural wastewaters [17]. The western end of the lake receives water intermittently from the channel La Cinta from Cuitzeo Lake, where there are records of the presence of metals such as arsenic, iron, and zinc, as well as discharges from industrial and domestic waste that inputs to the lake [18]. Also, at the western end, the lake receives input from the channel La Cienega from textile and tanning facilities which increases BOD$_5$, ammonia and total suspended solids, fats and oils, phenols, sulfur, chromium, and other heavy metals [19]. This lake is included in the list of Ramsar wetlands since 2004.

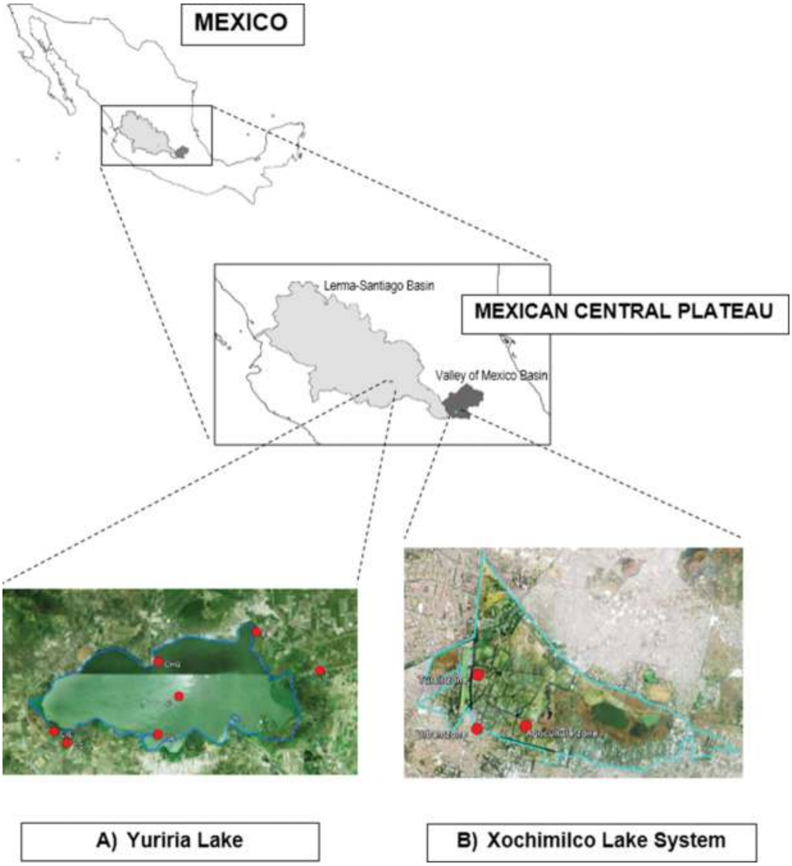

Figure 1. Location of (A) Yuriria Lake and (B) Xochimilco Lake. Circles represent the sampling sites.

The rainy season at Yuriria Lake is from May to September with mean values of 37.5–78.9 mm [20]. In the study period, the lake faced an atypical prolonged drought and the rainfall was delayed with monthly averages of 15 and 16.5 mm in May and August 2009, respectively, and lasted until November (with 10 mm), with values lower than the historical minimum average [21]. Moreover, during May 2010, the mean monthly rainfall was 7 mm; therefore, in this study, the rainy season included the months from May to November 2009 and the dry season from December 2009 to May 2010.

Monitoring was conducted in May, August, and November 2009 and February and May 2010. Seven study sites were established to collect water samples (**Figure 1**): three in the lake (C, CHG, ISL), the three tributaries of the lake (CC, CIE, the diversion channel of the Río Lerma L), and the effluent (E). Specimens of the native fish silverside *Chirostoma jordani* were collected in CHG and ISL with a net (1.5 m high and 4 m in length and 0.8 cm mesh) and a catch effort of 1 h. At each study site, 30 organisms were dissected in the field to get the gills, liver, and muscle for biomarkers assessment. The tissues obtained were transported in liquid nitrogen to the laboratory for later processing.

Xochimilco Lake is a shallow urban water body located in the south of Mexico City with an extension of 26.57 km^2 that belongs to the basin of Mexico. The climate is temperate, ranging from 13 to 25°C, with an average annual temperature of 16°C, an average annual rainfall between 700 and 900 mm, and the rainy season is during the summer (May to October). The lacustrine zone of Xochimilco has a long historical and cultural wealth that includes its high biodiversity and the floating gardens or "Chinampas" [22]. This lake has been one of the main water supplies for Mexico City. Nevertheless, since 1971, the inputs of wastewater in the lake from a treatment facility have provoked water quality depletion and the ecosystem deterioration [23]. Xochimilco Lake is included in the Ramsar list of wetlands since 2004. Three study sites were examined in Xochimilco Lake considering the main land uses that have impact on water quality and generate contrasting conditions: the urban zone (UZ), the tourist zone (TZ), and the agricultural zone (AZ) (**Figure 1B**). Monitoring was conducted in three periods of the year: rainy season (October 2008), the cold drought season (January 2009), and the warm drought season (May 2009). The sentinel organism studied in this lake was the "axolotl" *Ambystoma mexicanum*, a native amphibian that is neotenic and endemic of the basin of México. The species faces a reduction in its populations and is considered critically endangered [24]. The studied organisms were donated specimens that are cultivated in the Unit of Management from the Center of Biological and Aquaculture Researches (CIBAC). Data of oxidative stress biomarkers are from bioassays with the amphibian *A. mexicanum* exposed to elutriates from the three study sites.

3. Acclimatization of *Ambystoma mexicanum*

Acclimatization of organisms: Juvenile organisms of *A. mexicanum* (7 ± 1 cm length and weight of 4.0 ± 0.5 g) were used for the bioassays; organisms were donated by the CIBAC. For acclimatization, organisms were maintained in aquaria for 30 days with hard reconstituted water (100 mg/L $CaCO_3$) [25]. The oxygen saturation levels in the aquaria were maintained by aeration, the temperature was 17 ± 1°C with a photoperiod of 12 h light: 12 h dark. Organisms were fed with *Tubifex* sp. in a daily ration equal to 10% of the wet weight of the amphibians.

4. Sediment elutriation

Sediment elutriates have been useful for evaluating the risk that sediment contamination can exert on aquatic biota and the potential contributions from this matrix to the water column [26]. Elutriates were prepared following the procedure of the USEPA [27]. Water (hard reconstituted water with 100 mg/L $CaCO_3$) was added to sediments in a volume ratio of 1:4 at room temperature (20 ± 2°C). After the correct ratio was achieved, the mixture was stirred vigorously (100 rpm) for 30 min and then was allowed to settle for 1 h. The supernatant was siphoned off without disturbing the settled material and centrifuged to remove particulates prior to chemical analysis (2000 rpm, 30 min). This procedure was done for each study site (UZ, TZ, and AZ) for bioassays.

5. Water Quality Index

In the studied lakes, at each site and study period, dissolved oxygen (mg/L), conductivity (mS/cm), water and air temperature (°C), and pH were recorded *in situ* using a multiparameter probe quanta. Duplicate water samples were collected and transported for their laboratory analysis in dark and cool conditions. In order to quantify hardness ($CaCO_3$ mg/L), color (Pt–Co units) and nitrates (NO_3 mg/L) were determined in the laboratory with a spectrophotometer HACH DRL/2500. The biochemical oxygen demand (BOD_5 mg/L), alkalinity (mg/L), chloride (mg/L) and total and fecal coliform (most probable number, MPN) were quantified according to the APHA [25] procedures. Water Quality Index (WQI) was estimated according to [28], which evaluates on a scale of 0–100.

6. Oxidative stress biomarkers

The set of oxidative stress biomarkers in the sentinel organism of each studied lake includes the level of lipid peroxidation (LPO), and the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). LPO was determined using the method of [29] in homogenates of the study tissues, previously washed with Tris–HCl, pH 7.4. The results were determined by reading the absorbance of the pink adduct obtained at 535 nm using the molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. CAT was determined using the technique of [30], measuring the consumption of H_2O_2 at 480 nm. SOD activity was evaluated by reading absorbance at 560 nm by the method of [31]. The enzymatic activity of SOD was expressed as units (U) per mg protein. CAT activity was calculated as the rate constant of the first order of the decomposition of H_2O_2. GPx activity was measured with the method proposed by [32] using cumene hydroperoxide as a substrate, and the oxidation of NADPH was determined spectrophotometrically at 340 nm reading for 4 min. The enzyme activity was calculated as nmol NADPH oxidized per minute per mg protein, using the molar extinction coefficient of 6.22×10^6 M^{-1} cm^{-1}. All assays were assessed by triplicate.

7. Statistical analysis

Analysis of variance (ANOVA) was performed to determine the existence of differences: in biomarker responses between sites and periods of study. All statistical analyzes were performed using the software XLSTAT-Pro 2015.

8. The study case of Yuriria Lake

8.1. Water quality

Mean WQI values ranged from 55 to 70, with a global mean of 65.85 ± 4.74 for all sites and study periods. However, in the limnetic zone, mean values ranged from 63 to 70, and the center of the lake (C) was the site with the highest value. The tributaries, especially CC, showed the lowest average value (55), and the global mean of tributaries was 58.3 ± 3.5. The mean value for the effluent was 61 (**Figure 2A**). Mean values of WQI for periods of study varied from 57 to 66, with an overall mean of 61 ± 3.28; August was the month with the lowest WQI and May 2010 had the highest (**Figure 2B**).

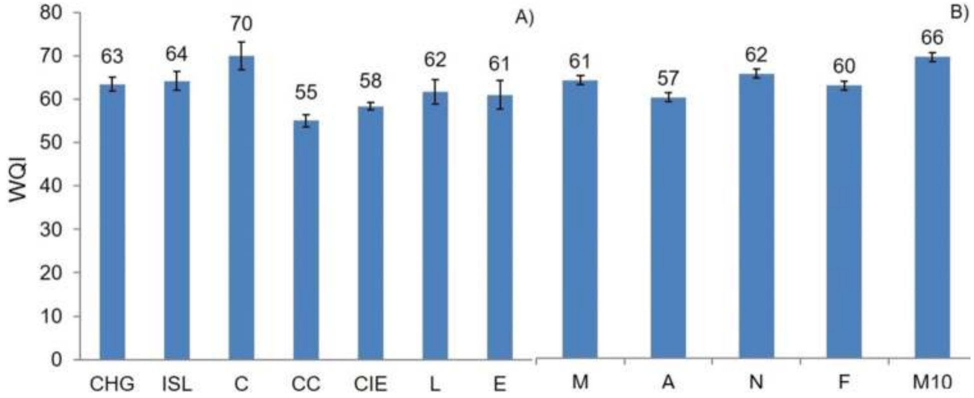

Figure 2. Mean values of WQI in Yuriria Lake. (A) Study sites and (B) periods of study.

8.2. Oxidative stress biomarkers in the fish silverside *Chirostoma jordani*

In general, the gills presented higher values of LPO than liver and muscle. In gill, May 2009 was the month with the highest levels and November the lowest ($p < 0.05$) (**Figure 3A**). In the liver, the level of LPO increased markedly and significantly during November and February in the ISL and May 2010 at both sites, compared to other seasons ($p < 0.05$) (**Figure 3A**). In muscle, the highest level of LPO was observed during February and May 2010 at both sites, while for the rest of the season, LPO values did not differ significantly (**Figure 3A**).

The activity of SOD and GPx significantly increased during November ($p < 0.05$), mainly in the gills, compared to the rest of the seasons (**Figure 3B, C**). CAT activity also showed higher values during November; however, its highest value was found in gills during February in CHG (**Figure 3D**). In general, for both sites, the activity of antioxidant enzymes decreased from February to May 2010 ($p < 0.05$) (**Figure 3B, C**).

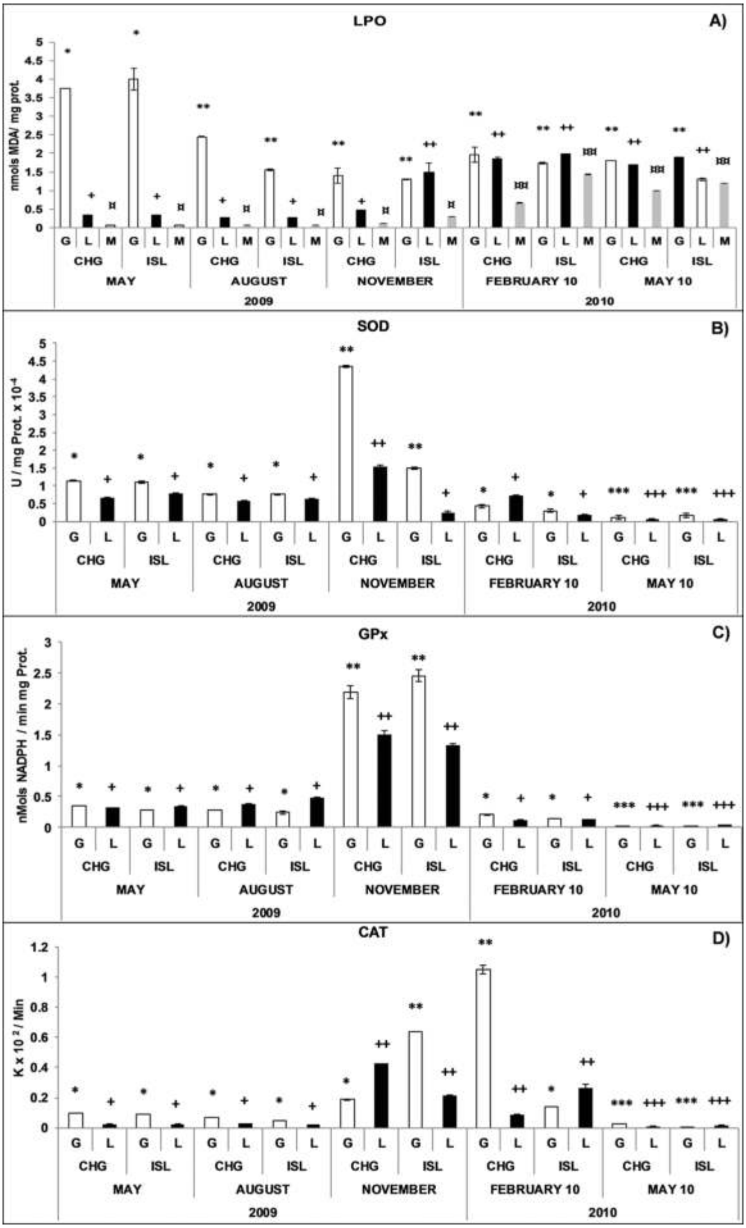

Figure 3. Oxidative stress biomarkers of *C. jordani*. (A) Lipid peroxidation (LPO) in gills (G), liver (L), and muscle (M); (B) superoxide dismutase activity (SOD); (C) glutathione peroxidase activity (GPx); and (D) catalase activity (CAT). * indicates significant differences between sites and seasons, gills (*), liver (+), and muscle (¤). The level of significance was put at $p < 0.05$.

8.3. Somatic indices

The assessment of somatic indices revealed that the condition factor (K) displayed small variation between sites and between periods with no significant differences ($p < 0.05$). The hepatosomatic index (HSI) showed significant differences between sites in August and between periods in May, and values were significantly lower than those of other periods ($p < 0.05$). The gonadosomatic index (GSI) showed the greatest variation, with a clear reproductive peak during May 2010, which differed significantly ($p < 0.05$) from the values found in the other seasons (**Figure 4**).

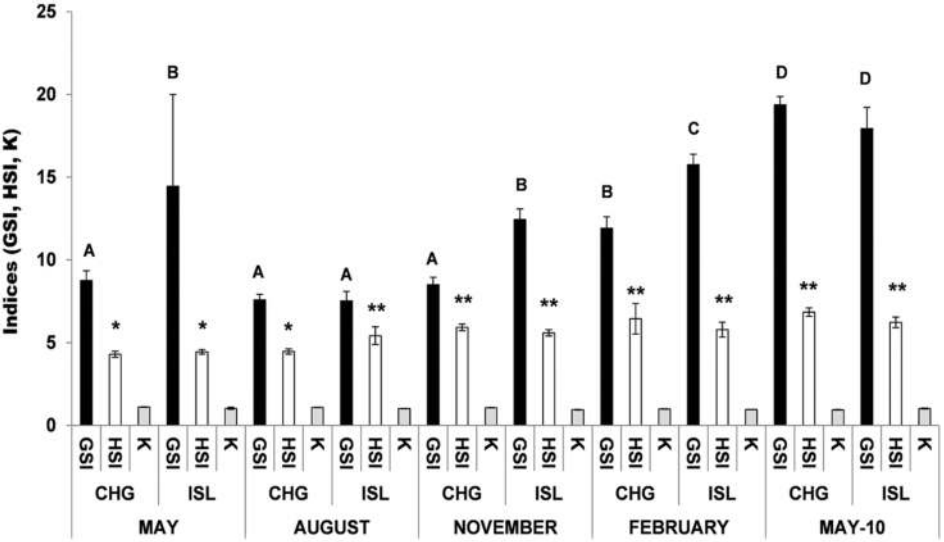

Figure 4. Somatic indices for *C. jordani*. The letters indicate differences statistically significant between GSI, and (*) indicates differences statistically significant between the HSI. The level of significance was put at $p < 0.05$.

9. The study case of Xochimilco Lake

9.1. Water quality

Xochimilco Lake has highly mineralized water and fertilized; conductivity showed higher values (mean value of 791 mS/cm), and the water is highly alkaline (137–288 mg/l), rich in carbonates ($CaCO_3$ 69–120 mg/l) and sulfates (38–67.5 mg/l). The WQI fluctuated from 45.78 to 61.87 in the UZ in June and in the AZ in January, respectively, with a global mean value of 54.48 (**Figure 5A, B**). There were no significant differences between study sites, neither between periods; however, the AZ showed a trend to reach the higher values, particularly in November and January (**Figure 5C, D**).

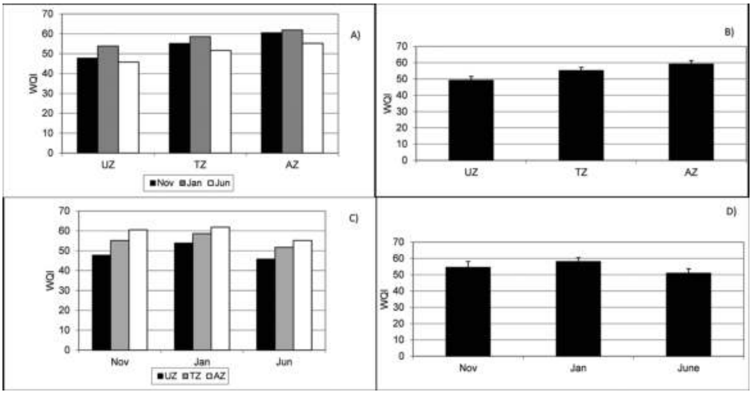

Figure 5. Mean values of WQI in Xochimilco Lake. (A) Study sites, (B) mean values by study sites, (C) periods of study, and (D) mean values by periods of study.

9.2. Oxidative stress biomarkers in the axolotl *Ambystoma mexicanum*

The LPO levels in gills showed the higher values in June, at the beginning of the rainy season, in the three study sites, while LPO levels in liver showed the higher values in AZ in November, in the UZ and TZ in January, and in TZ in June (**Figure 6A**). On the other hand, the antioxidant activities of SOD and GPx showed a depletion in January (during the winter) and June (rainy season) and an increase in activity in November, in all tissues and study sites (**Figure 6B**, **C**). However, CAT activities in gills showed a very slight fluctuation between periods and study sites, but CAT activity in liver showed a trend to increase in January (in TZ and AZ) and in June in TZ (**Figure 6C**).

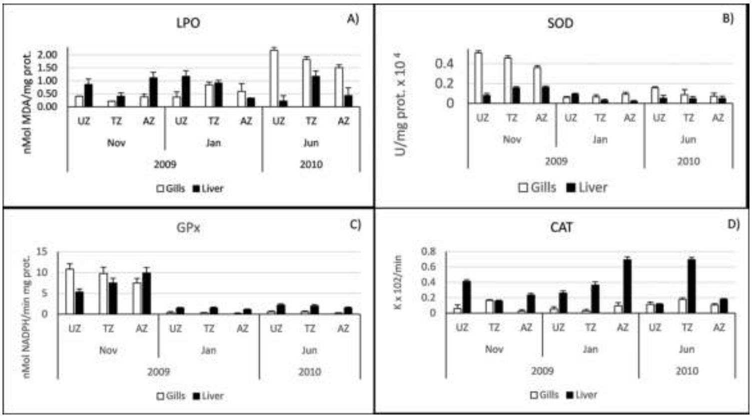

Figure 6. Oxidative stress biomarkers of *A. mexicanum*. (A) Lipid peroxidation (LPO) in gills (G) and liver (L), (B) superoxide dismutase activity (SOD), (C) glutathione peroxidase activity (GPx), and (D) catalase activity (CAT).

10. The importance of using biomarkers in sentinel organisms to assess environmental lake conditions

Yuriria Lake displays the general problem of water quality in the Mexican Central Plateau and the Lerma–Chapala basin, urban, and industrial wastewater discharges, and leachates of agrochemicals [33]. Particularly, the middle Lerma (where Yuriria Lake is located) is recognized as the most polluted area, with WQI scores between 41.1 and 54.2 in 1999 [8, 17]. In the Lake, the WQI ranged from 55 to 70, showing higher values than those of the Lerma River. All the tributaries showed lower WQI, and previous studies recorded that the type of pollution that inputs to the lake differs, forming a mix of xenobiotics with potential to exert damage to the aquatic biota. The tributaries CC and CIE receive discharges from a textile industry, which produces various alkaline residues [34]. The tributary L, which showed the lowest WQI scores, receives discharges from different pollution sources, including oil, mining, and waste disposal from various industries [18].

The toxic effects of the mixture of xenobiotics in aquatic ecosystems depend on the bioavailability and persistence of contaminants; the susceptibility of organisms to accumulate them; and the interference of these compounds with biochemical, physiological, and ecological processes [14]. In Yuriria Lake, our results suggest that the silverside *C. jordani* faces oxidative stress, indicating that the xenobiotics in the lake are able to produce pro-oxidants. The highest LPO levels were observed in gills and liver. Gills are the first organ of contact with water and thus are exposed directly to any xenobiotic in the aquatic environment.

Exposure to various xenobiotics can promote the formation of ROS and induce oxidative stress [16]. An increase in the level of LPO in liver was observed at the end of the rainy season (November) and during the dry season (February and May 2010), making evident the seasons when the pro-oxidant agents in Yuriria Lake trigged oxidative stress.

An increase in LPO levels in fish can trigger an antioxidant response as a defense mechanism to prevent cell damage caused by pro-oxidant agents [15] and could be expressed as increased or depleted in antioxidant enzyme activities [13]. In both cases, the result is impairment to the antioxidant system. In *C. jordani* inhabiting Yuriria Lake, both responses were detected: stimulation of the antioxidant activity in November, when LPO levels in liver and muscle began to increase; and depletion in antioxidant activity during May 2009 with the highest levels of LPO in the gills, and May 2010 when LPO in liver and muscle increased. Some authors [35] have indicated that complex mixtures of contaminants entering water bodies are not stable in time and space. Others [36] have found that a catfish exposure to metals such as isolated Cu, Zn, Pb, Cd, and As can provoke an increase in GPx activity and inhibition of CAT, but in combination, such as Cu and Zn, CAT activity increases. In addition, an increase in CAT activity has been found in fish exposed to polychlorinated biphenyls and polycyclic aromatic hydrocarbons and herbicides such as paraquat and glyphosate [37]. In our study, in November, the highest GPx and SOD activities were detected, but in May, these enzymes showed a decrease; these responses show that the fish is able to adapt to the fluctuating stressors in the lake by inducing additional synthesis of antioxidant enzymes to regulate oxidative stress.

The coupled biological responses of *C. jordani* with environmental fluctuations allowed the identification of critical periods in the health of *C. jordani*, which occurred in February and May 2010 (dry season), when the higher LPO, the lower the antioxidant response, and the lowest K was observed in contrast to the higher WQI scores detected in the same period. The WQI did not include xenobiotic assessment, and consequently, the biological responses assessed in *C. jordani* (biomarkers, somatic indices) are more sensitive as indicators of the lake condition. The drought has been recognized as one of the critical periods in fish health because during this period, the dilution capacity of aquatic ecosystems is low, which increases the risk of exposure to high concentrations of pollutants [38].

Xochimilco Lake is an urban lake that receives the inputs of pollutants from various sources. Urban lakes are impacted by atmospheric deposition, runoffs from urban areas, and rainfall containing a wide range of pollutants [39]. The present study highlights the damages exerted by sediment elutriates in the amphibian *A. mexicanum*, even when all the components of the mixture of pollutants in elutriates were not identified. The LPO reaction mechanisms can damage the lipids found in the cell membrane, altering their cohesion, flowability, permeability, and metabolic function and lead to membrane instability and cell death [14].

Various studies have reported that LPO is an indicator of the toxic action of contaminants, which leads to loss of cell function by oxidative stress. The LPO results in this study showed it is a suitable biomarker that provides important qualitative and quantitative information about the effects of toxic exposure from elutriated sediments from Lake Xochimilco. The most severe damage were detected in June (the rainy season), in the three study sites. During the rainy season in urban lakes, the runoff can lead to the input of several potential pro-oxidant contaminants that can be incorporated to the sediment and exert LPO. The depletion of the antioxidant enzymes SOD and GPx makes evident that January and June are periods were pro-oxidants are more bioavailable and that can exert a severe damage in the defense mechanisms of the sentinel species. Some authors [40] have found that exposure of amphibians to pollutants increases the production of ROS, causing subsequent alterations in the antioxidant defenses. Amphibians have distinctive characteristics, such as their aquatic–terrestrial life and the semipermeability of their skin, that make these species useful as bioindicators [41]; however, there are few studies evaluating amphibian ecotoxicology from urban ecosystems, as the case of Xochimilco Lake.

11. Concluding remarks

The data presented in the study of Yuriria Lake make evident that due to the shallowness of the lake the changes in the periods of dry and rains exert strong influence in the response of sentinel organisms detected by their biomarkers and bioindicators measurements. The input of xenobiotics in Yuriria lake, being a shallow water body, provokes a stronger response in sentinel organisms because dilution effects are minimal in a small water column; consequently, the health condition of the aquatic biota of this lake is more vulnerable to be affected during long dry periods or by the delay of the rainy season; furthermore, once the rainy season began, the lixiviation of xenobiotics from the basin can be incorporated in the lake and also can provoke stressful conditions, also by the scarce dilution potential of a shallow lake.

In the case of Xochimilco Lake, the results showed that the elutriated sediments from the three study sites of Lake Xochimilco were able to induce damages by LPO and in the antioxidant defense of the amphibian, which can compromise several physiological functions and ultimately the life cycle of the amphibian. Since the runoff of pollutants is a common event in urban lakes and sediment resuspension is a frequent process in shallow lakes, the biological responses observed in *A. mexicanum* after exposure to sediment elutriates reflect the risks that this amphibian faces in Lake Xochimilco, due to the toxicological potential of the sediments.

The integrated assessment of aquatic ecosystem approach (assessing environmental condition and biological responses) is more robust than just the physicochemical approach since offers information about the health condition of the aquatic biota. The studied lakes of the Mexican Central Plateau, a zone with high degree of endemisms of aquatic species, are facing stressful conditions that endangered the health of endemic species; thus, it must be a priority to provide measures of conservation to the improvement of the health condition of these aquatic ecosystems.

Acknowledgements

This study was financed by the Research and Postgraduate Studies Secretariat (SIP) of the National Polytechnic Institute (IPN-México) (Project 20100382) and Federal District government, México (ICyTDF/325/11).

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