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Freshwater Fish as Sentinel Organisms: From the Molecular to the Population Level, a Review

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1. Introduction

Fish around the world are found occupying almost any aquatic habitat. In particular, freshwater fish are severely threatened as the freshwater ecosystems are considered the most endangered of the world [1]. The ultimate destination of most contaminants is water; rivers, lakes, aquifers, or sea, are receptors of wastewaters with a complex mixture of xenobiotics. The variety of contaminants and their mixtures that daily reach the water bodies coupled with a multitude of irresponsible water management practices and destructive land uses, are currently threatening freshwater ecosystems [2], such is the case of discharge of municipal and industrial wastewaters, deforestation, increase of land crops, and water extraction from water bodies to human consumption and other uses. The impact of contaminants in an aquatic ecosystem is complex, therefore has increased the need for determining the ambient status in order to provide an indication of changes induced by anthropogenic activities and their influence on aquatic organisms. As physicochemical analyses shed no light on the biological status of ecosystems, a biological approach is needed to evaluate environmental health; moreover, the biological effects of contaminant interactions cannot be expressed by physicochemical investigations [3].The aquatic ecosystem health is often reflected by the health of organisms that reside in that system. Fish in their natural environments are typically exposed to numerous stressors including unfavorable or fluctuating temperatures, high water velocities and sediment loads, low dissolved oxygen concentrations, limited food availability, and among other types of natural episodic variables. In addition, anthropogenic stressors such as contaminant loading can add to the insults that fish may already experience in many systems. All these factors, individually or together, can impose considerable stress on physiological systems of fish and impair their health [4, 5]. Environmental contaminants are known

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to induce measurable biochemical changes in exposed aquatic organisms [6]. Likewise, stressors can load or limit physiological systems, reduce growth, impair reproduction, predispose fish to disease, and reduce the capacity of fish to tolerate additional stressors. Many species of fish, in particular those species near the top of the food chain, are generally regarded as integrators of environmental conditions and may reflect, therefore, the health of aquatic ecosystems [4] and therefore, they are excellent indicators of the relative health of aquatic ecosystems and their surrounding watersheds [7]. Thus, effects of contaminants on aquatic organisms may be manifested at all levels of biological organization (in a hierarchical scale that can be at cellular level, organisms, populations, communities, and ecosystems). In this way, the measuring of a suite of indicators across such levels of organization is often necessary to assess ecological integrity; these indicators also should include molecular, biochemical, physiological, population, community, and ecosystem responses.

The indicators allow us to isolate key aspects of the environment from an overwhelming array of signals [8]. Ecological indicators have been defined as measurable characteristics of the structure (e.g. genetic, population, habitat, and landscape pattern), composition (e.g., genes, species, populations, communities, and landscape types), or function (e.g., genetic, demographic/life history, ecosystem, and landscape disturbance processes) of ecological systems [9]. On the other hand, other authors [10], established that bioindicators are organisms or communities of organisms, which reactions are observed representatively to evaluate a situation, giving clues for the condition of the whole ecosystem; Gerhardt also indicate that bioindicators are species reacting to anthropogenical effects on the environment, concluding that a biological indicator would be: a species or group of species that readily reflects the abiotic or biotic state of an environment, represents the impact of environmental change on a habitat, community or ecosystem or is indicative of the diversity of a subset of taxa or the whole diversity within an area. In this sense, the primary role of ecological indicators is to measure the response of the ecosystem to anthropogenic disturbances [9]. A sentinel species can be defined as any domestic or wild microorganism, plant or animal, that can be used as an indicator of exposure to and toxicity of a xenobiotic that can be used in assessing the impact on human and/or environmental health because of the organism's sensitivity, position in a community, likelihood of exposure, geographic and ecological distribution or abundance [11].

The specific objective of this review is to provide a short framework of effects of xenobiotics on the responses of freshwater fish across molecular to population level when have been exposed to environmental stressors. Likewise, the present review considers the use of fish as sentinel organisms to assess the anthropogenic impacts over the freshwater ecosystems. The review asks whether fish can be able to reflect the environmental damage from molecular to population levels. Also, the present review offers a selection of examples of studies employing fish as sentinel organisms in ecological, toxicological and environmental risk assessments.

2. Suborganismal responses

When an organism is exposed to stressors like contaminants or a mixture of them, energy is demanded to deal with that stress [4]; stressors tend to impact ecosystems at lower levels of organization first [12]. One of the methods to quantify the exposure to xenobiotics and its potential impact on living organisms is the monitoring by the use of the so-called biomarkers [13]. Biomarkers have been defined by several authors, all of them, in reference to biological responses to contaminants exposure, as a) measurements in body fluids, cells or tissues indicating biochemical or cellular modifications due to the presence and magnitude of toxicants, or of host response [8]; b) a change in a biological response (ranging from molecular through cellular and physiological responses to behavioral changes) which can be related to exposure to or toxic effects of environmental chemicals [14]; c) any biological response to an environmental chemical at the subindividual level, measured inside an organism or in its products (urine, faeces, hair, feathers, etc.), indicating a deviation from the normal status that cannot be detected in the intact organism [15]; d) a xenobiotically induced variation in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or samples [16]; e) contaminant-induced physiological, biochemical, or histological response of an organism, and f) as functional measures of exposure to stressors expressed at the sub-organismal, physiological or behavioural level. Considering these definitions of biomarkers, we could adopt our own definition: "*any biological measurable response from an organism, induced by the exposure to a xenobiotic or complex mixture of them*". Biomarkers can provide valuable information in field or semifield testing and be used to measure a wide range of physiological responses to chemicals at the biochemical, cellular, or tissular level [17].

In concordance with [18] and other authors [19, 20], biomarkers have been classified in three different categories: a) biomarkers of exposure, which represent responses such as induction or inhibition of specific enzymes involved in biotransformation and detoxification as a consequence of chemical exposure [21], b) biomarkers of effect, are any changes in a biological system that reflects qualitative or quantitative impairment resulting from exposure [20], including responses measurable at level biochemical, physiological or some other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease [19], and c) biomarkers of susceptibility, which serve as indicators of a particular sensitivity of individuals to respond to the challenge of exposure to a effect of a xenobiotic or to the effects of a group of such compounds, in this case, individual changes included genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure [19]. However, other authors have been subdivided the biomarkers in exposure biomarkers, effects biomarkers and predictive biomarkers.

Responses of fish at suborganismal level to xenobiotic exposure are complex and varied and depending of type of contaminant and time of exposure. The most general effect of xenobiotics on fish is oxidative stress, which is experienced when antioxidant defenses are overcome by prooxidant compounds. Oxidative stress include a variety of oxidative reactions, usually started by free radicals and propagated by molecular oxygen, which results in the oxidation of lipids, proteins, and nucleic acids [22]. Free radicals are atoms, molecules, or ions with un-

paired electrons on an otherwise open shell configuration. These unpaired electrons are usually highly reactive due to which radicals are likely to take part in chemical reactions. Very often free radicals are confused with reactive oxygen species (ROS) such as molecular and singlet oxygen, superoxide anion, hydroxyl radical and some their derivatives; however, hydrogen peroxide is not a radical, but it is a reactive species because has higher activity than molecular oxygen [23]. Hydroxyl radical is the most important free radical of biological importance, because of its potent oxidative potential and indiscriminate reactivity with cellular components of enzymes and DNA [24, 25]; likewise, being oxidant, all ROS are agents which at high concentrations are toxic to cells. Oxidative stress is a risky condition in which increases in free radical production, and/or decreases in antioxidant levels can lead to potential damage. The antioxidant system in aerobic organisms includes several biochemical safety mechanisms such as antioxidant enzymes and other compounds like vitamins, glutathione, matallothioneins, and others.

Antioxidant defense enzymes are induced by various environmental pollutants under prooxydant conditions among these enzymes we can found superoxide dismutase (SOD), Catalase (Cat) and Glutation Peroxidase (GPx), Glutation reductase and Glutation S Transferase (GST, catalyze the nucleophilic conjugation of different biologically and potentially carcinogenic compounds [20]). The role of SOD is to catalyze the reaction of superoxide radical (O_2^-) to peroxide (H_2O_2). CAT detoxifies H_2O_2 to H_2O and O_2. GPx detoxifies mainly organic peroxides. CAT is an enzyme with high biological relevance because reduce the concentration of peroxide, a precursor of OH-, which is a highly reactive toxic form of ROS [26]. Cytochrome P450 monooxygenases (CYPs) are a multi-gene family of enzymes that play a key role in the biotransformation of pollutants, such as dioxins, pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). One of the most common and highly conserved is the CYP1A subfamily. The CYP1A biomarker is widely used as a biomarker of effect both in vertebrates and invertebrates for environmental biomonitoring, especially in marine bivalves and fish. The induction of CYP1A is triggered via the cytosolic aryl hydrocarbon (Ah) receptor due to exposure to pollutants, such as PCBs, dioxins, and numerous PAHs. CYP1A activity is typically measured using the substrate ethoxyresorufin, which is o-deethylated by ethoxyresorufin-O-deethylase (EROD) to a fluorescent product, resorufin, which can be easily measured. Because EROD activities are generally measured using liver homogenates that also tend to accumulate numerous CYP1A substrates, activity may be inhibited by residual substrates or metals [20].

3. Individual responses

Morphological alterations is one of the most individual level parameters that are measure to identified damage in sentinel organisms [27, 28]. In reference [28] identified severe histological damage in gills and liver of *Goodea atripinnis* a goodeid fish from Central Mexico, after the chronic exposure to Yerbimat, an herbicide with glyphosate. After 75 days of exposure to pesticide, they found lamellar hypertrophy and leukocyte infiltration in gills, and hepatocytes with vacuolization in the cytoplasm and piknotic nuclei in liver, concluding that Yerbimat

induces histological alterations in the gills and liver that might impair normal organ functioning that could lead to health damage in fish because of the important physiological roll of these organs.

Also, haematological parameters, such as erythrocyte and leucocyte count, erythrocyte indices and thrombocyte number vis-a-vis coagulation of blood has been considered bioindicators of toxicosis in fish following exposure to xenobiotics [29]. Any alteration in normal cellular components (morphology and number), and level of fluids of blood is named dyscrasia. Some authors [29] carry out an extensive review over dyscrasia in fish. They show several examples over alterations in morphological changes in blood cells, total count, haemoglobin content, thrombocytes and clotting time, concluding that haematological parameters are not specific to faced acute and chronic exposure of fish to xenobiotics, mainly organochlorinated, organophosphonate, pyrethroid and carbamate pesticides.

4. Population responses

The status of a fish population is a reflection of the overall condition of the aquatic environment in which that population resides. As such, fish population characteristics can be used as indicators of environmental health. Although changes in population structure may act as a sensitive indicator of changing environmental conditions, the timing, degree and nature of the feedback response to altered conditions will vary with the intensity, identity and the number of stressors, as well as the availability of energy [30].

Bioindicators are responses to environmental effects that occur at higher levels of biological organization than sub-organism (biomarkers); This kind of responses can be measured at different high levels of biological organization, from individual, through population (reproductive success, mortality, size distribution, reduction in abundance and biomass), community (primary production, disruption of the nutrient cycle) to ecosystem levels [31, 32], whose main characteristic is that the measure change with exposure to negative environmental factors. Organosomatic indices are common approaches for assessing fish health [4]. In this review we consider three organosomatic indices: Condition factor (CF), hepatosomatic index (HSI) and gonadosomatic index (GSI).

Since the nutritional status of fish can change according to different factors, such as season, food availability, and among they the exposure to xenobiotics, is important to have a measure of corporal condition of fish. The chronic exposure to contaminants may cause changes in feeding behavior, leading to a deterioration of the health. The CF is a frequently used index for fish biology study, as it furnishes important information related to fish physiological state [33]. It is a measure of corporal condition since measuring the body mass associated with the body length. A fish that is heavier for a given length (higher CF) is considered to be a healthier fish, because extra weight means extra energy reserves. While a lighter fish lack energy reserves and therefore, tend to be more susceptible to environmental stressors. A low body condition may also suggest muscle wasting (proteolysis) indicating a starvation response [34]. It has also

been suggested that females with a lower body condition reduce reproductive investment yet still have an increased risk of mortality.

The liver plays a major role in the metabolism of xenobiotic compounds with biochemical alterations occurring under some toxic conditions; likewise, the liver is a primary detoxification organ in fish [35]. Therefore, this strong activity can lead to an increase in liver size, from hypertrophy (an increase in size) to hyperplasia (an increase in number) of hepatocytes [35], or both. Studies evaluating the relative liver size of fishes from contaminated and reference sites often utilize the Hepatosomatic Index (HIS), which expresses the ratio of liver weight to body weight as a percentage.

Gonadosomatic index (GSI) is also a percentage relationship between the gonad weight and fish weight. Depending on the severity of exposure to xenobiotics, the sublethal effects can be to limit physiological capacity, reduce growth, and impair reproduction, therefore GSI is a convenient organosomaitc index [36].

5. Specific case studies about fish as sentinel organisms

In México some studies have analyzed the use of freshwater fish as sentinels. These studies include the use of biomarkers and sentinel fish and the use of the whole fish population as indicators of environmental change. Furthermore, these studies are in areas of contrasting environmental conditions, in the case of biomarkers studies are in: the course of a river in the Atlantic slope (Río Champotón), a Lake in the Central Plateau (Yuriria Lake), a spring and a reservoir in the upper portion of a river of the Pacific slope (Ameca river). These studies make evident the utility of freshwater fish as sentinels and are briefly exposed.

5.1. Case of study of *Astyanax aeneus* in the Champotón river

The Champotón river, located in the humid subtropics of southeastern Mexico in terrain with a high content of karstic material, is the main surface stream in the Yucatán Peninsula; is a coastal river with 48 km in length to its outlet with a drainage basin surface area of 650 km2. The fish studied inhabit the fresh water zone of the river with salinity up to 1.2 practical salinity units [37]. The climatic regime is hot subhumid with summer rain (June to September) and occasional winter precipitation as a result of the windy (northerly) and hurricane seasons. The main anthropogenic activity in the basin is agriculture and livestock raising [38]. During the study period (2007-2008) the region was affected by several hurricanes (mostly from August to October), that caused the river overflow. This study assessed the effects of the environmental conditions along the freshwater portion of the Champotón river on the native fish *A. aeneus*, analyzing responses between lower and higher levels of organization, and linking spatial and seasonal fish responses with water quality features. A Water Quality Index (WQI) was employed as an indicator of environmental conditions, a set of sub-organismal biomarkers in *A. aeneus* lipid peroxidation (LPO), GST, EROD, and lactate dehydrogenase (LDH)) was monitored to determine the Integrated biomarker response (IBR), and organosomatic indices: GSI, HSI and CF were characterized. Three study sites were analyzed: San Juan Carpizo (SJC) in

the upper portion of the river, San Antonio del Río (SAR) in the middle portion of the river (where there are a rustic swimming spot lacking of sanitary facilities), and downstream Ulumal (U); the study periods were: April, July, and November 2007 and February 2008.

The WQI scores exhibited spatial and temporal variations (from 53.21 to 78.49) on a scale of 0 to 100. The lowest value was recorded in July at site SAR, where the lack of sanitary facilities provoke fecal materials are swept away by runoff during the rainy season (July), increasing coliform numbers and lowering the WQI. In addition, this river flows through a region in which calcareous substrates predominate; with a high content of calcium carbonate and increase in conductivity both provoked decreases WQI scores, particularly during the drought. However, WQI scores were higher in November and February, following the hurricane season that brought large amounts of precipitation and increased river flow favoring dilution. As a result, the values of several WQI parameters (including hardness and conductivity) decreased while the river was in flood. Several studies reported similar WQI fluctuation patterns. WQI scores in the Lerma-Chapala Basin, Mexico, indicated severe degradation of the basin, particularly during the dry season, when its rating ranged from contaminated to highly contaminated; however, WQI improved during the wet season, [39, 40].

Although the WQI scores indicated that the Champotón river had acceptable water quality, some pollutants (residues of persistent organic compounds (POCs), such as PCBs, hexachlorocyclohexanes, aldrin-related pesticides, heptachlor, and dichlorodiphenyltrichloroethane) have been detected in the Champotón river [41]. In reference [42] reported that sediments from several Champotón river sites contained two or more of the 16 PAHs considered by the Environmental Protection Agency United States as priority pollutants that represent a potential threat to exposed organisms. Seasonal variations in POCs were found by [41]; PCBs and hexachlorocyclohexanes reached their highest values during the rainy season, while dichlorodiphenyltrichloroethane, drines, and heptachlor peaked during the dry season. Additionally, high episodic loadings of contaminants have been detected in aquatic ecosystems following flooding events [43].

Regarding biomarkers in the sentinel fish *A. aeneus*, the highest LPO values were detected in November and February (post-hurricane and windy seasons), while the lowest values were detected in July at all study sites. GST activity was highest in November at site U and lowest in July and April at all study sites. EROD activity in general was highest in April, while the lowest means occurred in July at all sites. Spatial analysis revealed that SAR recorded the highest mean EROD activity, while the lowest occurred at sites SJC and U. LDH values peaked in November and February following the hurricane and windy seasons, while minimum LDH levels occurred in April during the dry season. Spatial variability occurred; sites in the middle reaches (U, SAR) had the highest LDH values, while the lowest were recorded at headwater site SJC [44].

The increased LPO values in the post-hurricane season (November) and in the windy season (February) was associated with the hurricane season in the Champotón river which provokes flooding of adjacent areas where field crops are treated with agrochemicals that, along with the POCs and PAHs detected in sediments by [41], may be incorporated into the aquatic system. Similar results were found by [45] that detected the mobilization of agriculture-related

xenobiotics, during the flooding of the river Elbe. Oxidative stress in fish after extensive flooding was also detected by [43] in the Pamlico Sound estuary. The observed increase in LPO levels in *A. aeneus* may be related to climate-induced stress and by exposure to the mixture of xenobiotics that may be mobilized during that period.

Produced ROS are detoxified by antioxidant defense mechanisms, which are essential for protection of cellular systems against xenobiotic-induced oxidative stress [46]. GST is particularly interesting, since it is involved in elimination of reactive compounds and is the transport system for glutathione [22]. GST activity in *A. aeneus* peaked during the same period that LPO increased, following the hurricane season, which may reflect a defense of the fish against oxidative stress, as reported by [47] in *Prochilodus lineatus* in Argentina and by [48] in three subspecies of *Salmo truta* in Turkey. The liver usually contains high levels of antioxidant enzyme activity, which may be due to high rates of free radical generation in this tissue [49].

A. aeneus exhibited seasonal fluctuations in EROD activity, peaking in April at the SAR site. Several authors [43, 50] point out that POCs (including PAHs) typically cause elevated levels of EROD activity, and fossil fuel spills and infiltrations are a major source of PAH input [51]. In *A. aeneus* EROD activity was highest at the SAR site, a recreational spot reached only by unpaved road where motor vehicles park on the riverbank. EROD induction may thus be due to surface runoff carrying PAHs.

LDHs are cytoplasmic enzymes that catalyze the reversible reduction of pyruvate to lactate [52], an important step in the energy processes of many animal groups. Its use as a biomarker is based on the assumption that organisms subjected to chemical stress must obtain additional energy rapidly, thus increasing anaerobic glycolysis. The LDH response is apparently time-dependent and may vary with the pollutant and organism involved [41]. LDH in *A. aeneus* increased at all sites in November and February, coinciding with the period of maximum LPO as well as the post-hurricane season and highest river flow.

The IBR enables evaluation of the global variations of biomarkers, taking into account the contributions and variations in the biomarkers assessed [17]. IBR data revealed seasonal fluctuations, the maximum total IBR values occurred in April and November (April, 15.73; November, 14.73) and the minimum values in February (2.53). Response in April and November suggested that *A. aeneus* was exposed to greater stress during this period, one at the end of the dry season and the other coinciding with the post-hurricane season that also led to high LPO values (oxidative stress), high GST levels (antioxidant responses), and high levels of LDH (high energetic need). Multiple stressors are involved during flooding, such as altered habitat, changes in hydrological regime, and mobilization of pollutants. Responses of biota to environmental stressors are the integrated result of natural and anthropogenic stressors that can be ultimately manifested in biotic changes at several levels of organization [53]. IBR differences among the study periods may reflect a compensatory mechanism by which the fish regains homeostasis following the period of highest stress. Fish display a large variety of physiological stress responses that manifest as an increase in certain biomarkers after a stressful event [54]. These responses are considered adaptations of the fish to adjust itself to the disturbance and regain homeostasis [55]. Fish have also been found to exhibit recovery responses dependent on stress duration and magnitude. If the stressors are too severe or persistent and the fish is

unable to regain homeostasis, the responses may become maladaptive and may pose a risk to the health and wellbeing of the fish [54].

Regarding somatic indices, GSI displayed the reproductive period of *A. aeneus* occurred from April (end of the dry season) to July (early rainy season), with the reproductive peak in July. HSI was highest in *A. aeneus* during periods of reproductive inactivity, which is interpreted as an increase in liver-stored reserve materials for later use during gametogenesis. Furthermore, low HSI values prior to and during reproduction may result from the transfer of liver energy reserves toward gonadal maturation and the reproductive event, with consequent depletion of these reserves and a decrease in HSI values [56]. CF remained constant among sites and study periods, with maximum values during July. CF reflects the interactions between abiotic and biotic factors in the physiological conditions of the fish [57]. Observations demonstrate that *A. aeneus* maintains a stable, robust condition. CF and HSI trends may reflect physiological conditions in *A. aeneus* that enable oocyte maturation and release, suggesting that oocyte production relies on energy stored in the liver and not on energy stored in the musculature [58, 59]. GSI, HSI, and CF revealed that the reproductive success of the sentinel species had not been affected, since the GSI values concurred with those reported for other species in the genus *Astyanax*. HSI values showed the transfer of energy during the reproductive period, and CF remained stable throughout the study, evidencing no effects on the general condition of the fish during periods of higher stress and indicating that the stress was temporal and the fish were able to compensate for it.

Despite WQI scores suggest that the Champotón river water is not highly polluted, the set of *A. aeneus* biomarkers constitutes a more sensitive and effective tool for identifying periods of environmental conditions adverse to fish health. Markers of oxidative damage (LPO), energy processes (LDH), detoxification (EROD), and antioxidant activity (GST) suggested that two stress periods affected the health condition of *A. aeneus* in different ways. These biomarkers can be used as early warning signals of environmental change prior to the onset of irreversible damage at the population level. Indeed, the IBR values highlighted the two periods of high biomarker response. Overall, this study provides evidence supporting the use of a biomarker set in assessing the health of aquatic systems, corroborating the suitability of *Astyanas aeneus* as a sentinel species.

5.2. Study case of *Chirostoma jordani* in Yurira Lake

Chirostoma jordani is an Atherinopsid fish endemic of Central México. Data presented are from a population living in Yuriria Lake, one of the most important lakes in México, located in the Central Plateau (hydrologic region Lerma-Chapala-Santiago 20 ° 20'24'' -19 ° 04'48'' N and 101 ° 55'48'' -100 ° 48 '36'' W). It is an artificial, small and shallow lake (area= 66 km^2 and maximum depth of 3.2 m) feed by a diversion of Lerma river (their main tributary), that carries wastes from mining activities, livestock, industrial, urban and rural areas [60]. In the western end the lake receives water from two small an intermittent tributaries. Yuriria Lake supplies water for the surrounding farming areas, harbors migratory bird populations, and supports fisheries and tourism of several human settlements on the littoral zone.

This study shows an assessment of water quality by means of a WQI and a battery of oxidative stress: LPO, and the activity of antioxidant enzymes, SOD, CAT, and GPx along with somatic indices, such as the GSI, HSI, and CF were analyzed to assess the health condition of *C. jordani* in Yuriria Lake. The study was carried on in a period with scarce pluvial precipitation and prolonged drought (May, August, November 2009 and February and May 2010).

Yuriria Lake is characterized by a high deterioration in their water quality, WQI values ranged from 55 to 70, with a global mean of 65.85. The lake has spatial differences in water quality, the limnetic zone has higher scores (63 to 70), and the tributaries have the lower quality (55 to 58). Yuriria Lake being located in the Central Plateau, one of the most highly populated areas in México, displays the general problem of water quality of the basin (the Lerma-Chapala basin), where urban and industrial wastewater discharges, and leachates of agrochemicals are the main pollutants that diminish the water quality [61]. Particularly the middle Lerma (where Yuriria Lake is located) is recognized as the most affected area, with WQI scores between 41.1 and 54.2 in 1999 [40, 61, 62]. Furthermore, previous studies have recognized the entry of pollutants in Yuriria Lake [61]; however, the effect of the mixtures of these pollutants on the aquatic biota inhabiting the lake had not been analyzed.

The biomarker assessment suggests that the lake conditions exert stress on the fish *C. jordani*. The biomarker response showed pronounced seasonal variations. The gills presented higher values of LPO. May 2009 displayed the highest levels and November the lowest. In the liver the higher levels of LPO were detected during November and February and May 2010 (the end of the rainy season and the dry season). In muscle, the highest level of LPO was observed during February and May 2010. Gills being the first organ of contact with water are exposed directly to any xenobiotic in the aquatic environment and their biomarker responses are the result of the exposure to stressors. In addition, toxics can also enter via the intake of water and food and be absorbed and transported by the portal system to the liver before entering the general circulatory system; in consequence, the liver is one of the most sensitive organs to environmental stressors [63].

The activity of the antioxidant enzymes in liver and gills also showed a marked seasonal variation. SOD and GPx significantly increased during November, mainly in the gills, compared to the rest of the seasons. CAT also showed higher values in activity during November; however, its highest value was found in gills during February. In general, the activity of antioxidant enzymes decreased from November to May 2010.

Exposure to various xenobiotics, such as metals and organic compounds that enter water bodies, can promote the formation of ROS and induce oxidative stress [12]. The increase in the level of LPO in liver observed in November 2009 and February and May 2010, suggests the existence of pro-oxidant agents in Yuriria Lake and indicates increased oxidative stress in these seasons. Seasonal variation in LPO values could be related to the rainy and the dry seasons; the rainy season can promote dilution of xenobiotics that induce less stress during this season. Rainfall also increases leaching and runoff that enhance the entry of xenobiotics (chemicals), in this study results show that the damage generated in the fish liver became evident from November (the end of the rainy season) until February and May 2010 (dry season), when the processes of evaporation and consequent concentration of xenobiotics could be higher. Fur-

thermore, in May 2009, there were higher levels of LPO in gills, which may indicate that water in the lake at the beginning of the rainy season provoke oxidative stress in gills.

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 [19, 64] and could be expressed as increased or depleted CAT, SOD, and GPx activities [12]; in both cases, the result is damage to the antioxidant system. In *C. jordani* inhabiting Yuriria Lake, both responses were detected: 1) stimulation of the activity, when LPO levels in liver and muscle were higher (November and February) and antioxidant activity showed the highest values; and 2) depletion in antioxidant activity during May 2009 with high levels of LPO in the gills, and May 2010 with increased LPO in liver and muscle. These highly variable responses of the antioxidant system depend on the type and concentration of contaminant to which fish are exposed, as well as on the intensity and duration of exposure [65]. The clear decrease in the activities of CAT, SOD, and GPx in *C. jordani* is extremely important because it suggests severe damage to the antioxidant system of fish. These damages have been previously documented by [66], who recorded a collapse of the antioxidant defense system of *Liza aurata* in the Ria de Aveiro, Portugal, with exposure to mercury. In addition, [67] reported damage to the antioxidant system of *Oreochromis niloticus* from acute and chronic exposure to Cd, Cr, Cu, Zn, and Fe.

At the population level the assessment can reveal changes in the fish biology and ecology resulting from natural fluctuations and/or ecosystem changes caused by environmental degradation. The standard length of *C. jordani* ranges from 21.16 to 77.61 mm. Three size classes were determined: Class I with a mean size of 26 mm, represented only in the month of May 2010; Class II with a mean size of 56 mm; and class III with a mean size of 62 mm. There was a gap in the size frequencies in the class of 32–50 mm from May to August 2009, whose abundances were not sufficient to form this cohort. The lack of smaller sizes from May to November 2009 can be interpreted as a possible reproductive failure during that year, resulting in low recruitment and consequently precluding estimation of the cohort [68]. These gaps or missing cohorts have been previously documented for other fish as a result of overfishing and/or environmental degradation [69]. Several causes could explain this event. According to [70], the hydrological cycle plays an important role in the development of different biological attributes such as gonadal maturation, migration, spawning, larval development, growth, and feeding. Prolonged periods of drought are also associated with failures in the recruitment and subsequently reduced adult stocks and serious effects on fisheries.

The somatic indices revealed that K displayed small variation between sites and between periods. HSI showed significant differences between sites in August, and between periods in May, values were significantly lower than those of other periods. The GSI showed the greatest variation, with a clear reproductive peak during May 2010. There was a positive correlation between the GSI and HSI. By size class, only the GSI showed variations between seasons. Class I was significantly lower than the rest of the classes. Classes II and III the GSI showed a reproductive peak in May 2010.

In fish, the cost of reproduction may be considerable; thus, fish can express different patterns of energy storage and depletion in relation to reproductive cycles, with an alternation in energy storage (56). The comparison of K and the HSI with the GSI could therefore be useful for

estimating the possible balances or energy transfer between the reproductive period and nutritional status [71]. *C. jordani* in Yuriria Lake show that K was maintained at stable levels. Furthermore, there was no alternation of energy storage between the liver and gonads; GSI and HSI correlated positively, indicating that the reproductive period did not compromise energy reserves or the liver or the soma.

According to [72], fish living in waters contaminated with domestic sewage exhibit higher K and GSI, these authors suggest it is because these sites have more available food for fish, enabling them to compensate for the environmental impairments. This scenario is likely occurring in Lake Yuriria, where *C. jordani*, having enough food, can complete reproductive cycles and reach larger sizes despite the presence of stressors in the lake.

According to the results critical periods in the health of *C. jordani* occur from November, February to May 2010 (end of the rainy season and the dry season) because in this period, the higher LPO, the lowest antioxidant response, and the lowest K were observed in contrast to the higher WQI scores detected in the same period. This result indicates that fish health assessed by biological indicators as oxidative stress biomarkers and the lack of a cohort, are highly sensitive to environmental conditions imposed by the dry season. 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 [73].

The findings suggest that *C. jordani* faces oxidative stress resulting from the presence of pro-oxidant agents in Yuriria Lake. At the population level, *C. jordani* has adapted to the conditions in Yuriria Lake, with mean values of HSI and GSI greater than those in other sites and K values stable throughout the year. Changes were observed in recruitment and reproductive success associated with low water levels in the lake in 2009 which shows that the fish population is highly dependent on water levels in the lake and climate changes. Previous studies indicate that Yuriria Lake receives various xenobiotics with levels that vary both spatially and temporally. Biomarkers of oxidative stress, somatic indices, and monitoring of the size classes in the fish *C. jordani* are appropriate indicators of Yuriria Lake conditions.

5.3. Study case of *Ameca splendens* and *Goodea atripinnis* in Ameca river

The Ameca River is located in the western slope of Mexico which drains to the Pacific Ocean. It is a river characterized by their great fish biodiversity, however, the upper portion of Ameca River, is affected by several environmental disturbers: the construction of a reservoir, the inputs of wastewater from a sugar-processing facility and water extraction, which have resulted in a drastic reduction in fish biodiversity [74, 75]. In addition, some endemic fish species such as *Ameca splendens*, have suffered a reduction in their range, and have become more prone to extinction (NOM-059, 2002) than those with a broad distribution (like *Goodea atripinnis*).

The authors of this paper [32] analyze biomarkers and bioindicators of two viviparous fish species, *A. splendens* and *G. atripinnis* living in a reservoir of the upper portion of the Ameca River, which receives wastewater, and in a spring of the same river that is free from such polluting water. In this study a comparison of the biomarker responses and bioindicators in

two fish species were assessed, according to the main objectives: a) to assess water quality of a spring (ER reference site) and De LaVega reservoir (LV impacted site) where *G. atripinnis* and *A. splendens* coexist; b) to examine the health of both fish species in the reference and impacted sites by means of a battery of biomarkers; and c) to analyze physiological condition indices and population level assessment by mean of bioindicators (population measurements). A WQI was assessed. The set of biomarkers was composed by: enzyme activities of gamma-glutamyl-transpeptidase (γ-GTP), acetylcholinesterase (AChE), EROD and the LPO were determined. Additionally, somatic index were analyzed: CF, HSI and GSI.

Data of WQI scores showed spatial and temporal variations, the spring ER achieved the highest scores in all months over the course of the study and the highest values occurred in March in both sites.

Regarding biomarkers highest values of LPO were found in September (rainy season); the highest values were detected for female livers and gills of *A. splendens* in the impacted site LV and for female livers and male gills of *G. atripinnis* in LV. The LPO activity in the upper Ameca River displayed the stress to which organisms are subjected, since in the reservoir LPO showed the highest values, being *A. splendens* the most affected species. Many environmental pollutants and their metabolites have shown to exert toxic effects associated to oxidative stress, producing free radicals that initiate the LPO and cause damage to membrane proteins [76].

Results of γ-GTP showed less marked seasonal differences than LPO. In this study, in most comparisons between sites, γ-GTP activity was slightly higher at the spring; the inhibitory effect of this activity at LV could indicate a diminution in the amount of membrane proteins caused by LPO [77]. On the other hand, an increase of γ-GTP activity towards March in LV could be to prevent increases in LPO [76]; this increase coincides with the rainy season. Several authors have found seasonal variations in the response of this enzyme as a result of exposition to alkylphenols, the final degradation products of pesticides, detergents and other formulated products [78].

There was a seasonal variation in AchE activity. Organophosphates and carbamates, as well as PAHs, have been widely recognized for causing AchE inhibition, through their reaction with the serine at active site of the enzyme [79]. In nervous tissue AchE is responsible for the breakdown of acetylcholine (Ach) during transmission of an impulse; if the enzyme is inhibited, Ach is accumulated and thus a prolonged transmission of impulses could result in tetani and often in respiratory failure and death. In September in *A. splendens* living at LV, AchE exhibited lower values related to reference site that could have resulted from fish exposure to diverse pesticides used in the adjacent agricultural lands that run off in the rainy season. In March during the dry season, in *G. atripinnis*, living at LV, AchE also exhibited lower values related to ER, may be the effect of evaporation and consequently the concentration of total solid dissolved at LV, including pesticides and other xenobiotics.

Regarding EROD, the hydrocarbons discharged by the sugar-processing facility and other effluents into LV from December to June, could be responsible for activating EROD detoxification mechanism in fishes living there [80]. This mechanism is considered as the main measure of the CYP1A activity, which in turn constitutes a part of the enzyme complex of

the Mixed-Function Oxidase (MFO). Since MFO facilitates the excretion of aromatic contaminants from the body induction of this complex is an effective biomarker of exposure [81, 82]; there are several studies reporting elevated levels of MFO activity in liver fishes as a result of exposure to organic contaminants, such as PAHs, dioxins, PCBs and agricultural and urban wastewater [83]. Also, there are other factors, such as UV radiation, that causes increase in MFO activity [84], moreover, damaged livers, like those of LV organisms, are less capable to MFO induction [85].

Responses to environmental stress also were reflected in bioindicators in both species studied. The major HSI values in LV concurs with [86] who reported major HSI values, related with higher EROD induction, at contaminated sites in comparison with a reference site. Moreover, in our study, higher LSI in LV concurs with higher IBR values. High values of HSI could have resulted from exposition to hydrocarbons which cause hypertrophia in liver [86].

Species may differ in the nature of their physiological response and reproductive consequences to stressors [87]. Tolerant species to environmental stress, like *G. atripinnis*, are more abundant in more disturbed environments, like LV [74, 88]; on the other hand, *A. splendens* is more abundant in a more stable environment, like ER. In LV females/male ratio for *A. splendens* could be affected by environmental estrogens, like pesticides [89]; these could act by merging receptor binding properties of estradiol, alteration of estradiol/testosterone ratios or estrogen receptor levels [12].

The higher SL, weight and CF values in LV are in concordance with [71], that found higher CF and GSI in fishes living in waters polluted with untreated domestic sewage; they suggested that fishes in these sites could find abundant food availability, and they are able to compensate for environmental changes caused by untreated domestic sewage discharges. Fishes could have major GSI values, higher fecundity and lower maturity age even under conditions of high pH [89]; in the present study, *A splendens* in LV presented these features; only in *G. atripinnis* organisms GSI was higher in the ER. [90] found a decrease in GSI as a result of the exposition of chubs to effluents carrying out organic pollutants and metals. [71] revealed a negative relation between CF and HSI with GSI, but this relation was observed only in *G. atripinnis*. The larger size, higher growth, longevity and reproductive success of organisms living at LV suggest a tactic to compensate for the stress to which the populations of both species studied are subjected; however, offspring is smaller and has a lower weight.

Throughout this study, water quality was higher in ER than in LV due to human activities; but in both sites there were different spatial and temporal factors that produced stress on fishes living there. Therefore, fishes had responses at biomarker and population levels of biological organization. Every biomarker and IBR in this study showed seasonal variation and they were useful environmental tools to demonstrate that, as consequence of pollution, LV is a more stressing place to organisms living them in comparison with ER. In general, organisms in LV presented oxidative stress by the LPO levels, and then neurotoxic impacts by the AchE and some detoxification mechanisms were evident by the γ-GTP and EROD activities. Bioindicators showed evidences of physiological changes due to contaminants exposure and make evident the plasticity of the organisms to survive in this site, in turn the responses should be considered as tactics to survive under stress condition. Moreover, both biomarkers and bio-

indicators revealed that *A. splendens* is a less tolerant species than *G. atripinnis* to environmental stress. Differences in biological response could be attributed to different physiological status of each fish species during the wet and dry season as well as to differences in the type and quantity of the xenobiotics that input at LV due to the period of maximum and minimal activity of the sugar industry; the lixiviation of the agrochemicals from the adjacent lands to the water bodies and also to the complexity of the mixtures of pollutants that are conform at LV that provoke several biological responses.

6. Holistic approach

Environmental stressors can cause several and different damages over aquatic organisms. These damages could be from molecular to population levels, likewise community and ecosystem levels. Through the biomarkers such as defined in this document (any biological measurable response from an organism, induced by the exposure to a xenobiotic or complex mixture of them) we can determine only some of possible causal relationships. Therefore, it is necessary always, measure a set of biomarkers to identify different stressors or damage on sentinel organisms. Several indices have been proposed to try to integrate the multi responses of different biomarkers in a single number that is indicative of the severity of the damage or stress. Such is the case of the IBR proposed by [17]. In this index, the biomarker data must first be normalized and standardized; then the score is represented by the area of a star plot. IBR considers the responses of activation or inhibition of the biomarkers assessed. IBR is an exploratory tool and should be appropriate only if an a priori justification exists for each biomarker used and if the physiological significance of the changes to each biomarker is well known [17].

An other case of index based on a battery of biomarkers was proposed by [91]. This biomarker index was obtained by summing the biomarker values expressed in term of classes. Classes were determined by a distribution-free approach derived from the theory of rough sets. No synergistic or antagonistic assumptions were incorporated into this index.

In [4] the authors proposed a quantitative health assessment index for rapid evaluation of fish condition in the field named Health Assessment Index (HAI). This index is not based on a battery of biomarkers; however, it is a quantitative index that allows statistical comparisons of fish health among data sets. Index variables are assigned numerical values based on the degree of severity or damage incurred by an organ or tissue from environmental stressors.

The Bioeffect Assessment Index (BAI), is based on the integration of several pathological endpoints measured in the liver of fish [21]. The BAI represents a modification of the HAI since it includes solely validated biomarkers reflecting toxically induced alterations at different levels of biological organisation in order to quantify the effects of environmental pollution. BAI is able to reflect deleterious effects of several classes of xenobiotics such as heavy metals, organochlorines, pesticides, PAHs, and therefore is also considered as an integrative index of health in aquatic ecosystems.

When we use sentinel organisms, a key point is the study of baseline or natural variation of responses of the sentinel organism selected, or characterizing the response of the same sentinel organism in reference sites.

The use of fish as sentinel organism is feasible for pollution monitoring in aquatic systems; however, the survey should consider the application of a suite of measurable responses (biomarkers and bioindicators) to identify potential sources of stress and damage to which organisms are exposed, as shown in the case studies presented above. The set of biomarkers or bioindicators should also, consider several levels of biological organization in order to identify effects of environmental stressors, spatio temporal trends in environmental conditions and to identify early warning signals to prevent that damage continue from low biological organizing levels to higher levels of organization.

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