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Assessing the health condition profile in the freshwater fish *Astyanax aeneus* in Champoton river, Mexico

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Abstract

The use of biomarkers for monitoring aquatic environmental quality has gained considerable interest worldwide. The effects of the environmental conditions of Río Champotón, México, in the hotspot of Mesoamerica, were assessed in *Astyanax aeneus*, a native fish of the tropics of southwestern México. Pollution from agrochemical residues is a major problem in Río Champotón. Three study sites along the freshwater portion of the river were monitored in April, July, and November 2007 and February 2008. This study includes a water quality index, a set of biomarkers (hepatic glycogen levels and lipid peroxidation in liver, gills, and muscle) to assess the integrated biomarker response, and population bioindicators (gonadosomatic and hepatosomatic indices and Fulton's condition factor). Although the water quality index suggested low level of contamination in the Río Champotón, biomarkers indicated that *A. aeneus* is exposed to stressors that impair biological responses. The integrated biomarker response showed stress periods with higher biomarker response and recovery periods with decreasing biomarker values. The somatic indices did not indicate severe effects at the population level. This study illustrates the usefulness of lipid peroxidation evaluation in the assessment of aquatic health conditions and corroborates the suitability of *A. aeneus* as a sentinel species.

Key words

Astyanax, Early warning biomarkers, Health condition assessment, Native fish, *Río* Champoton

Introduction

In aquatic ecosystems, pollution appears as a complex mixture of xenobiotics (van der Oost *et al.*, 2003). Recently, the production of contaminant-stimulated reactive oxygen species and the resultant oxidative stress (disturbance of the pro-oxidant–antioxidant balance in favor of the former, which potentially provokes damage) have been indicated as a mechanism of toxicity in aquatic organisms exposed to pollution (Livingstone, 2003) and are known to play a large role in the pathology of several diseases and longevity in a number of species, thereby establishing ecological relevance. The most general effect of xenobiotics on fish is oxidative stress, which includes variety of oxidative reactions that impair the health conditions of fish (van der Oost *et al.*, 2003).

Although conventional tools for environmental monitoring assess contaminant levels, they do not reveal interactions between pollutants. However, the use of biomarkers can unveil the global effects of the mixture of contaminants in organisms. Biomarkers of oxidative stress include changes in antioxidant enzyme activity, damage in DNA bases, protein oxidation products and lipid peroxidation (LPO) (Livingstone, 2003). LPO is one of the most commonly used biomarkers for evaluating oxidative stress, as it reflects the action of reactive oxygen species on lipids (van der Oost, *et al*., 2003).

Studies in fish species have largely been carried out on the major organs of biotransformation (liver) and respiration (gills). The liver plays an important role in intermediary metabolism, the storage of reserve compounds such as lipids and glycogen, the biotransformation and detoxification of lipophilic organic compounds (xenobiotics), and reproduction (vitellogenesis). Fish gills are efficient tools for biomonitoring potential impacts;a large area of the fish gills is in contact with the water and is highly permeable, and thus the environmental impacts of pollutants may affect fish gills (Coutinho and Gokhale, 2000). Muscle has been also studied as a target of pollutants due to the accumulation of some pollutants in this tissue (Solé *et al*., 2008). Basic energy reserves in the form of glycogen comprise1% of total body weight. The amount of glycogen stored in the liver depends on the physical, chemical and biological factors faced by the fish. Rapid movements, stress factors, and hypoxia are able to decrease carbohydrate reserves, beginning with glycogen in the liver and muscles. Various studies have shown that hormonal changes in fish affect the conversion of liver glycogen into blood glucose (Coban and Sean, 2011). Taken together, these observations indicate that hepatic glycogen is an important biomarker for the assessment of fish health (Sehgal and Goswami, 2001).

Organisms are subject to multiple stressors in the environment, both natural and anthropogenic, inducing an integrated response that is reflected in growth and reproductive success. The sum of these individual responses results in a population-level response. For this reason, evaluation of responses at different levels of biological organization aids the assessment of the health conditions of fish (Adams *et al*., 1999).

México encompasses diverse aquatic ecosystems of great importance particularly in their Southeastern region due to the productivity and biodiversity. Río Champotón, the main surface stream of the Yucatán Peninsula, is located in this area in terrain with a high content of karstic material that is classified as a priority hydrological region by the National Commission for the Knowledge and Use of Biodiversity. This river is within the so-called hotspot of Mesoamerica (Myers *et al.*, 2000), whose main problems are agricultural waste input, discharges from a sugar mill and contamination by domestic sewage at the mouth of the river. Río Champotón is particularly relevant due to attributes associated with ecosystems amenable to conservation, although it faces major challenges from deforestation and non-point source pollution (López-López *et al*., 2009).

The current study assessed the health condition of the fish *Astyanax aeneus* by evaluating a set of biomarkers, the level of LPO in three tissues (in liver, gills, and muscle to assess an integrated biomarker response, IBR), hepatic glycogen levels and various somaticindices (gonadosomatic index, GSI; hepatosomatic index, HSI; Fulton's condition factor, K). Analysis of fish sampled at three points along Río Champotón, in four periods allowed us to assess the spatial and seasonal changes of the biomarkers and their relationships with environmental factors.

Materials and Methods

Three study sites were selected in the upper and middle reaches of the freshwater portion of Río Champotón: San Juan Carpizo (SJC) in the upper portion of the river, San Antonio del Río (SAR) in the middle portion (including a rustic swimming spot and camping area lacking sanitary facilities), and the downstream site Ulumal (U) (Fig. 1). Four surveys were conducted at the study sites in April (dry season), July (rainy season), and November 2007 (post-hurricane season) and February 2008 (windy or northerly season). Diverse environmental factors were recorded at each site using a Quanta multiparametrics onde.

Fish were collected with sweep nets 5 and 10 m long and 5 m deep (0.03m mesh size) and a 0.05-cm mesh-size casting net. Nets were cast for 1 hr at each site. An average of 60 specimens were collected and fixed in 10% formaldehyde at each study site. These samples were analyzed for somatic indices, while an average of 60 non-breeding specimens were dissected immediately to extract the liver, gill, and muscle for biomarker evaluation. A total of 1,286 specimens were measured for standard length, total weight, eviscerated weight, liver weight, and gonad weight. GSI, hepatosomatic index and condition factor K were estimated in three groups: immature individuals, females, and males.

The WQI was calculated using the multiplicative weighted index (Dinius, 1987). Thirteen parameters were considered in the

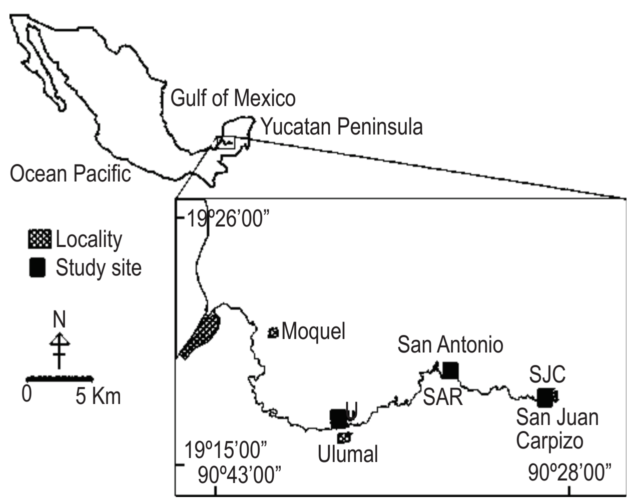


Fig. 1 : Location of Río Champotón and study sites. SJC, San Juan Carpizo; SAR, San Antonio del Río; U, Ulumal

calculation of WQI: dissolved oxygen, conductivity, air and water temperatures, pH, nitrate, color, hardness, biochemical oxygen demand (BOD_5), alkalinity, chloride, and total and fecal coliform counts.

LPO was determined by the procedure of Buege and Aust (1978); the malondialdehyde produced was measured as n mol malondialdehyde per mg protein. Hepatic glycogen levels were determined by the method of Morris (1948) and were expressed as mg g^{-1}. Protein levels were measured by the method of Bradford (1976).

The IBR assessment for LPO for the three tissues was carried out after a normalization and standardization process following the method of Beliaeff and Burgeot (2002). Each standardized biomarker was plotted as a vector in star plots; the IBR was the area enclosed by the triangle formed when the end of each vector was joined. Total IBR per site and per period were assessed by summing the IBRs for all sites in one period (total IBR per period) and for all periods in one site (total IBR per site).

Values are reported as mean± standard error unless otherwise indicated. Mean biomarker values and the WQI for each study site were computed considering the data from all study periods; mean values per study period were computed using data for all study sites. Analysis of variance was used to compare differences in biomarker responses among sites and study periods. Mean differences within each group were compared using Fisher's test, with significance set at $p< 0.01$. A canonical correspondence analysis was used to integrate data for somatic indices, biomarkers, and WQI.

Results and Discussion

The WQI values throughout the study period remained below 80 (range 53.21-78.49) on a scale of 0-100, with a global mean of 64.58±2.43 (Fig. 2a). The WQI scores exhibited spatial and temporal variations; the highest values occurred downstream in the U and SAR sites (Fig. 2b), while seasonally the highest scores were recorded in November and February during the windy and post-hurricane periods (Fig. 2c). Following the hurricane season that brought large amounts of precipitation and increased river flow, the values of several WQI parameters (hardness, conductivity, coliform levels) decreased. WQI scores in the Lerma-Chapala Basin, México, indicated a severe degradation of the basin, particularly during the dry season, when its scores ranged from contaminated to highly contaminated; WQI improved during the wet season, ranging from contaminated to moderately contaminated (Sedeño-Díaz and López-López, 2007). WQI in Río Champotón is affected by the calcareous nature of the basin (hardness, conductivity), in contrast to the Lerma Chapala, where pollution by industries and large cities affects water quality (Sedeño-Díaz and López-López, 2007).

In Río Champotón, the major sources of pollution were non-point sources from agriculture (mainly sugarcane), livestock-related chemical residues, and the input of organic matter from small human settlements near the river. Quetz*et al.* (2009) reported that sediments from several Río Champotón sites contained two or more of the 16 polycyclic aromatic hydrocarbons considered by the Environmental Protection Agency of the United States to be priority pollutants that represent a potential threat to exposed organisms. Rendón von Osten *et al.* (2008) found seasonal variations in persistent organic compounds (POCs) in Río Champotón; polychlorinated biphenyls and

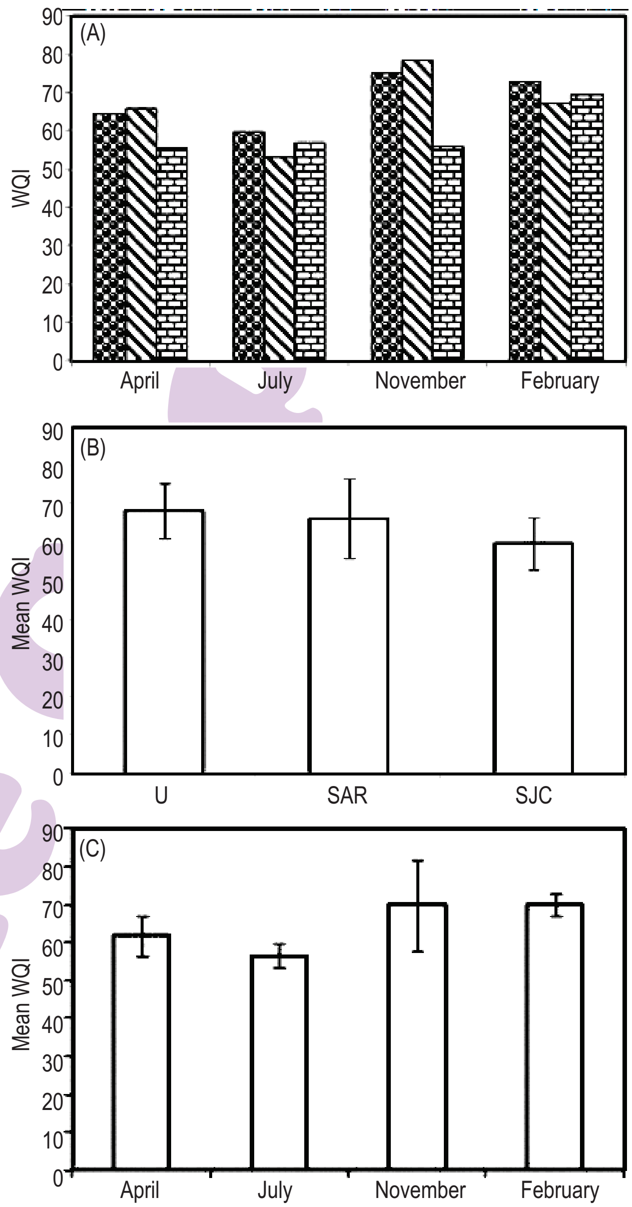
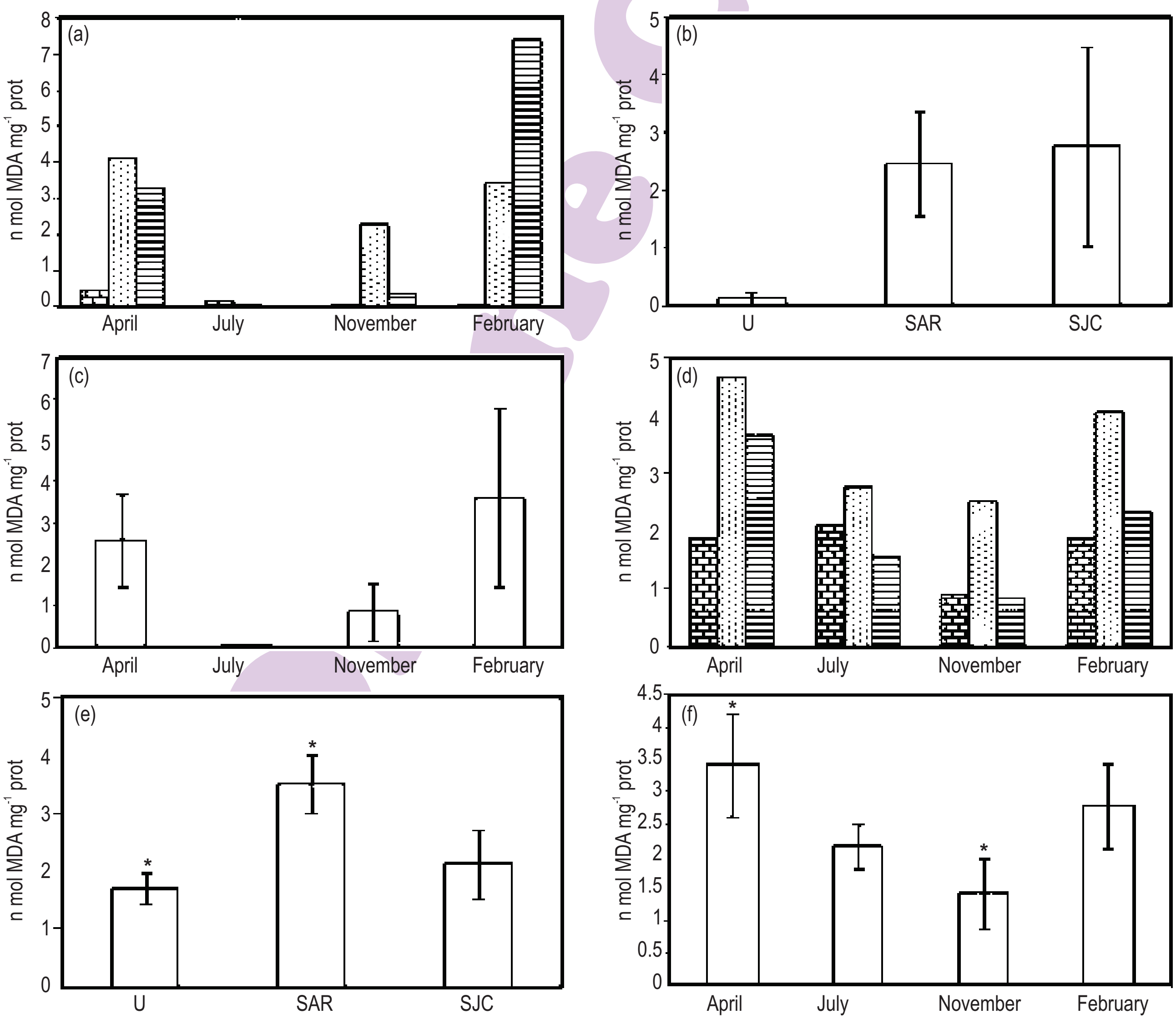


Fig. 2 : Water quality index scores (WQI) at Río Champotón. (a) Scores variation between the study sites and periods. Mean values between sites (b) and mean values between periods (c) SJC, San Juan Carpizo; SAR, San Antonio del Río; U, Ulumal. The bar represents the standard deviation

hexachlorocyclohexanes reached their highest values during the rainy season, while dichlorodiphenyltrichloroethane, drines, and heptachlor peaked during the dry season. However, studies about the effect of those pollutants in aquatic biota are scarce. Additionally, high episodic loadings of contaminants have been detected in aquatic ecosystems following flooding events (Adams *et al*., 2003). In Río Champotón, hurricanes provoke flooding of flood plains where some agrochemicals are used.

The highest LPO-Liver values were detected in February, while the lowest values were observed in July and November at all study sites except at SAR in November (Figs. 3a and c). Site U had the lowest levels (Fig. 3b). Differences between sites were not significant (p> 0.01). LPO-Gill values were highest in April and February and lowest in November at all study sites except at SAR in November (Figs. 3d and f). Site U had the lowest LPO-Gill levels (Fig. 3e). Significant differences were detected between SAR and U and between April and November (p< 0.01). LPO-Muscle values were highest in November, while the lowest values were detected in April and February at all study sites except at SAR in November (Fig. 3g, h and i). Differences between sites were not significant (p> 0.01). Mean glycogen values were highest in July and November (Fig. 3j and l); site U had the lowest mean glycogen levels and SJC had the highest (Fig. 3k). No significant differences between sites and study period were detected (p>0.01).

IBR data revealed seasonal fluctuations, as the maximum total IBR values were measured in April and November and the minimum values occurred in July and February (Fig. 4). Spatial variations were also recorded, as the maximum total IBR was detected at SAR (Fig. 4), while the middle site of U had the lowest total IBR value (Fig. 4). The star plots also revealed greater contributions of the LPO-Liver and LPO-Gill vectors (SAR in April,



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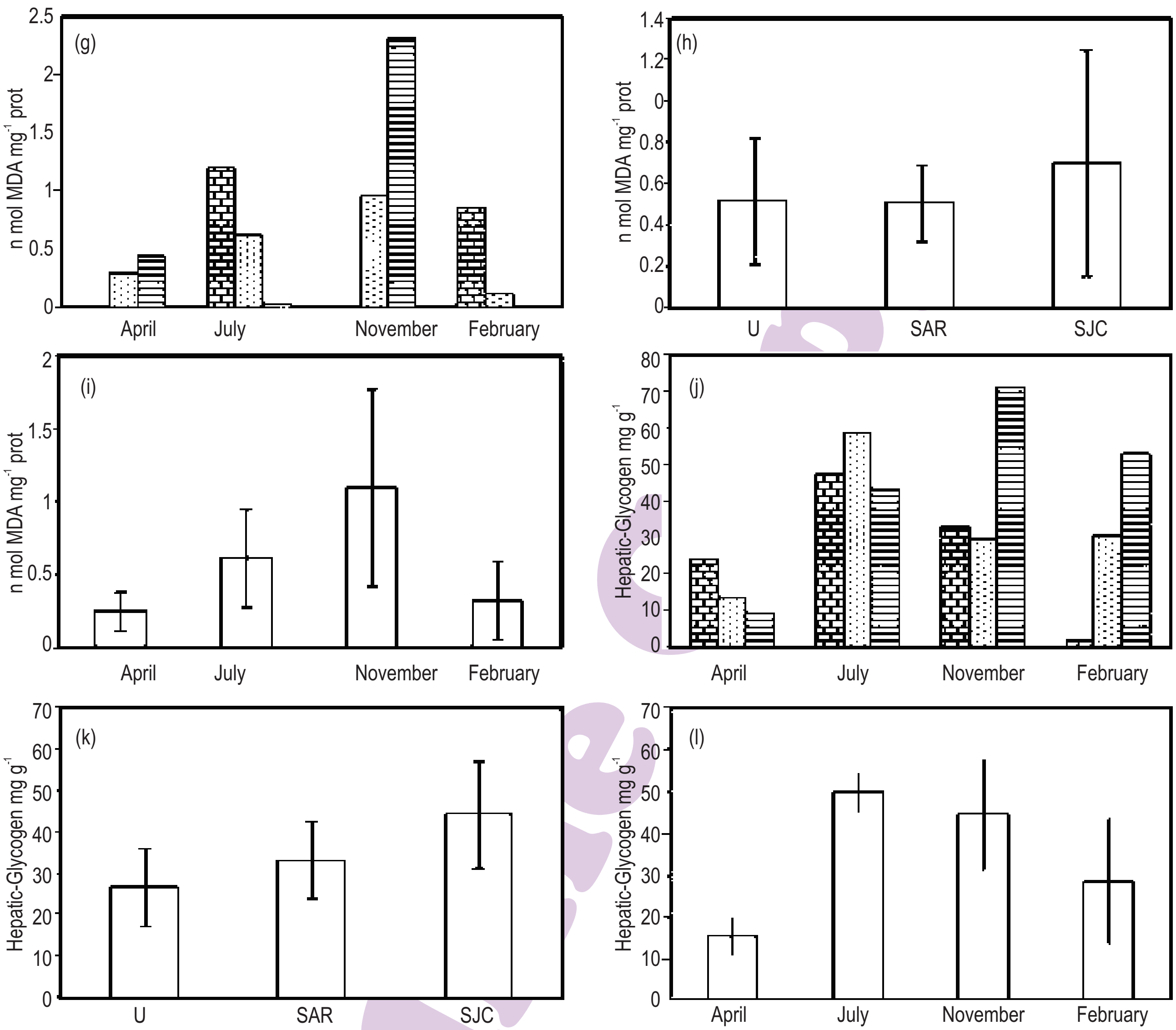

Fig. 3 : Spatial and temporal variations in biomarkersofAstyanaxaeneus in Río Champotón. Lipid peroxidation: Liver (a, b, c), Gill (d, e, f); Muscle (g, h, i) and hepatic glycogen (j, k, l). The bar represents the standard error. * Significant differences at $p<0.01$. SJC, San Juan Carpizo; SAR, San Antonio del Río; U, Ulumal, b, e, h, k = mean values per study site; c, f, i, l = mean values per study period

SAR and SJC in February) to the IBR, while the LPO-Muscle vector was important on few occasions (U in February and SAR in November; Fig. 4).

Our observations of increased LPO values during spring (April) and windy seasons (November), as well as higher IBR values in the same periods, were associated with two events. First, high temperature during spring and dry seasons may have concentrated the xenobiotics previously recorded by other investigations (Ballesteros *et al*., 2009); furthermore, temperature has been reported to influence LPO levels in fish (Chien and Hwang, 2001). Second, the high LPO values in February may be associated with the post-hurricane season, which causes the flooding of adjacent areas in which field crops are treated with agrochemicals that, along with the POCs in sediments (Rendón von Osten *et al.*, 2008), may be incorporated into the aquatic system; both events led to an increase in oxidative stress in *A. aeneus*. The high values of LPO in liver and gill should be considered as indicators the presence of contaminant-stimulated reactive oxygen species and environmental health degradation.

Given the relative fragility of the gills compared to other surface tissues and their constant exposure to the fish's external environment, it is remarkable that these structures are able to compensate for the damages induced by the chemical and physical assaults to which they are invariably subjected. Consequently, the gills appear to be a frequent target organ for

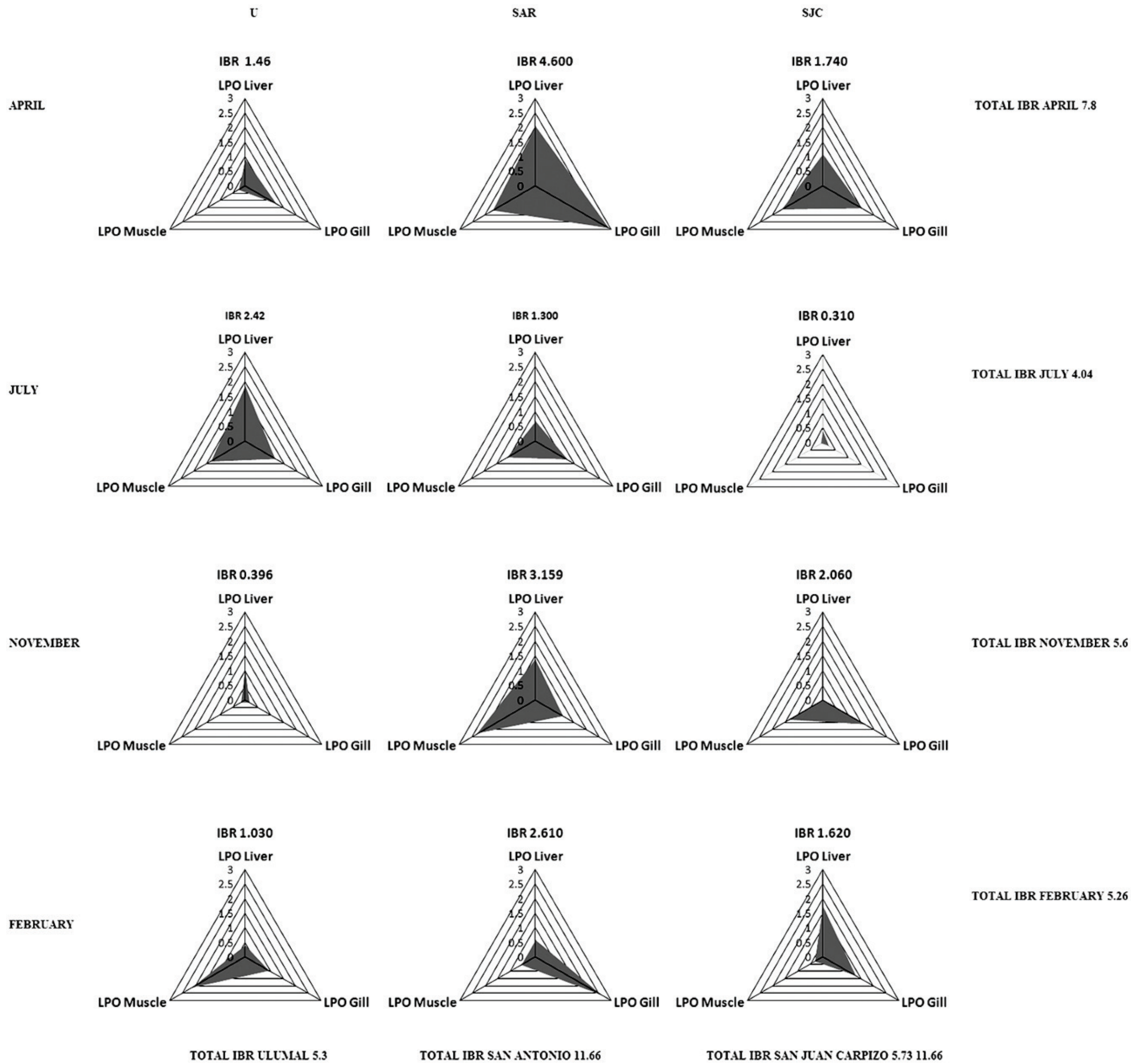

Fig. 4 : Star plots of the integrated biomarker response (IBR) of sites and study periods in the Río Champotón

stress responses (Harper and Wolf, 2009). Unlike the gills, the liver is clearly protected from physical exposure to the external environment, at least under normal circumstances (Coutinho and Gokhale, 2000).

Immature individuals were found only at U in January and November and at SAR in November, with low GSI values. HSI values were high at SAR in November, and K values fluctuated from 1.69± 0.05 at SAR to 2.02±0.04 at U, both in November (Fig. 5a). GSI values in females at SJC ranged from 0.64 ± 0.04 in January to 2.93 ± 0.48 in July, while males were associated with lower values in February and July. At SAR, both females and males attained peak values of GSI in July, and both sexes had relatively low values in other months. Females at U had high GSI values in April and July, while males attained their maximum value in April (Figs. 5b and c). Based on GSI, *A. aeneus* has a reproductive peak during July, a trait that was previously noted in *A. fasciatus* (Carvalho *et al*., 2009).

HSI in females at SJC ranged from 1.94±0.08 (February) to 2.98±0.41 (November), while males achieved their minimum HSI value in January and their maximum HSI value in November. At SAR, females had their minimum value in April, and the minimum value for males occurred in January and April. Both

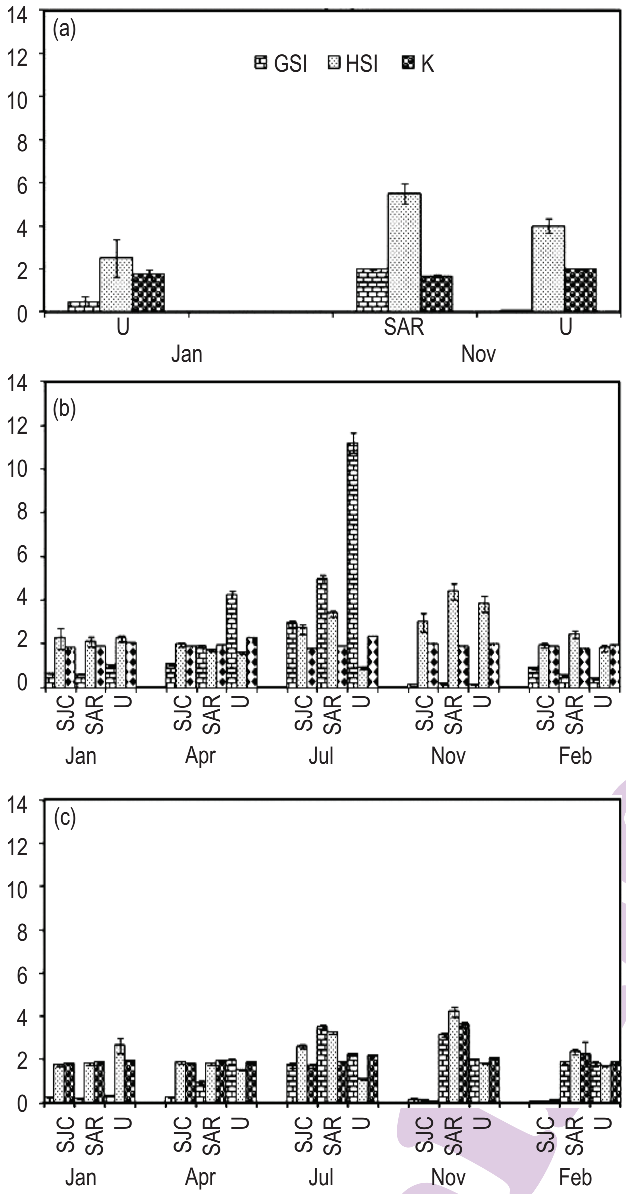

Fig. 5 : Somatic indices in (a) Immature (b) Female (c) Male *Astyanax aeneus*. GSI: gonadosomatic index; HSI: hepatosomatic index; K: condition factor.

sexes at U in July and November had higher values (Figs. 5b and c). In *A. aeneus*, higher HSI values were recorded during period of reproductive inactivity, which we interpreted as an increase in the materials stored in the liver for subsequent use in gamete production. Low HSI values prior to and during reproduction may result from the transfer of energy materials stored in the liver to gonad maturation and the breeding event, depleting energy reserves in the liver and reducing HSI values (Maddock and Burton, 1999). *A. henseli* in previous studies was associated with higher HSI values before the breeding period and with lower values at its reproductive peak, suggesting greater use of liver reserves for vitellogenesis and gonadal maturation (Carvalho *et al*., 2009).

The lowest K values at SJC for both sexes occurred in July, while the highest were observed in January for females and in November for males. For both sexes at SAR, the lowest K value was recorded in February and the highest value in April. At U, the lowest K value in females was recorded in November and February and the highest value was observed in July, while in males the lowest values occurred in April and February and the highest value occurred in July (Figs. 5b and c). Our K calculations demonstrate that *A. aeneus* maintained a robust and stable condition between study periods in the upper river, and spatial differences were evident during the breeding season, when organisms with higher K and GSI values were located in the SAR and U sites. Santos and Costa (2008) stated that K values in two *Astyanax* species were higher during the wet season, which may be related to a greater supply of allochthonous food during this period. In the present study, highest K value occurred in July at site U for both sexes, and may be closely related to a greater supply of allochthonous food. At this study site, fish were cultured in hanging cages and provided with food pellets. Native fish such as *A. aeneus* may ultimately access the pellets, obtaining nutrients that help them attain higher K values.

The first two axes of the canonical correspondence analysis accounted for 86.58% of the observed variance (62.23% in the first axis and 24.35% in the second axis). The sampling sites were scattered throughout the four quadrants in the biplot of the first two components. During the months of February and April, plotted in the lower-left quadrant, the three study sites were characterized by higher values for LPO-Liver; higher values of BOD_5, hardness, and alkalinity were also detected during these months. Additionally, in April (the dry season) the three sites were associated with higher values of LPO-Gill. The SAR and U study sites in the month of July appeared in the upper-left quadrant and were characterized by higher GSI values. During this period, higher levels of nitrates and total and fecal coliforms as well as higher LPO-Gill values were detected. November (post-hurricane period) is plotted in the upper-right quadrant and was characterized by higher values of LPO-Muscle, hepatic glycogen, HSI, K and color (Fig. 6).

Hepatic glycogen and K values started decreasing in April when the GSI value was the highest, and this decrease proceeded until June. During the period of low reproductive activity, nutrition reserves such as fat and glycogen accumulated in the body, and K tended to increase during this period. On the other hand, when gonad development began, K was very high, as reserve materials such as fat and glycogen began to be consumed and transferred to gonads for the formation of oocytes.

We detected an inverse relationship between GSI and liver glycogen; when GSI values were lower (repose phase),the highest glycogen levels in the liver were observed, allowing the

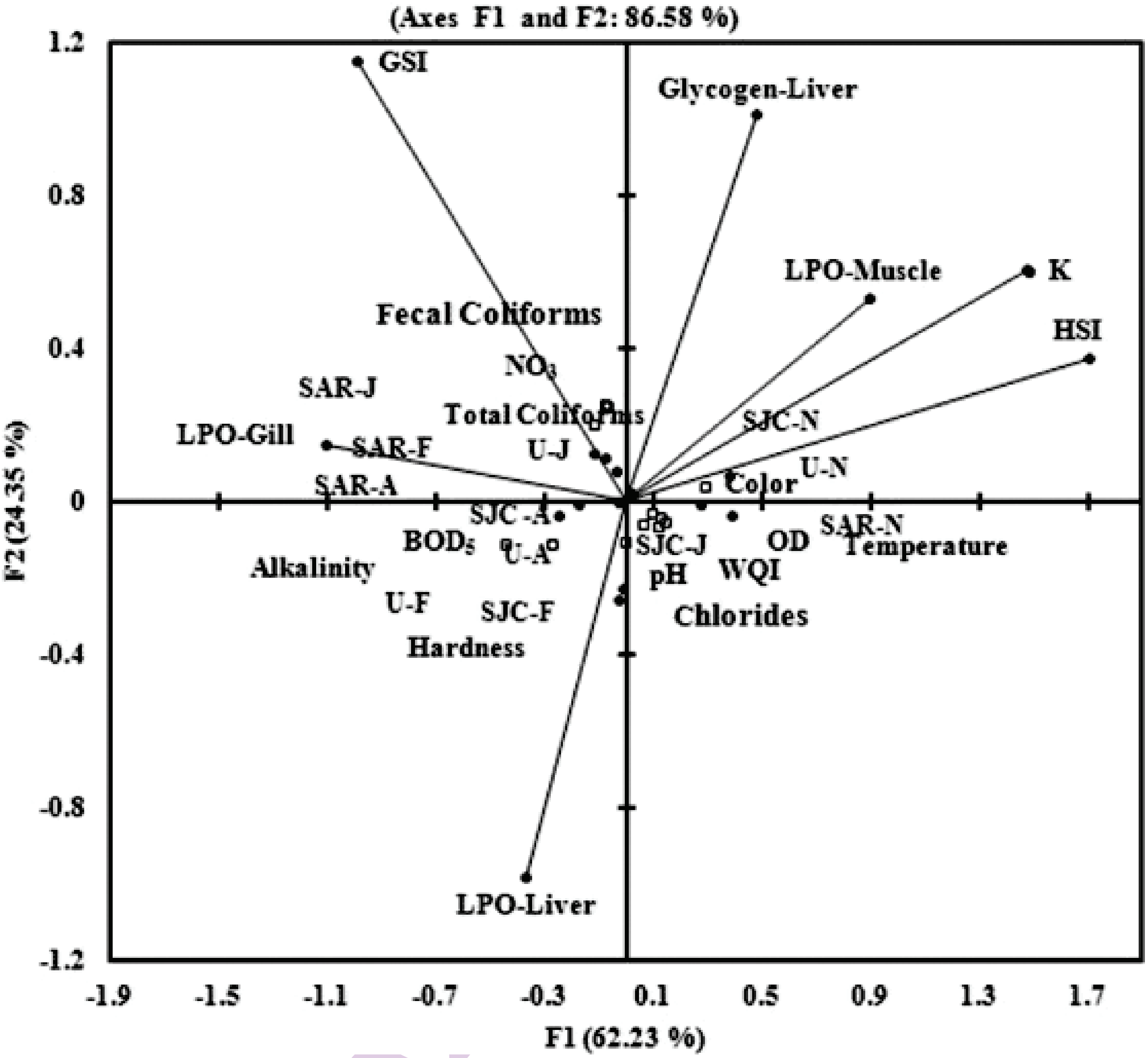

Fig. 6 : Canonical correspondence analysis biplot.Sites, environmental variables, biomarkers and somatic indices: Letters after the study site (SJC, San Juan Carpizo; SAR, San Antonio del Río; U, Ulumal) indicate the month: F, February; A, April; J, July; N, November. Biomarkers and somatic indices, WQI, water quality index; LPO, lipid peroxidation; OD, dissolved oxygen; NO3, nitrates; BDO5, biochemical demand oxygen

fish to use these reserves during gonad development. Sehgal and Goswami (2001) reported decreasing levels of hepatic glycogen during the reproduction period, an observation that was obtained in this study as well. Treberg *et al*. (2002) studied the fish *Osmerus mordax* and found that hepatic glycogen levels and K were lowest during reproduction and increased after the reproductive period.

In addition to the energy required to complete the life cycle of the fish, environmental stressors, such as contact with xenobiotics, may also deplete hepatic glycogen reserves (Coban and Sean, 2011). In *A. aeneus*, the LPO values in gill and liver as well as the IBR indicate that environmental stressors during the dry season (April) and in the post-hurricane season (November) exert strong stress effects in the fish and are also related to low level of hepatic glycogen. Wiseman and Vijayan (2011) reported a significant drop in liver glycogen content in *Oncorhynchus mykiss* exposed to polychlorinated biphenyls. In addition, Vijayan *et al.* (1997) observed an immediate increase in plasma glucose as a result of the breakdown of stored glycogen in post-stressed fish.

Despite WQI score ssuggest that Río Champotón is not highly polluted, an increase in LPO was detected during dry season and after hurricane-induced flooding. The biomarker lipid peroxidation assessed here in *A. aeneus* constitutes a sensitive and effective tool for identifying periods of environmental conditions that exert stress. Markers of oxidative damage (LPO) and hepatic glycogen suggested that two stress periods affected the health condition of *A. aeneus* in different ways. In addition to biomarkers, the use of somatic indices (GSI, HSI, and K) allowed the detection of periods of energy demand (during reproduction and stress). Overall, the present study provides evidence supporting the use of a lipid peroxidation and bioindicators in assessing the health of aquatic organisms, corroborating the suitability of *A.aeneus* as a sentinel species.

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