*Evaluation of the Health Status of the Silverside (*Odontesthes bonariensis*) at a RAMSAR Site in South America*

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EDITORIAL

Editor's Note
C.J. Schmitt 1

FOCUSED REVIEW

A Review of Organophosphate Esters in the Environment from Biological Effects to Distribution and Fate
A.K. Greaves · R.J. Letcher 2

ORIGINAL PAPERS

Domoic Acid Poisoning as a Possible Cause of Seasonal Cetacean Mass Stranding Events in Tasmania, Australia
S.M. Bengtson Nash · M.C. Baddock · E. Takahashi · A. Dawson · R. Cropp 8

Persistent Mercury Contamination in Shooting Range Soils: The Legacy from Former Primers
M. Stauffer · A. Pignolet · J.A. Corcho Alvarado 14

Application of Biotic Ligand Model in Predicting Copper Acute Toxicity to Carp (*Cyprinidae*)
W. Wang · Q. Liang · J. Zhao · R. Chen 22

Potential of the Biotic Ligand Model (BLM) to Predict Copper Toxicity in the White-Water of the Solimões-Amazon River
G.D. Pont · F.X.V. Domingos · M. Fernandes-de-Castilho · A.L. Val 27

Biochemical and Histopathological Changes Induced by Nickel in the Striped Mullet, *Mugil cephalus* (Linnaeus 1758)
A. Nagarjuna · D. Mohan 33

Partial Gene Sequencing of CYP1A, Vitellogenin, and Metallothionein in Mosquitofish *Gambusia yucatana* and *Gambusia sexradiata*
R. Vázquez-Euán · K.S. Escalante-Herrera · G. Rodríguez-Fuentes 41

Multi-Biomarker Responses After Exposure to Pollution in the Mediterranean Mussels (*Mytilus galloprovincialis* L.) in the Aegean Coast of Turkey
D. Ozkan · M. Dagdeviren · S. Katalay · A. Guner · N.Ü.K. Yavaşoğlu 46

A Cautionary Note: *Ceriodaphnia dubia* Inter-Laboratory Test Variability
L. Pacholski · P. Chapman · A. Hood · M. Peters 53

Do Varying Aquatic Plant Species Affect Phytoplankton and Crustacean Responses to a Nitrogen-Permethrin Mixture?
R.E. Lizotte Jr · M.T. Moore 58

Polychlorinated Biphenyl Bioaccumulation Patterns Among Lake Erie Lower Trophic Level Consumers Reflect Species Ecologies
J.A. Pitt · K.G. Drouillard · G. Paterson 65

Bioaccumulation of PCBs in a Wild North American Felid
E. Boyles · C.K. Nielsen 71

Characteristics, Sources and Health Risk Assessment of Trace Metals in PM_{10} in Panzhihua, China
X. Cheng · Y. Huang · Z. Long · S. Ni · Z. Shi · C. Zhang 76

Contents continued on back cover

128 BECTA6 98(1) 1–146 (2017) ISSN 0007-4861

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Evaluation of the Health Status of the Silverside (*Odontesthes bonariensis*) at a RAMSAR Site in South America

M. L. Ballesteros[1] · A. C. Hued[1] · M. Gonzalez[2] · K. S. B. Miglioranza[2] · M. A. Bistoni[1]

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Abstract The objective of this work was to evaluate the health status of an economic and ecologically important fish species from Mar Chiquita Lake, a RAMSAR site located in Cordoba, Argentina, relative to the levels of selected persistent organic pollutants (POPs) in lake water and fish tissues. *Odontesthes bonariensis* was used as a model species, and its health was estimated by means of histological indices in gills and liver. Sampling was performed according to rainy and dry seasons (i.e. dry, rainy and post-rainy). Gill and liver histopathology were evaluated by semi-quantitative indices and morphometric analysis. Although epithelial lifting in gills and lipid degeneration in liver were frequently registered, they are considered as reversible if environmental conditions improve. During rainy and post-rainy seasons fish presented significantly higher scores of liver and total indices. These higher index scores were correlated with increased levels of POPs in gill and liver tissue. Therefore, preventive measures are needed to mitigate the entry of these compounds into the lake.

Keywords *Odontesthes bonariensis* · Histological indices · Persistent Organic Pollutants · Health status

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Wetlands are among the most important ecosystems and many of their functions (e.g. nutrient cycling, sediment retention, flood control, etc.) are considered as important ecosystem services for humans (Mitsch and Gosselink 2000). Mar Chiquita Lake is the biggest saline lake of South America. It has belonged to the Western Hemisphere Shorebird Reserve Network since 1993 and was declared a RAMSAR site (wetlands with international importance by RAMSAR Convention) in 2002. It is a legally protected environment, with three endorheic rivers discharging into it: Suquía, Xanaes and Dulce Rivers. The occurrence of a great variety of contaminants in the water phase (up to 100 ng/L) such as atrazine, α endosulfan, β endosulfan, chlorpyrifos, polychlorinated biphenyls, polybrominated diphenyl ethers and heavy metals have been reported in the Suquía River Basin (Monferrán et al. 2011; Bonansea et al. 2013; Ballesteros et al. 2014). The exposure to these compounds is frequently associated with many deleterious effects in the biota, at different levels of biological organization including histological effects in fish after acute and chronic exposure (Ballesteros et al. 2007; Rautenberg et al. 2014). In this sense, histological alterations have been widely used to detect contaminant induced effects (Stentiford et al. 2003). Semi-quantitative histopathological methods provide more sensitive and useful insights than descriptive histology alone because they take into account the frequency and the biological importance of alterations (Bernet et al. 1999). Fish are considered as suitable indicators for environmental pollution biomonitoring as they can absorb and concentrate pollutants in different tissues depending on the exposure route (either water or diet). They also enable the assessment of potential pollutant transfer through the trophic food web (Fisk et al. 2001; Boon et al. 2002; Erdogrul et al. 2005).

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The silverside *Odontesthes bonariensis* (Atheriniformes, Atherinopsidae) has been widely used as a model species under field and laboratory conditions (Cazenave et al. 2005; Carriquiriborde et al. 2009). In a previous work we examined the occurrence and distribution of persistent organic pollutants (POPs) in water, sediments, suspended particulate matter and silversides from Mar Chiquita (Ballesteros et al. 2014). It was thereby uncovered, that during the post-rainy season the levels of polychlorinated biphenyls (PCBs) in muscle were above the acceptable daily intake for 70 kg (ATSDR 2000). Also the levels of hexachlorocyclohexanes (γ HCH mainly) in water and sediments exceeded the regulatory acceptable levels (Subsecretaria de Recursos Hidricos 2005). However, no evaluation on the adverse effects of POPs on aquatic organisms was performed. Therefore, in the present work our main goal was to evaluate possible histological effects in *O. bonariensis* relative to measured levels of POPs in the water and fish from the Mar Chiquita Lake RAMSAR site.

Materials and Methods

Mar Chiquita Lake (30–30°55′S y 62–63′W) is a hypersaline shallow lake (salinity > 35 g L^{-1}) with a surface of 5770 m^2, located 150 km northeast of Cordoba City (Argentina) (Fig. 1). Two monitoring stations were selected at the south coast of the lake: 1- Laguna del Plata (LP, S 30° 50′ 09.6″/W62° 53′ 21.6″), close to Suquía River mouth, where many kind of contaminants (agricultural and/or industrial) reach the lake and 2- Campo Mare (CM, S 30° 48′ 49.4″/W 62° 52′ 02.8″) where the soybean fields extend to the shore of the lake. Three samplings over 1 year were carried out at each station during 2008, covering dry (September), rainy (March) and post-rainy (May) seasons.

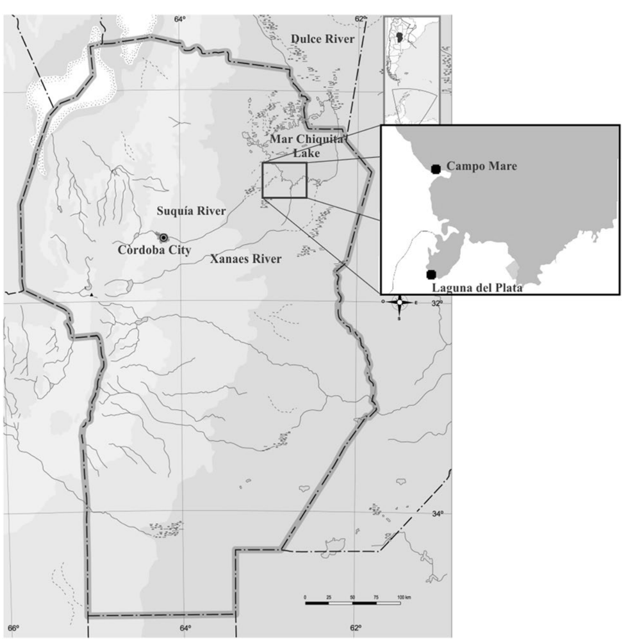

Fig. 1 Study area and location of sampling sites in Mar Chiquita Lake

Individuals of *O. bonariensis* (n = 15) were sampled simultaneously with the sampling of water, sediments and suspended particulate matter for screening of POPs (data previously reported in Ballesteros et al. 2014). Fish were collected following standard fishing procedures using a seine net (25 m length, 1.2 height, 15 mm mesh at both sides and 6 mm in the middle). After collecting, fish were killed immediately by transecting the spinal cord. This experimental procedure was approved by the Committee on Animal Bioethics and Welfare Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, 2005, Res 1047). Fish were transported to the laboratory in ice and stored in buffered 10% formaldehyde solution using monobasic (0.03 M) and dibasic (0.05 M) sodium phosphate to reach pH 7. Once in the laboratory, each fish was weighed individually (mean = 20.2 ± 3.7 g, accuracy = 0.0001 g) and the standard length measured as follows: the distance from the tip of the longest jaw to the end of caudal peduncle (mean = 114.6 ± 7.4 mm, accuracy = 0.5 mm). The Fulton condition factor [K= (total weight × 100,000)/total $length^3$] and the liver somatic index [LSI= (liver weight × 100)/total weight] were also calculated (Van der Oost et al. 2003). Afterwards, individuals were dissected and gills (GLS, second gill arch) and liver (LVR) removed for histological preparations. Samples were stained with Hematoxylin-Eosyn (H&E). Tissue lesions were examined with a light microscope and photographed with a digital camera (Panasonic®, Model DMC-FH20, Xiamen, China). The observation of the slides were carried out using a blind approach in order to avoid any bias during the quantification process. Digital images were analyzed through the Image J software 1.44p (Rasband, 1997–2012). The histological characteristics of each organ were analyzed through the application of semi-quantitative indices for gills (I_{GLS}) and liver (I_{LVR}) proposed by Bernet et al. (1999) with modifications as suggested by Rautenberg et al. (2014). Briefly, in order to calculate those indices, alterations were classified into four major reaction patterns (RP): RP1, circulatory disturbances (GLS: hemorrhage, aneurysm, edema; LVR: dilatation of sinusoids, vascular congestion, hemorrhage), RP2, regressive (GLS: epithelial lifting, lamellar disorganization and shortening; LVR: vacuolar degeneration, nuclear alteration, fibrosis, necrosis), RP3, progressive (cell hypertrophy and hyperplasia) and RP4, inflammatory changes (leukocyte infiltration). Since the relevance of a lesion depends on its pathological importance, an Importance Factor (W) (ranging from 1 to

3) was assigned to each alteration. A high importance factor represents a greater potential of an alteration to impact fish health (Bernet et al. 1999).

For each organ, the percentage of gill filaments or liver areas affected were assessed using a score ranging from 0 to 8, according the degree and extension for a particular alteration: 0- unchanged; 2- mild occurrence; 4- moderate occurrence; and 6- severe occurrence and 8-very severe occurrence. The importance factors and the score values were multiplied to give an index for a particular alteration. Then, they were summed to give an index for each reaction pattern (reaction pattern index for an organ). The indices for each reaction pattern of an organ were summed to give an overall organ index (I_{GLS}= gill histopathological index; I_{LVR}= liver histopathological index). In order to determine the overall health status based on the histological lesions, a total histopathological index ($I_{[T]}$) was calculated by adding up gills and liver indices of an individual fish (Bernet et al. 1999). The higher the index values, the more severely the organs were affected.

Morphometric analyses of GLS were performed following Nero et al. (2006), with modifications according to Hued et al. (2012). Briefly, ten secondary lamellae of five primary filaments were randomly selected and photographed for each fish. Then, secondary lamellae length (SLL), width (SLW), interlamellar distance (ID), and basal epithelial thickness (BET) were measured. In order to evaluate the gills functionality for gas exchange, the proportion of the secondary lamellae available for gas exchange (PAGE) was calculated as: PAGE (%) = 100*[mean SLL/(mean BET + mean SLL)]; another index was calculated as a function of lamellae width: $PAGE_W$(%) = 100*[mean ID/(mean ID + mean SLW)]; and finally, an integrative PAGE: $PAGE_T$ (%)=(PAGE * $PAGE_W$)/100 was considered. The last two PAGE indices were proposed by (Maggioni et al. 2012).

With the purpose of assessing possible relationships between POP levels and histological indices, data for different groups of POPs that were accumulated by *O. bonariensis* (n = 35) in GLS and LVR, and the levels in water samples, were taken from Ballesteros et al. (2014). These groups were (a) ΣOCPs (organochlorine pesticides, as the sum of endosulfans, DDTs-dichlorodiphenyltrichloroethane, HCHs-hexachlorocyclohexanes) and (b) ΣPCBs (polychlorinated biphenyls) + ΣPBDEs (polybrominated diphenyl ethers), indicating pollution from agricultural and industrial activities, respectively.

Statistical analyses were carried out using the Infostat Software Package (Di Rienzo et al. 2016). Differences among sampling periods or between sampling stations were assessed by a one way analysis of variance (ANOVA), followed by the Student Newman Keuls (SNK) multiple comparison test. When parametric assumptions were not fulfilled, the Kruskal–Wallis followed by the multi-comparison Dunn's tests were used. Significance level was set at $\alpha = 0.05$. Because of the absence of significant differences between sampling stations, all the following analyses were carried out to evaluate differences among sampling seasons. Also, two multivariate analysis were carried out. First, in order to establish possible correlations among POPs levels in GLS, LVR and water, histological indices, and gill morphometrics a canonical correlation analysis was carried out. Secondly, a discriminant analysis was performed with the purpose of elucidating which combination of single variables (POP levels in GLS, LVR and water, histological indices, and gills morphometrics) measured in fish could discriminate among groups of interest (in this case sampling seasons).

Results and Discussion

Because none of the selected variables in this work showed differences between the two sampling stations ($p > 0.05$), all data were grouped in order to assess for differences among sampling periods (i.e. dry, rainy and post-rainy seasons). The absence of significant differences in the accumulation of POPs in fish between sites is coincident with a previous work (Ballesteros et al. 2014) where the levels of POPs in the abiotic matrices (water, sediments and suspended particulate matter) in the sampling stations were not significantly different. As it is well known, most of these contaminants were forbidden decades ago with the exception of the pesticide endosulfan that was still being used during the rainy season at the time of this study. Likewise, in that previous work, the authors registered the presence of POPs in the stomach content of the silverside and they did not find significant differences between sampling stations. Finally, the high mobility of silverside, should be considered. Although the displacements of this species in Mar Chiquita Lake is still unknown, Bucher and Etchegoin (2006) pointed out that larger specimens were captured on the center of the lake during the non-reproductive season and they appeared on the shore of the lake during the spawning season, suggesting displacements of this species in the lake. Furthermore, the anatomy of this species (a compressed and elongated body with high pectoral fins located right behind the head), is associated with high mobility and speed (Ringuelet et al. 1967).

When the levels of POPs were analyzed according to season, significantly higher ΣOCPs and Total POPs were measured in the post-rainy period compared with the other seasons, whereas industrial ΣPCBs + PBDEs were not significantly different (Table 1). During the rainy season, contaminants enter the lake through many routes such as atmospheric deposition, aerial spraying, surface runoff

Table 1 Mean levels of POPs (ng g wet wt^{-1}) in gills and liver of *O. bonariensis* and water (ng L^{-1}) from Mar Chiquita Lake

	Dry	Rainy	Post-rainy
Gills			
ΣOCPs	75.7 ± 12.8^a	171.1 ± 36.7^c	213.7 ± 44.1^b
ΣPCBs + PBDEs	27.8 ± 5.9^a	39.3 ± 11.1^b	63.4 ± 8.4^c
Total POPs	76.1 ± 12.9^a	171.9 ± 36.8^b	213.8 ± 44.6^b
Liver			
ΣOCPs	32.6 ± 9.8^a	194.0 ± 35.6^c	134.7 ± 19.2^b
ΣPCBs + PBDEs	14.7 ± 9.3^a	34.3 ± 7.0^c	28.7 ± 6.1^b
Total POPs	40.6 ± 10.8^a	198.7 ± 35.6^c	150.6 ± 35.6^b
Water			
ΣOCPs	6.9 ± 3^a	18.8 ± 2.7^a	35.2 ± 11.6^b
ΣPCBs + PBDEs	3.8 ± 0.9^a	2.2 ± 1^a	4.9 ± 2.7^a
Total POPs	14.1 ± 2.8^a	21.0 ± 3.0^a	40.1 ± 14.3^b

Means not sharing the same letter in each row are significantly different at p <0.05. Data taken and modified from Ballesteros et al. (2014).

Table 2 Condition factor, hepatosomatic index, gill and liver pathological indices and gill measurements in *O. bonariensis* from Mar Chiquita Lake (mean ± SE, n = 15)

	Dry	Rainy	Post-rainy
K	1.1 ± 0.1a	1.0 ± 0.2a	1.4 ± 0.1 A
LSI	1.8 ± 0.2a	1.8 ± 0.2a	2.1 ± 0.3 A
I_{GLS}	14.8 ± 3.1a	15.5 ± 4.7a	15.0 ± 4.9 A
I_{LVR}	9.6 ± 2.5a	11.3 ± 2.4b	29.0 ± 2.4 C
I_T	24.4 ± 3.1a	26.7 ± 5.5b	44.5 ± 3.6 C
PAGE	69.7 ± 1.8a	71.7 ± 4.5a	69.4 ± 4.6 A
$PAGE_w$	56.0 ± 1.5a	51.1 ± 3.6a	54.5 ± 4.3 A
$PAGE_T$	38.9 ± 0.8a	36.9 ± 4.6a	38.4 ± 5.4 A

Means not sharing the same letter in each row are significantly different at $p < 0.05$

K condition factor, *LSI* liver somatic index, I_{GLS} Gill histological index, I_{LVR} Liver Histological Index. I^T Total Index, *PAGE* proportion of the secondary lamellae available for gas exchange, $PAGE_w$ proportion of the secondary lamellae available for gas exchange based on width, $PAGE_T$ Total proportion of the secondary lamellae available for gas exchange

and from the tributary of the rivers. Contaminants remain in the lake during the post-rainy period adsorbed to suspended particulate material, sediment. Fish may ultimately uptake the contaminants through their gills or via food consumption, allowing for the contaminants to enter the blood stream and body tissues, with the possibility for causing histological damage in the different tissues.

Although the K index is considered a general biomarker of the health fish status, it also reflects the effects of non-pollutant factors (e.g. season, diseases and nutritional level) and gives information about energy reserves (Linde-Arias et al. 2008). In the present work, K values did not show any changes among sampling periods (Table 2) and varied between 1.1 and 1.4 These results are coincident with those obtained by Mancini et al. (2009) for the same species in Los Charos Lagoon (Córdoba, Argentina), where the K index varied around 0.9–1.3. The mentioned authors indicated that these values are close to the standard curve for the species. Moreover, all fish assessed in the present work contained food in their guts suggesting a sufficient availability of food throughout the year (Sagretti and Bistoni 2001). Similarly, the LSI index, which informs about the liver status, also did not vary significantly between sampling periods.

The histological study showed that I_{GLS} and gill morphometric analysis values were similar at all sampling periods. The most frequent alterations registered in GLS corresponded to RP2, being the epithelial lifting and the shortening of secondary lamellae present in 93% and 60% of all the individuals analyzed, respectively. These alterations are considered as defense mechanisms of fish to avoid contaminant uptake, increasing the distance between blood and the surrounding environment (Mallat 1985). The alterations related to RP3 (progressive changes) such as chloride and pavement cell hyperthrophy and hyperplasia were the least frequent (<27%) while circulatory disturbances (RP1) were only represented by aneurisms and they were found in 40% of samples. On the other hand, leukocyte infiltration was not registered (RP4). These histopathological alterations in GLS could be considered as a general response to the exposure to environmental contaminants. The vital functions performed by gills, their permanent contact with the aquatic environment and the extremely large surface area of the secondary epithelium are the main reasons for the particular sensitivity of this organ to pollutants (Wood 2001). The score obtained by the I_{GLS} are around 8%–10% of maximum value that the index can assume as the poorest condition of the organ. According to this, gills of fish inhabiting Mar Chiquita Lake were slightly affected. Also, the PAGE index values calculated in this study (between 69% and 72%) is coincident to the values obtained for *Prochilodus lineatus*, from moderately polluted sites in the Salado River Basin (Santa Fe, Argentina) (Troncoso et al. 2012). In contrast, POP levels in GLS showed significant differences among sampling periods for all the studied pollutants (agricultural ΣOCPs, industrial ΣPCBs + PBDEs and total of POPs, Table 1). The highest levels of ΣOCPs and industrial contaminants (ΣPCBs + PBDEs) were found during the post-rainy season followed by the rainy season. In all cases the lowest levels were found in the dry season.

The RP1 was the most frequent group of alterations registered in LVR. Dilation of sinusoids and vascular congestion were found in 100% and 93% of the fish sampled, respectively. Alterations that correspond to RP2 were found in 27% of the samples, with lipid degeneration being the only histopathological damage registered for RP2. Progressive (RP3) and inflammatory alterations (RP4) were not observed. Thus, the histological changes detected in our work can be considered as reversible if the environmental conditions improve (Ballesteros et al. 2007; Costa et al. 2009). The liver histopathological index (I_{LVR}) and Total Index (I_T) differed significantly between sampling periods, showing the highest scores during the post-rainy season (Table 1). The ΣOCPs, ΣPCBs + PBDEs and the Total POP levels in liver were significantly higher during the rainy season followed in order by the post-rainy season and the dry season (Table 1). These results suggest that fishes were chronically exposed during the rainy season. However, the main effects were seen in the post-rainy season where fishes presented the highest values of the histopathological indices, possibly indicating the consequences of the previous exposures during the rainy season.

Special attention should be given to the pesticide endosulfan, because it was the only POP used in the area while this study was carried out. This insecticide is applied for soybean and wheat cultures which represent 50% of the agricultural areas in the country (15 million ha) (González et al. 2012) The other POPs were forbidden in the 1990s and 2000s and they were also included in the POPs list by the Stockholm Convention (UNEP. Stockolm Convention 2005). Their presence in the environment is due to their high persistence and past use. During rainy and post-rainy seasons Ballesteros et al. (2014) registered the highest levels of endosulfan in water (11–12 ng/L during rainy season) and suspended particulate material (37–57 ng/g dry wt during post-rainy season). It is well known that endosulfan and other POPs cause histological damage in gills (e.g. epithelial lifting, aneurisms and shortening of secondary lamellae) and liver (e.g. hydropic degeneration, vascular congestion, etc.). Fish species like *Jenynsia. multidentata, Cichlasoma dimerus* and *Corydoras paleatus* exposed to endosulfan and lindane presented these alterations under acute exposure to high concentrations of endosulfan (µg/L, Ballesteros et al. 2007; Pesce et al. 2008; Da Cuña et al. 2011). Therefore it is expected that fish chronically exposed to this contaminant and others not measured here could present similar alterations in their organs. Also, lipid degeneration in silversides was reported by Silva Barni et al. (2016) in a field study from La Pelegrina lake during during a post-pesticide application, where the levels of endosulfan in water and liver were in the same order of magnitude as those reported in Ballesteros et al. (2014) and in this study.

Canonical correlation is a multivariate technique widely used to establish correlations between groups of variables. In this study, it was used to determine the relationships between the levels of POPs in fish organs and histological indices measured in fish grouped together and the levels of contaminants in water. The results showed a significant relationship between both groups (Table 3), demonstrating the likelihood that the levels of contaminants measured in water, as well as in the studied organs, exerted effects at the histological level. It is important to note that even when the individual analysis of the variables did not demonstrate changes, the combination of them in one multivariate analysis can elucidate what could be actually happening in the environment.

Table 3 Canonical correlation analysis between group of biological variables and POPS measured in water

	L(1)	L(2)
R	0.814	0.629
R²	0.663	0.395
λ	33.411	10.554
df	14	6
p-value	0.002	0.103

L(1) and L(*2*) canonical variables generated through the analysis, *R* Canonical correlation coefficient, R^2 Proportion of variance explained by each pair of canonical variables, statistic used in the analysis; *df* degrees of freedom

Table 4 Discriminant analysis output as cross-classification table of assigned cases to the different groups (apparent error rate %)

Groups	Dry	Rainy	Post-rainy	Total	Error (%)
Dry	8	0	0	8	0
Rainy	0	7	0	7	0
Post-rainy	0	1	11	12	8.3
Total	8	8	11	27	3.7

Linear discriminant analysis (LDA) was carried out considering the sampling season as the grouping variable (Fig. 2). The variables ΣOCPs, ΣPCBs + PBDEs in gills and liver, I_{GLS}, $I_{LVR,}$ PAGE (%), $PAGE_W$ (%) and $PAGE_T$ (%), were used as independent variables. Total POPs and I_T were not used in the LDA as they are carried redundant information. On the other hand, the LDA allowed us to evaluate if the individuals of the samples were correctly assigned to the pre-assigned groups (in this work, the sampling stations) according to the combination of variables taken into account for the analysis. Thus, the classification matrix afforded 97% of right assignations of the individuals of the sample to the groups (Table 4), there being only one fish from post-rainy season group assigned to the rainy season group incorrectly. This analysis indicated that fish from the three groups had clearly different health conditions according to the combination

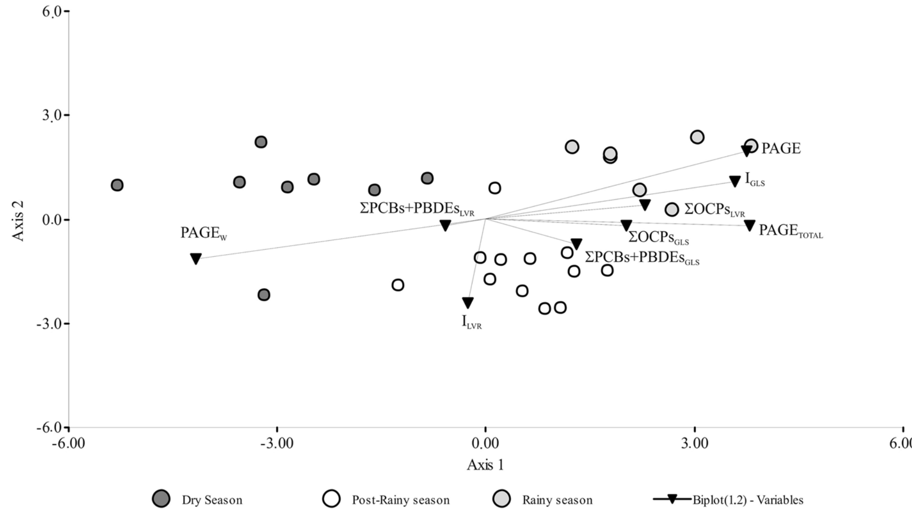

Fig. 2 Discriminant analysis plot between sampling periods (grouping variables) and combinations of biological and chemical variables from Mar Chiquita Lake. The length of the *dotted lines* in the variables indicates the weight of each variable on each axis

of variables in multivariate space. Figure 2 shows those dry and rainy seasons can be discriminated along axis 1 by the variables $PAGE_w$ to the left side and $PAGE_{total}$ to the right side. Also, the histopathological index I_{LVR} was the variable that contributed the most on axis 2 in the discrimination of individuals of the Post-rainy season. Thus, this multivariate analysis allowed us to determine the overall health condition of fish during the studied sampling periods in an integrative manner where the study of variables individually was not sensitive enough to achieve it.

The present study demonstrated the occurrence of histological damage in the organs of fish at the RAMSAR site of Mar Chiquita Lake. The histological alterations in gills and liver were related to POP levels found in these organs and also with the concentrations registered in the water environment. Although the liver presented reversible damage, the chronicity of the exposure to which fish are subjected, may compromise the health of individuals and population integrity, since the possible consequences are unknown in the long term. As POPs are persistent for decades, preventive measures to mitigate the entry of these compounds into the aquatic environment should be taken to preserve the biota that inhabit Mar Chiquita Lake. Such measures might include the control of pesticide and industrial emissions, control of legal and illegal discharge of residues into the lake and its tributaries, restriction in the cultivated areas near the lake, and the establishment of riparian areas and artificial wetlands as natural filters.

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References

Agency for Toxic Substances and Disease Registry (ATSDR) (2000) Toxicological profile for polychlorinated biphenyls (PCBs). Atlanta, GA. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=142&tid=26

Ballesteros ML, Bianchi GE, Carranza M, Bistoni MA (2007) Endosulfan acute toxicity and histomorphological alterations in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). J Environ Sci Health B 42:351–357

Ballesteros ML, Miglioranza KSB, González M et al (2014) Multimatrix measurement of persistent organic pollutants in Mar Chiquita, a continental saline shallow lake. Sci Total Environ 490:73–80

Bernet D, Schmidt H, Meier W et al. (1999) Histopathology in fish: proposal for a p.rotocol to assess aquatic pollution. J Fish Dis 22:25–34

Bonansea RI, Amé MV, Wunderlin DA (2013) Determination of priority pesticides in water samples combining SPE and SPME coupled to GC-MS. A case study: Suquía River basin (Argentina). Chemosphere 90:1860–1869

Boon JP, Lewis WE, Tjoen-A-Choy MR et al (2002) Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. Environ Sci Technol 36:4025–4032

Bucher EH, Etchegoin M (2006) El pejerrey como recurso. In: Bucher EH (ed) Bañados del río Dulce y Laguna Mar Chiquita (Córdoba, Argentina), Primea. Academia Nacional de Ciencias (Córdoba, Argentina), Córdoba, pp 201–217

Carriquiriborde P, Díaz J, López GC et al (2009) Effects of cypermethrin chronic exposure and water temperature on survival, growth, sex differentiation, and gonadal developmental stages of *Odontesthes bonariensis* (Teleostei). Chemosphere 76:374–380

Cazenave J, Wunderlin DA, Bistoni M de los, et al (2005) Uptake, tissue distribution and accumulation of microcystin-RR in *Corydoras paleatus, Jenynsia multidentata* and *Odontesthes bonariensis*: A field and laboratory study. Aquat Toxicol 75:178–190

Costa PM, Diniz MS, Caeiro S et al (2009) Histological biomarkers in liver and gills of juvenile *Solea senegalensis* exposed to contaminated estuarine sediments: a weighted indices approach. Aquat Toxicol 92:202–212

Da Cuña RH, Rey Vázquez G, Piol MN et al (2011) Assessment of the acute toxicity of the organochlorine pesticide endosulfan in

Cichlasoma dimerus (Teleostei, Perciformes). Ecotoxicol Environ Saf 74:1065–1073
Di Rienzo JA, Casanofes F, Balzarini MG, et al (2016) InfoStat Software Page, InfoStat, Córdoba
Erdogrul Ö, Covaci A, Schepens P (2005) Levels of organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in fish species from Kahramanmaras, Turkey. Environ Int 31:703–711
Fisk AT, Hobson KA, Norstrom RJ (2001) Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. Environ Sci Technol 35:732–738
González M, Miglioranza KSB, Shimabukuro VM et al (2012) Surface and groundwater pollution by organochlorine compounds in a typical soybean system from the south Pampa, Argentina. Environ. Earth Sci 65:481–491
Hued AC, Oberhofer S, Bistoni MA (2012) Exposure to a commercial glyphosate formulation (Roundup) alters normal gill and liver histology and affects male sexual activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). Arch Environ Contam Toxicol 62:107–117
Linde-Arias AR, Inácio AF, Novo LA et al (2008) Multibiomarker approach in fish to assess the impact of pollution in a large Brazilian river, Paraiba do Sul. Environ Pollut 156:974–979
Maggioni T, Hued AC, Monferrán MV et al (2012) Bioindicators and biomarkers of environmental pollution in the middle-lower basin of the Suquía River (Córdoba, Argentina). Arch Environ Contam Toxicol 63:337–353
Mallat J (1985) Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can J Fish Aquat Sci 42:630–648
Mancini M, Nicola I, Salinas V, Bucco C (2009) Biology of silverside *Odontesthes bonariensis* (Pisces, Atherinopsidae) in the shallow lake "Los Charos" (Córdoba, Argentina). Rev Peru Biol 15:65–71
Mitsch WJ, Gosselink JG (2000) Wetlands, Third edn. John Wiley & Sons, Inc, New York
Monferrán MV, Galanti LN, Bonansea RI et al (2011) Integrated survey of water pollution in the Suquía River basin (Córdoba, Argentina). J Environ Monit 13:398–409
Nero V, Farwell A, Lee LEJ et al (2006) The effects of salinity on naphthenic acid toxicity to yellow perch: gill and liver histopathology. Ecotoxicol Environ Saf 65:252–264
Pesce SF, Cazenave J, Monferrán MV et al (2008) Integrated survey on toxic effects of lindane on neotropical fish: *Corydoras paleatus* and *Jenynsia multidentata*. Environ Pollut 156:775–783
Rasband WS (2011) ImageJ. v.1.46r. https://imagej.nih.gov/ij/
Rautenberg GE, Amé M V., Monferrán M V., et al (2014) A multilevel approach using *Gambusia affinis* as a bioindicator of environmental pollution in the middle-lower basin of Suquía River. Ecol Indic 48:706–720
Ringuelet RA., Arámburu RH., Alonso de Arámburu A (1967) Los peces argentinos de agua dulce. Com. Inv. Cient. Prov. Bs. As., La Plata, Buenos Aires
Sagretti L, Bistoni MA (2001) Alimentacion de *Odontesthes bonariensis* (Cuvier y Valenciennes 1835) (Atheriniformes, Atherinidae) en la laguna Salada de Mar Chiquita (Cordoba, Argentina). Gayana 65:6–11
Silva Barni MF, Ondarza PM, Gonzalez M et al (2016) Persistent organic pollutants (POPs) in fish with different feeding habits inhabiting a shallow lake ecosystem. Sci Total Environ 550:900–909
Stentiford GD, Longshaw M, Lyons BP et al (2003) Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar Environ Res 55:137–159
Subsecretaría de Recursos Hídricos de la Nación (2005) Niveles guía nacionales de calidad de agua ambiente correspondientes a Lindano
Troncoso I, Cazenave J, Bacchetta C, Bistoni MA de los (2012) Histopahological changes in the gills and liver of *Prochilodus lineatus* from the Salado River basin (Santa Fe, Argentina). Fish Physiol Biochem 38:693–702
Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ Toxicol Pharmacol 13:57–149
Wood CM (2001) Toxic responses of the gill. In: Schlenk D, Benson WH (eds) Target organ toxicity in marine and freshwater teleosts. Taylor and Francis, USA, pp 1–67
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (2005) Resolución D Nº 1047: Marco Etico para las Investigaciones Biomédicas
UNEP. Stockolm Convention (2005) Eliminando los COP del mundo: guia del convenio de Estocolmo sobre contaminantes orgánicos persistentes