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Fishes as indicators of untreated sewage contamination in a Mexican coastal lagoon

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ABSTRACT

Lagoons are important nursery habitats for fishes but are often sites of intense human activity including wastewater discharges. The goal of this research was to compare stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes, total mercury (THg) and other metal levels in four selected fish species among sites with different levels of untreated sewage discharge inside Barra de Navidad coastal lagoon in the Mexican Pacific. Three species from sites heavily impacted by sewage showed higher $\delta^{15}N$ and $\delta^{13}C$ compared to those from non-impacted sites. In addition, the highest concentrations of THg were present in fish of two species (*Sciades guatemalensis* and *Diapterus brevirostris*) collected at the two most impacted sites, and exceeded the 0.2 μg/g ww threshold believed to be protective of adult and juvenile fish. No individuals of *Achirus mazatlanus* and *Mugil curema* exceeded this threshold, and liver somatic index and condition did not distinguish high from low impacted sites for all species. In general, the metal levels differed among species but not sites, and were lower than what has been measured in fishes elsewhere. The study also provides the first information on several fish species for coastal areas of Mexico, suggests that THg and isotopes can distinguish sewage-impacted sites, and can serve as a baseline for future studies.

1. Introduction

Human populations, urban development and land use changes are increasingly impacting the world's estuarine ecosystems through nutrient loading and chemical contaminants (Millennium Ecosystem Assessment, 2005). Non-treated sewage discharges are important sources of particulate organic matter and nutrients in estuarine environments and can induce significant changes in water quality and jeopardize the health of aquatic organisms due to habitat loss, contamination, the presence of disease-causing organisms and other factors (Pitt et al., 2009; Rožič et al., 2014). Impacts of sewage on aquatic biota are diverse and have been identified at all levels of ecological organization (Schlacher et al., 2005). Some of the particulate and dissolved nutrients discharged in sewage incorporated by organisms at the base of the food web and their use and transfer through the food web can be traced with carbon and nitrogen stable isotopes ratios (Rogers, 2003; Vizzini and Mazzola, 2006; Barros et al., 2010; Davias et al., 2013). Other impacts of sewage discharge include high concentrations of mercury (Hg) and other metals in species (Alonso et al., 2000; Canli and Atli, 2003; Marcovecchio, 2004; Rocha et al., 2014).

Organisms used as pollution monitors have numerous advantages over the chemical analyses of abiotic compartments because biota only accumulates the bioavailable forms of the contaminants (Azevedo et al., 2009). Fishes have frequently been used to assess aquatic habitat degradation and their numerous advantages, and some disadvantages, as indicator organisms have been summarized by Whitfield and Elliott (2002). In addition, an understanding of contaminants in fishes can be used to determine the risks to fish-eating wildlife and humans. This is a relevant point for coastal areas and particularly in coastal lagoons because many of them support important artisanal fisheries.

Coastal lagoons are considered to be among the most productive ecosystems in the world and provide a host of socioeconomic benefits for humans (Pérez-Ruzafa and Marcos (2012). These habitats have been recognized as important nursery grounds for many fish species (Cowan et al., 2013). Barra de Navidad lagoon is an important coastal wetland in Jalisco State, on the western coast of Mexico. The lagoon provides important ecological services and offers exceptional opportunities for developing tourism, but also can lead to resource conflicts. Previous studies have identified more than 20 species of coastal fishes which use

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the Barra de Navidad lagoon as a nursery area (González-Sansón et al., 2014). Some of these species are caught regularly as adults in the adjacent shelf waters in a successful artisanal fishery. In 2008 the Barra de Navidad lagoon ecosystem was declared a Wetland of International Importance according to the standards of the Ramsar Convention (www.ramsar.org) by the National Commission of Protected Natural Areas (www.ramsar.conanp.gob.mx/lsr.php). The lagoon is also included among the 81 priority mangrove sites in Mexico by the National Commission for the Protection of Biodiversity (CONABIO, 2009). The lagoon's ecological functions are currently impacted by erosion, and changes in land use during last 40 years have been identified in the basins associated with this coastal wetland (Silva-Bátiz et al., 2012) and have altered its hydrological regime in a way which is not well understood.

Considerable anthropogenic stressors are present in the Barra de Navidad lagoon, including residential/wastewater, touristic, agricultural and fisheries impacts. Residential impacts come from a human settlement of ca. 4300 persons on the northwest coast of the lagoon, which discharges untreated sewage, and from a large resort with a hotel, golf course, and marina for large sports boats located along the southwest shore of the lagoon. During the winter, many sailing ships (70–90) use the lagoon as anchorage with low control by harbor authorities, and without waste treatment facilities. Agriculture occurs in the catchment of the lagoon and is a source of residual pesticides and fertilizers, and artisanal fisheries harvest juveniles or preadult species within the lagoon. There has not been any systematic assessment of the impacts of the stressors on the lagoon ecosystem or coastal fish populations. The goal of this research was to conduct a comparative study of selected fish species among sites of Barra de Navidad lagoon with different levels of human impact. We hypothesized that stable isotope levels, mercury and other metals in fish would reflect their proximity to human inputs at sites where high quantities of untreated sewage are discharged into the lagoon.

2. Materials and methods

2.1. Study area and sampling sites

Barra de Navidad lagoon is located in the southern coast of Jalisco State, Mexico (19°11′25″ N–104°39′53″ W). It has a surface area of 334 ha and is surrounded by a well-developed mangrove forest (571 ha) mainly in its northeastern and southeastern margins. Freshwater inputs are through the Arroyo Seco River and an artificial channel connecting the Marabasco River with the lagoon. The lagoon exchanges water continuously with the sea through a 100 m wide channel.

The research was done in January and February 2014 at four sites of Barra de Navidad lagoon along a gradient of human impact (Fig. 1). Sampling sites were: inside Cabo Blanco Marina's channels (CAN), a site affected by boat anchorages and docking facilities, as well as untreated sewage discharge coming from Barra de Navidad town (ca. 4300 habitants) and many restaurants; Colimilla (COL), a small town (ca. 230 habitants) with untreated sewage from the town and several restaurants, as well as a gas station for boats; a reference site (ATR) which is not impacted directly by sewage discharge but receives runoff from agricultural activities near the lagoon's margins; and, a reference site (TEP) with a low level of impact by sewage discharge and other kinds of human activities (Fig. 1). All sites have mud-sand sediments rich in organic matter (10–20% of dry weight).

2.2. Sampling and methods for chemical and biological analyses

Four species were selected (Latin names after Eschemeyer et al., 2016): Blue sea-catfish, *Sciades guatemalensis* (Ariidae, Siluriformes); Pacific lined sole, *Achirus mazatlanus* (Achiridae, Pleuronectiformes); Short-snout mojarra, *Diapterus brevirostris* (Gerreidae, Perciformes) and White mullet, *Mugil curema* (Mugilidae, Mugiliformes). The main criteria for selection were: abundance, maximum reported size, feeding habits (including species with different feeding habits), and possibilities of capture (Table 1). Fish were captured using a cast net (3 m, 2.5 cm stretched mesh size), gill nets (60 m, 7.0 cm mesh size) and a small trawl net (5 m mouth, 2.5 cm mesh size). Individuals were collected between January and February of 2014 from each site. During each sampling operation salinity and temperature were measured using a YSI-30 probe. Fishes were sacrificed in the field and transported on ice (approximately 20–30 min) to the laboratory at the Department of Coastal Studies from the University of Guadalajara. At the laboratory, fish were individually measured for total length (± 1 mm) and total mass (± 1 g); sex was determined, viscera were removed and the carcass mass (± 1 g) and liver weight (± 0.001 g) were determined. Calculations were made from raw data for liver somatic index (LSI; 100 $\times$ liver

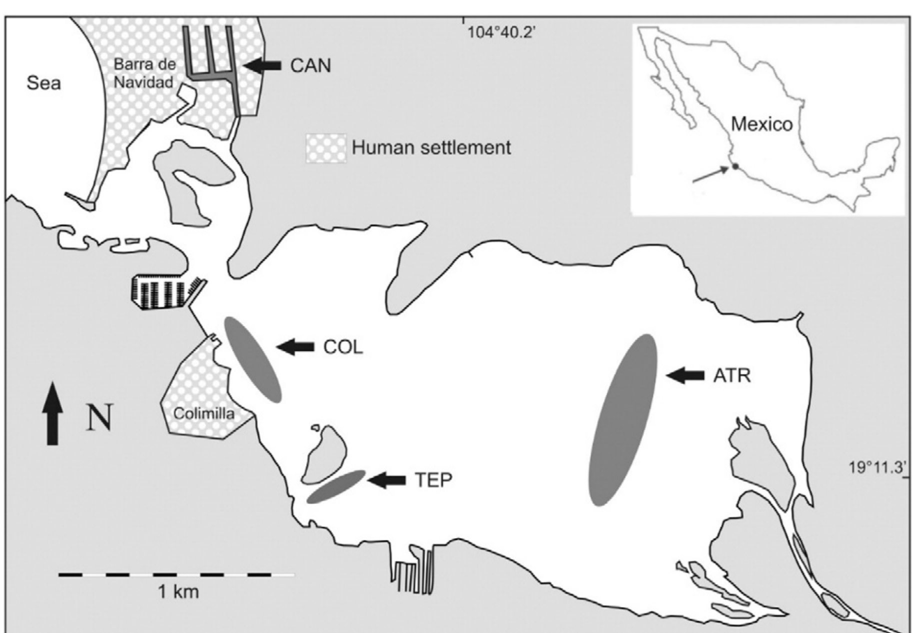

Fig. 1. Sampling sites in Barra de Navidad lagoon.

Table 1
Characteristics of selected species (based on González-Sansón et al., 2014; Froese and Pauly, 2014; Fischer et al., 1995). TL = total length in cm.

Characteristics	*Achirus mazatlanus*	*Diapterus brevirostris*	*Mugil curema*	*Sciades guatemalensis*
Abundance[a]	Abundant	Highly abundant	Abundant	Occasional
Life span (years, approx.)[b]	3	5	20	47
Reported maximum TL[b]	20.9	31.5	45.5	58.6
Estimated first reproduction TL[b]	12.8	18.5	25.8	32.3
Mean TL in study area[a]	13.7 ± 0.11	10.6 ± 0.12	15.4 ± 0.34	24.3 ± 0.91
TL range in study area[a]	1.4–22.5	1.7–24.7	3.4–38.5	6.6–51.5
Main food[c]	Benthic invertebrates and fishes	Small benthic invertebrates	Detritus	Benthic invertebrates and fishes
Trophic level[b]	3.2 ± 0.2	3.7 ± 0.2	2.0 ± 0.01	3.6 ± 0.5

[a] González-Sansón et al. (2014).
[b] Froese and Pauly (2014).
[c] Fischer et al. (1995).

weight / body weight), condition factor (CF; 100,000 × weight [g]) / (length^3 [mm]), and adjusted CF (CFadj; 100,000 × (body weight − gonad weight [g]) / (length^3 [mm]).

2.3. Stable isotope analyses

A piece of white dorsal epaxial muscle (skinless) was dissected from each fish and kept at −20 °C until tissues were processed. The samples were oven-dried at 60 °C for 48 h and then ground into a fine powder with a mortar and pestle. For the stable isotope analyses an aliquot of 1 mg was taken from each sample and packed into a 3.5 × 5 mm tin cup. The individual fish samples were combusted using a Thermoquest NC 2500 (ThermoQuest Corp. Austin, TX), and gases were submitted by way of a continuous flow of helium to a Finnigan MAT Delta Plus isotope-ratio mass spectrometer for $\delta^{13}C$ and $\delta^{15}N$ analyses at the Stable Isotopes in Nature Laboratory, University of New Brunswick, Fredericton, NB, Canada. Precision and accuracy of the method was verified by analyzing replicates of commercial isotopes standards (N_2, sucrose, acetanilide, nicotinamide, SMB-M, BLS; International Atomic Energy Agency). Duplicates of some fish were analyzed every 10th sample. Stable isotope ratios were reported as follows:

$$\delta X = \left[\left(R_{samples}/R_{standard}\right) - 1\right] \times 1000$$

where X = ^{13}C or ^{15}N and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (expressed in per mil or ‰).

2.4. Mercury analyses

The same muscle samples were analyzed for both THg and isotopes. Approximately 10 ± 0.01 mg of dried, homogenized muscle was weighed into a precleaned quartz boat. Samples were analyzed on a Milestone DMA-80 Direct Mercury Analyzer by thermal decomposition, amalgamation, and atomic absorption spectrophotometry according to United States Environmental Protection Agency Method 7473 (US EPA, 2007a). Standards and certified reference material (CRM) (DORM-4; National Research Council of Canada) were analyzed after every 10th sample. Mean (±SD) recovery of the CRM was 91.0 ± 4.2% for DORM-4. A 10 ng calibration standard yielded recoveries of 103 ± 11.2%. Relative percent differences of 14 duplicate samples ranged from 0.8 to 12.7%. The limit of detection (LOD) for these analyses was 0.025 μg/g dry weight (dw); the LOD was determined by averaging all the method blanks run in the batch and adding 3 times the SD of the method blanks. For comparisons of THg concentrations with known toxicity thresholds for fish, dry-weight concentrations were converted to muscle wet-weight (ww) concentrations using a mean water content in fish muscle of 80% (Kannan et al., 1998; Rodriguez-Sierra and Jimenez, 2002). All calibration standards (Ultra Scientific, N. Kingstown, RI, USA) were certified with a certificate of analysis.

2.5. Metal analyses

Sample digestion and analysis of metals followed a test method based on US EPA standard testing protocols 3051A (US EPA, 2007b), 200.7 (US EPA, 1994), and 6010C (US EPA, 1998). A 0.5 g aliquot of homogenized, dried fish tissue was processed using a microwave digestion (CEM Mars 5) and 10 mL of metal grade nitric acid (Fisher Scientific, Canada). After the digestion process, 3.0 mL of 2% lithium nitrate ionization buffer (SCP Science, QC) and 37 mL of Milli-Q water was added along with a known amount of Yittrium (Y) (SCP Science, QC) as an internal standard. The 22 elements were quantified using an inductively coupled plasma-optical emissions spectrophotometer (ICP-OES, iCAP 6500 Duo, Thermo Fisher Scientific) using an internal standard calibration method. Limit of quantification (LOQ; see below for explanation) and wavelengths used for quantification are listed in Table 2.

Quality assurance/quality control (QA/QC) procedures included the following: each batch of 11 samples included a method blank (MB), certified reference material (CRM) [National Institute of Standards & Technology (NIST) Standard Reference Material (SRM); 2976 Mussel Tissue], and sample duplicate. The MB consisted of Ottawa sand (Fisher Scientific, Ottawa, ON) which was run through the entire testing process. The target MB value was equal to or less than the LOQ. For instances where the MB was greater than the LOQ, the LOQ was increased to the level found in the blank. CRM and calibration check results were reported as percent recovery based on the certified and calculated target

Table 2
Summary of LOQs and wavelengths for individual elements.

Element	Symbol	LOQ (mg/kg dw)	Wavelength (λ)
Aluminum	Al	<0.31	396.0
Arsenic	As	<0.85	193.7
Cadmium	Cd	<0.08	226.5
Chromium	Cr	<0.12	267.7
Cobalt	Co	<0.15	228.6
Copper	Cu	<0.19	324.7
Iron	Fe	<0.64	259.9
Lanthanum	La	<1.00	333.7
Lead	Pb	<0.77	220.3
Magnesium	Mg	<1.14	279.0
Manganese	Mn	<0.03	257.6
Nickel	Ni	<0.13	221.6
Phosphorus	P	<0.81	178.2
Rubidium	Rb	<0.86	780.0
Selenium	Se	<1.08	196.0
Silver	Ag	<0.22	328.0
Strontium	Sr	<0.004	407.7
Sulphur	S	<1.11	180.7
Thallium	Tl	<0.55	190.8
Uranium	U	<3.8	385.9
Vanadium	V	<0.15	292.4
Zinc	Zn	<0.04	202.5, 206.2, 213.8

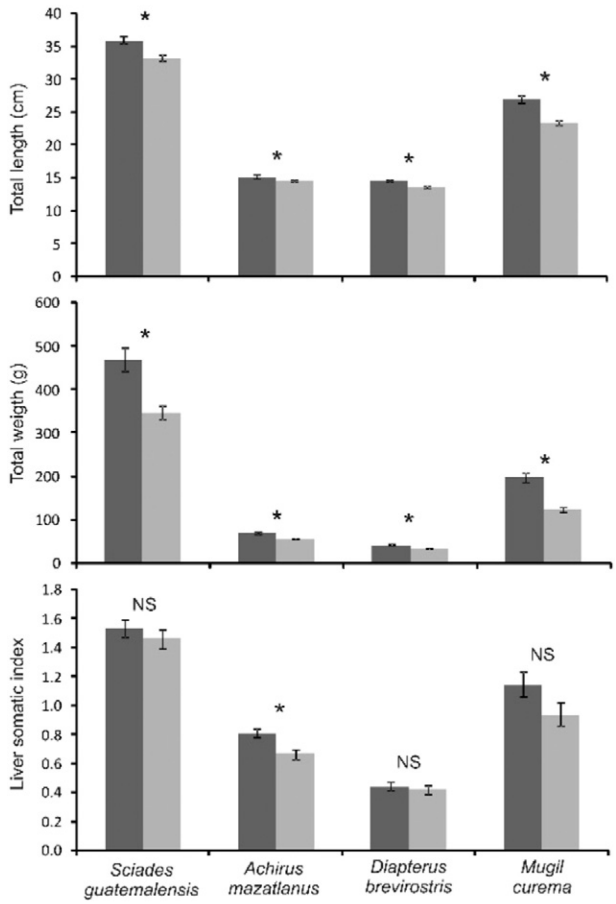

Fig. 2. Mean values (±SE) of total length, total weight and LSI for both sexes in studied species. Star indicates significant differences; NS = nonsignificant difference. Dark bars = females; light bars = males.

values. The duplicate samples were reported as relative percent differences. Instrument blanks and calibration checks were routinely done throughout the analysis. All standards (SCP Science, QC), calibration checks (SCP Science, QC), and reference materials were certified with a certificate of analysis. Instrument detection limits (IDL) were determined by running 20 repeats of a blank (IDL = average$_{blanks}$ + 3 × SD$_{blanks}$; based on US EPA, 1994). The LOQs were calculated as 5 times the IDL (Montaser and Golightly, 1992; see Table 2 for the LOQs).

2.6. Statistical analyses

Before statistical analyses, normality of the data and homogeneity of variances were examined according to the criteria of Zar (2010) and Underwood (1997). In cases where the criteria were not met, data were transformed to achieve the assumptions. Analysis of variance (ANOVA) was used to determine whether stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and metal concentrations varied among species, sexes and sites. In cases where ANOVA yielded significant F values, the Student-Newman–Keuls (SNK) post hoc procedure was used to compare all pairs of means. Linear discriminant analysis (LDA) on log-transformed metal concentrations was used for assessing the differences in metal patterns (excluding mercury) among species and sampling sites. Pairwise comparisons among all groups were made using Hotelling's test based in Mahalanobis distances. The significance levels of the multiple pairwise tests were adjusted using the sequential Bonferroni procedure. The significance level for all tests was 0.05. All data are expressed as mean ± SE. STATISTICA version 7.1 (Statsoft Inc., Tulsa, OK) was employed for data processing.

3. Results

Most individuals of the species *Diapterus brevirostris* (99%) and a high proportion of the individuals of *Mugil curema* (62%) were under their reported maturation length (Table 1) and as such did not have well developed gonads. On the contrary, only a few specimens of *Achirus mazatlanus* (11%) and *Sciades guatemalensis* (17%) were under their reported maturation length, but only a few of those above maturation lengths had developed gonads. Sex of the fish could still be determined in almost all specimens. Sampling sites showed little difference in salinity (32–34) and temperature (27–28 °C). This is likely because sampling took place during the dry season at a time when lagoon waters were strongly dominated by the ocean.

The differences among mean values of morphologic and organosomatic indices within each species were mostly due to sex (Fig. 2). Significant differences between sexes were found in *Sciades guatemalensis* for mean total length ($F_{1,60}$ = 8.83, p = 0.004) and

Table 3
Length, weight, and relative liver size (LSI) for fishes from sites sampled in Barra de Navidad lagoon. Data are shown as mean ± SE (n). Bold indicates that significant differences were found comparing sites. Values sharing a superscript letter are not significantly different.

Species	Sex	Site	Length (cm)	Weight (g)	LSI
Sciades guatemalensis	F	CAN	36.09 ± 0.49 (29)	472.28 ± 21.70 (29)	1.48 ± 0.07 (29)
		TEP	36.38 ± 1.56 (12)	488.08 ± 76.04 (13)	1.61 ± 0.11 (13)
	M	CAN	33.54 ± 0.43 (11)	356.36 ± 16.77 (11)	1.38 ± 0.11 (11)
		TEP	32.96 ± 1.00 (7)	338.71 ± 30.70 (7)	1.59 ± 0.05 (7)
Achirus mazatlanus	F	ATR	14.66 ± 0.42 (22)	63.27 ± 5.70 (22)	0.87 ± 0.03 (22)
		CAN	16.07 ± 0.56 (13)	77.31 ± 7.20 (13)	0.76 ± 0.06 (13)
		COL	15.41 ± 0.46 (12)	73.83 ± 6.49 (12)	0.77 ± 0.06 (12)
	M	ATR	14.61 ± 0.20 (16)	57.38 ± 3.02 (16)	0.68 ± 0.05 (16)
		CAN	14.89 ± 0.40 (18)	59.22 ± 4.63 (18)	0.66 ± 0.06 (18)
		COL	14.43 ± 0.43 (19)	56.26 ± 4.30 (19)	0.67 ± 0.11 (19)
Diapterus brevirostris	F	ATR	14.49 ± 0.38 (22)	43.57 ± 3.37 (21)	**0.40 ± 0.03 (21)^{b}**
		CAN	14.41 ± 0.41 (16)	44.50 ± 4.07 (16)	**0.57 ± 0.06 (16)^{a}**
		COL	14.75 ± 0.30 (22)	43.41 ± 2.59 (22)	**0.40 ± 0.05 (22)^{b}**
	M	ATR	14.16 ± 0.32 (21)	39.00 ± 2.24 (20)	0.36 ± 0.03 (20)
		CAN	13.05 ± 0.34 (19)	32.79 ± 2.69 (19)	0.47 ± 0.04 (19)
		COL	13.77 ± 0.20 (31)	34.77 ± 1.48 (31)	0.43 ± 0.06 (31)
Mugil curema	F	CAN	26.93 ± 0.51 (36)	197.72 ± 12.76 (36)	1.18 ± 0.10 (36)
		COL	26.15 ± 2.56 (4)	182.25 ± 57.66 (4)	1.00 ± 0.09 (4)
	M	CAN	23.15 ± 0.89 (6)	124.83 ± 13.69 (6)	0.84 ± 0.17 (6)
		COL	23.56 ± 0.37 (14)	125.57 ± 5.67 (14)	0.98 ± 0.08 (14)

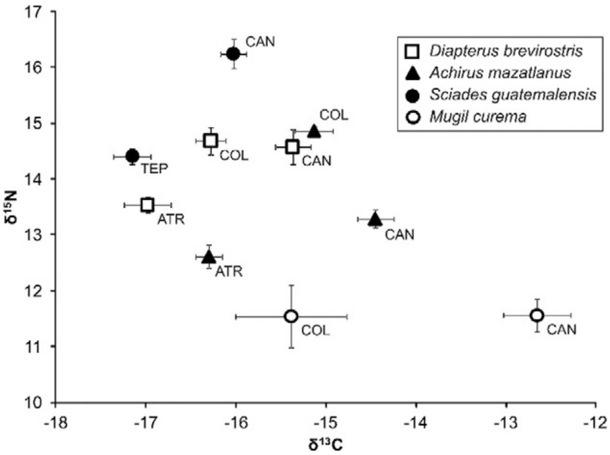

Fig. 3. Mean values (±SE) of $\delta^{13}C$ and $\delta^{15}N$ (n = 10 in all cases). See site acronyms in Fig. 1.

mean total weight ($F_{1,61} = 8.50$, $p = 0.004$); in *Achirus mazatlanus* for mean total length ($F_{1,99} = 3.82$, $p = 0.032$), mean total weight ($F_{1,99} = 7.92$, $p = 0.005$) and mean LSI ($F_{1,99} = 6.96$, $p = 0.009$); in *Diapterus brevirostris* for mean total length ($F_{1,129} = 11.10$, $p = 0.001$) and mean total weight ($F_{1,127} = 15.07$, $p < 0.001$); and in *Mugil curema* for mean total length ($F_{1,60} = 21.07$, $p < 0.001$) and mean total weight ($F_{1,60} = 16.42$, $p < 0.001$). In all species females were longer and heavier than males. No between sex differences were observed for condition factor or adjusted condition factor.

Within species, analyses of length, weight and LSI differences among sites were therefore made for each sex separately (Table 3) and significant differences were found only for *D. brevirostris* females ($F_{2,56} = 4.23$, $p = 0.019$), with specimens in the channels site (CAN) having relatively heavier livers compared to those from COL and the reference ATR sites. Mean condition factor and adjusted condition factor calculated by pooling data for males and females were not significantly different among sites within each species.

Mean values of $\delta^{13}C$ (Fig. 3) varied from -17.15 ± 0.20‰ (*S. guatemalensis* at site TEP) to -12.65 ± 0.37‰ (*M. curema* at site CAN). There was a significant interaction among species and sites ($F_{3,89} = 5.53$; $p = 0.002$). Therefore, differences among sites were tested separately for each species. Values were significantly different among sites for all species (Table 4). In all cases the $\delta^{13}C$ values of fish from the most contaminated site (CAN) were highest and followed by those at site COL, when the species was sampled at this site. Lowest values of $\delta^{13}C$ were consistently found for fishes collected at the two reference sites TEP and ATR.

Table 5
THg concentrations in muscle (μg/g dw) for fishes collected from sites sampled in Barra de Navidad lagoon. ANOVA analyses were made on log-transformed data. Data are shown as mean ± SE (n). Bold indicates that significant differences were found comparing sites for each species and superscripts indicate significant differences among sites within a species.

Species	Site	n	Length (cm)	Weight (g)	Hg
Sciades guatemalensis	CAN	12	36.03 ± 0.57	483.36 ± 25.82	**2.17 ± 0.180[a]**
	TEP	12	35.88 ± 1.61	472.00 ± 80.87	**0.70 ± 0.075[b]**
Achirus mazatlanus	ATR	13	14.76 ± 0.22	62.18 ± 3.45	0.30 ± 0.023
	CAN	12	15.59 ± 0.34	71.25 ± 5.64	0.30 ± 0.031
	COL	11	15.00 ± 0.33	69.27 ± 5.59	0.26 ± 0.032
Diapterus brevirostris	ATR	12	14.27 ± 0.19	36.11 ± 1.44	**0.14 ± 0.007[b]**
	CAN	12	14.38 ± 0.29	44.30 ± 2.70	**0.64 ± 0.186[a]**
	COL	12	14.11 ± 0.21	37.33 ± 2.09	**0.30 ± 0.174[ab]**
Mugil curema	CAN	14	25.34 ± 0.53	161.83 ± 10.51	0.03 ± 0.002
	COL	15	24.33 ± 0.93	143.83 ± 19.81	0.03 ± 0.002

Mean values of $\delta^{15}N$ (Fig. 3) varied from 11.54 ± 0.56‰ (*M. curema* at site COL) to 16.23 ± 0.26‰ (*S. guatemalensis* at site CAN). There was a significant interaction among species and sites ($F_{3,89} = 3.98$; $p = 0.010$); for this reason, differences among sites were tested separately for each species. $\delta^{15}N$ values were significantly different among sites for three species and some spatial patterns emerged (Table 4). *A. mazatlanus* showed highest values at site COL, followed by site CAN, with lowest values at site ATR. Values for *D. brevirostris* were highest at sewage-exposed sites COL and CAN, with no significant difference between these sites, and lowest at the reference site ATR. *S. guatemalensis* had $\delta^{15}N$ values that were significantly higher at site CAN when compared to site TEP. No significant difference between sites was found for *M. curema*, a species that was only collected at the two more human-impacted sites. A size effect on isotope differences was not examined, as no species yielded significant differences in mean total length among sites (Table 4). In spite of the interaction present among sites and species, mean values of $\delta^{15}N$ were calculated for each species by pooling the data across all sites to obtain a relative trophic level within the lagoon. The lowest mean value was found for *M. curema* (11.54 ± 0.30‰) followed by *A. mazatlanus* (13.59 ± 0.19‰), *D. brevirostris* (14.26 ± 0.16‰) and *S. guatemalensis* (15.32 ± 0.25‰).

Values of THg were highest for *S. guatemalensis* with a mean of 1.33 ± 0.20 and a range from 0.02 to 3.20 μg/g-dw; *D. brevirostris* had a mean concentration of 0.38 ± 0.11 and a range of 0.08 to 2.42 μg/g-dw; *A. mazatlanus* showed a mean value of 0.29 ± 0.02 with a range of 0.07 to 0.48 μg/g-dw; values for *M. curema* were the lowest with a mean of 0.03 ± 0.001 and a range from 0.02 to 0.04 μg/g-dw. Mean values of THg did not show significant differences between sexes for any species (*S. guatemalensis*: $F_{1,22} = 2.58$, $p = 0.127$; *D. brevirostris*: $F_{1,34} = 0.15$, $p = 0.701$; *A. mazatlanus*: $F_{1,34} = 1.55$, $p = 0.221$; *M. curema*: $F_{1,27} = 0.05$, $p = 0.824$). Therefore, data of both sexes

Table 4
ANOVA results for differences among sites in mean total length and stable isotope values ($\delta^{13}C$, $\delta^{15}N$). SNK column summarizes pairwise comparisons results (only for significant F tests).

		DF effect	DF error	F	*p*	SNK
Total length	*Achirus mazatlanus*	2	27	2.168	0.134	
	Diapterus brevirostris	2	27	0.292	0.749	
	Sciades guatemalensis	1	18	1.085	0.331	
	Mugil curema	1	18	0.967	0.339	
$\delta^{13}C$	*Achirus mazatlanus*	2	27	24.67	<0.001	CAN > COL > ATR
	Diapterus brevirostris	2	27	14.51	<0.001	CAN > COL > ATR
	Sciades guatemalensis	1	18	20.10	<0.001	CAN > TEP
	Mugil curema	1	18	15.07	0.001	CAN > COL
$\delta^{15}N$	*Achirus mazatlanus*	2	27	52.79	<0.001	COL > CAN > ATR
	Diapterus brevirostris	2	27	6.80	0.004	(COL = CAN) > ATR
	Sciades guatemalensis	1	18	38.38	<0.001	CAN > TEP
	Mugil curema	1	18	0.00	0.974	

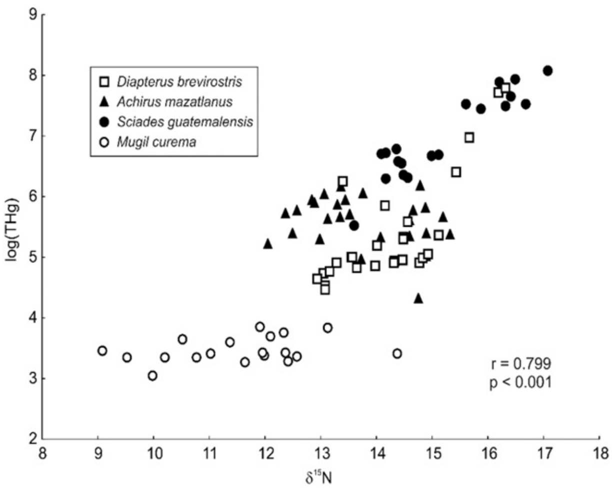

Fig. 4. Scatterplot of log (THg) vs $\delta^{15}N$ for all species pooled.

were pooled for each site and differences among sites were tested separately for each species (Table 5). Two species did not show significant differences among sites, but *S. guatemalensis* from the highly exposed channels site (CAN) had THg concentrations which were 300% higher than those at the reference TEP site, and *D. brevirostris* showed higher concentrations at site CAN compared to the reference site ATR. Log-transformed THg concentrations were significantly correlated with $\delta^{15}N$ across all species (Fig. 4). Analyses made separately for each species found highly significant correlations for *D. brevirostris* and *S. guatemalensis* (Table 6). A size effect was not considered for this relationship, as no correlation was present between total length and log-transformed THg concentrations for any species (Table 6).

Significant differences among sites and within species were detected only in a few cases for some metals other than mercury (Table 7). However, most metals with detectable concentrations showed significant differences among species (Table 8). LDA allowed a better understanding of the response patterns in metals (Fig. 5). The three first axes were significant and explained 95.6% of the variability in the data. Axes 1 and 2 explained 84% of the total variation and showed well-defined patterns in a biplot. All species were clearly separated along the first axis, as this axis had high positive loadings for Mg and Zn and high negative loadings for Fe, As and Rb (Table 9). Along the second axis, a further separation occurred among *D. brevirostris*, *S. guatemalensis* and *M. curema*, which mainly presented positive scores, and *A. mazatlanus* with negative scores. This axis had high negative loadings for Mn and Se (Table 9). Pair-wise comparisons of groups formed by combinations of species and sites yielded significant differences among centroids for all cases involving two different species while comparisons of sites within species were mostly not significant with the exceptions of CAN vs COL for *A. mazatlanus* and ATR vs COL for *D. brevirostris* (Table 10). In summary, differences occurred among species in their patterns of metals and were related to subsets of correlated metals. Samples of *A. mazatlanus* presented high concentrations of Se and Mn. *D. brevirostris* had high concentrations of Mg and Zn. *S. guatemalensis* and *M. curema* both presented high concentrations of Rb and Fe relative to the other metals that were measured.

Table 6
Correlations of log (THg) with total length (TL) and $\delta^{15}N$. Bold values indicate statistical significance.

Species	n	TL vs log (THg)		$\delta^{15}N$ vs log (THg)	
		r	*p*	*r*	*p*
Diapterus brevirostris	27	−0.086	0.671	**0.776**	<**0.001**
Mugil curema	19	0.017	0.945	0.317	0.186
Achirus mazatlanus	27	0.239	0.228	−0.227	0.256
Sciades guatemalensis	19	0.302	0.208	**0.927**	<**0.001**

4. Discussion

In this study the fish that were collected were mainly juvenile or young adults. This is a consequence of the role the lagoon plays as an important nursery area for coastal fishes (González-Sansón et al., 2014). Only a few individuals longer than the reported maturation length for each species had developed gonads. This suggests that these species were not in their reproductive seasons during the short sampling period of our study. Although no studies reporting reproductive periods for these species were found, information for other species of the same families supports our assumption. de Oliveira and Fávaro (2010) reported that *Achirus lineatus* in south Brazil was spawning during the spring while Sánchez-Gil et al. (2008) reported that two species of Pleuronectiformes (*Etropus crossotus*, *Cytharichthys spilopterus*) spawn during the rainy season in the southern Gulf of Mexico. Several authors have found that species of the family Ariidae have spawning periods in spring and/or summer (Mendoza-Carranza and Hernández-Franyutti, 2005; Muro and Amezcua, 2011; Amezcua and Muro-Torres, 2012; Segura-Berttolini and Mendoza-Carranza, 2013).

Morphologic and organosomatic indices showed no differences among sites in this coastal lagoon, suggesting that exposure to raw sewage was not affecting energy storage in these species and life stages. Differences due to sex were present for weight and length in all species while only *A. mazatlanus* showed a significant difference due to sex for LSI. This is not unexpected as size and organ differences would be most pronounced in mature species and closer to spawning times.

Differences in $\delta^{15}N$ among species agree well with the trophic level expected based on their general trophic preferences (Table 1). It is important to emphasize that although *S. guatemalensis* and *D. brevirostris* have similar estimated trophic levels (Froese and Pauly, 2014), sampled individuals of the former species were much larger than those of the latter and this should be, and was, reflected in the actual trophic level being higher in *S. guatemalensis* compared to *D. brevirostris* within sites, as has been demonstrated for other predator species (Cocheret de la Morinière et al., 2003).

Sites heavily impacted by sewage (COL, CAN) showed higher $\delta^{15}N$ when compared to non-impacted sites (ATR, TEP) within three species. This result agrees well with findings by other authors (McClelland et al., 1997; Hadwen and Arthington, 2007; Davias et al., 2013) and can be explained by a well-documented ^{15}N enrichment of the organic matter in sewage discharges (Constanzo et al., 2001; Vizzini and Mazzola, 2006). $\delta^{15}N$ differences among species were partially masked by the significant differences among sites for each species, which can be explained by the contribution of organic matter enriched in ^{15}N due to sewage discharges. *M. curema* was collected only in sewage-impacted sites. A similar result was found for *D. brevirostris* when comparing sites CAN vs COL, although this last species showed significant lower values of $\delta^{15}N$ in site ATR, which is not impacted by sewage discharge.

Values of $\delta^{13}C$ found in our research are similar to those reported by other authors for fishes in estuarine habitats. Winemiller et al. (2011; see Table 1) found a range of −10.24 to −16.70‰ for most fish species collected in mangrove-dominated areas at the mouth of the Monkey River, Belize. Garcia et al. (2007) found more than 90% of fish species in estuarine habitats of a subtropical coastal lagoon having $\delta^{13}C$ values from −12.37 to −19.35‰.

Our results provide strong evidence for higher (less depleted) values of $\delta^{13}C$ in fishes from more polluted sites. Most of the values found in this study fall in the range −17.8 to −15.3‰ reported by Vizzini and Mazzola (2006) for consumers in sewage-impacted coastal waters of Sicilia. The same was found by Spies et al. (1989) for three fish species in a marine coastal area and by Rogers (2003) for seaweed (*Ulva lactuca*) samples in sites impacted by a coastal sewage outfall. An explanation for this pattern could be the differences in primary sources of organic

Table 7
Metal concentrations by sites (mg/kg-dw) in muscle samples of each species. Only metals with significant differences among sites and within species are shown. ANOVAs were performed on log-transformed values.

Species	df	F	*p*	Metal	CAN	COL	ATR
Achirus mazatlanus	2,27	4.42	0.022	Cr	0.45 ± 0.05[a]	0.26 ± 0.04[b]	0.33 ± 0.04[ab]
Diapterus brevirostris	2,27	5.20	0.012	As	2.63 ± 0.48[b]	4.81 ± 0.65[a]	2.39 ± 0.85[b]
	2,27	5.25	0.011	Ni	0.53 ± 0.15[a]	0.52 ± 0.04[a]	0.34 ± 0.03[b]
Mugil curema	1,17	5.48	0.031	As	3.70 ± 0.36[a]	2.79 ± 0.19[b]	

matter among sites, with a higher input of δ^{13}C-elevated organic matter in the more sewage-impacted sites (Faganeli et al., 1988). δ^{13}C has been showed to be higher (less depleted) in sewage organic matter compared to autochthonous organic matter in marine systems (Spies et al., 1989). However, the δ^{13}C values of primary consumers will be determined by the composition of their diets. In general terms, each primary consumer feeds on a combination of primary producers (getting their carbon from CO_2) and particulate organic matter (detritus) which has a higher sewage component in polluted sites. On the other hand, important differences in δ^{13}C are to be expected for different species from the same site due to their different position in the food web as a result of the consistent increase in δ^{13}C of ~1‰ from prey to predator (Peterson and Fry, 1987). Our results match well with this expectation.

Our results showed that the highest concentrations of THg were present in the two fish species collected at the two most impacted sites. At site CAN, 67% of *S. guatemalensis* and 17% of *D. brevirostris* individuals had THg concentrations greater than the 0.2 μg/g-ww threshold (assuming 80% moisture in tissues) believed to be protective of adult and juvenile fish (Beckvar et al., 2005); 8% of the individuals of *D. brevirostris* exceeded this threshold at site COL. Fish with concentrations greater than this threshold are at risk of Hg intoxication, and the effects can include decreased growth, development, and reproduction as well as changes in behavior (Beckvar et al., 2005). No individuals of *A. mazatlanus* and *M. curema* or any fish collected at sites TEP and ATR exceeded the threshold.

On the other hand, THg concentrations found in our study are below the limits currently defined for fish traded for human consumption (Nauen, 1983; Chatterjee et al., 2014). Canadian standards (maximum levels) for mercury in retail fish are 0.5 mg/kg-ww for most fish species and 1.0 mg/kg-ww for some large pelagic (e.g. tuna, sharks) as defined by Health Canada (www.hc-sc.gc.ca, consulted 12 February 2016). The same limits were proposed by the FAO/WHO food standards programme (FAO/WHO, 2011). All values found in our study are below 0.5 μg/g-ww (= mg/kg-ww), suggesting a lower risk of Hg to humans consuming the fish from this lagoon.

Significant correlations were found between THg concentrations and δ^{15}N when all species were pooled. There are two potential explanations. First, higher-trophic-level species have more enriched values of δ^{15}N and should have also higher Hg concentrations due to biomagnification (Lacerda and Malm, 2008; Adams and Paperno, 2012). Second, δ^{15}N values are higher at sites with elevated sewage discharges (Vizzini and Mazzola, 2006; Pitt et al., 2009; Winemiller et al., 2011), which also are expected to have higher discharges of Hg (Alonso et al., 2000; Mirlean et al., 2003; Azevedo et al., 2011, 2012). The significant correlations between THg concentrations and δ^{15}N found in our study for two species and for all species combined support this expectation well. A third potential cause for the correlation of THg with δ^{15}N could be that both variables increase with size (Jackson, 1991; Marcovecchio, 2004; Cocheret de la Morinière et al., 2003 and references therein), but this was discarded because our results did not show significant correlation between total length and THg concentrations for any species. Overall, the positive relationships between THg and δ^{15}N within and among fishes supports what has been found previously in both marine and freshwater species (Kidd et al., 1995).

Our results showed weak evidence of increased concentrations for a few metals at the more polluted sites in the lagoon, as just four metals showed this trend in three species. Nickel was the only metal that was higher in *Diapterus brevirostris* at the two more contaminated sites than the reference site, suggesting that this element may be a good tracer of sewage exposure in the lagoon. Notably, arsenic was high in fish from the site with a gas station (COL), perhaps due to local contamination by fossil fuels. Overall, species with the highest metal concentrations varied depending on the metal, making interpretations based on single metals and generalizations across metals difficult. However, differences among species were strongly supported with the LDA and related analyses and were present for all metals with detectable

Table 8
Metal concentrations by species (mg/kg-dw) in muscle samples and pooled across sites. ANOVAs performed on log-transformed values and superscript letters indicate significant differences among species within a metal. SG: *Sciades guatemalensis*; AM: *Achirus mazatlanus*; DB: *Diapterus brevirostris*; MC: *Mugil curema.*

	$F_{3,96}$	*p*	SG	AM	DB	MC
Ag	–	–	<0.22	<0.22	<0.22	<0.22
Al	2.11	0.103	9.92 ± 3.11	6.28 ± 0.57	8.73 ± 1.27	5.46 ± 1.23
As	**18.9**	**<0.001**	**7.28 ± 0.64[a]**	**10.24 ± 1.63[a]**	**3.28 ± 0.43[b]**	**3.27 ± 0.23[b]**
Cd	–	–	<0.08	<0.08	<0.08	<0.08
Co	–	–	<0.15	<0.15	<0.15	<0.15
Cr	**9.45**	**<0.001**	**0.26 ± 0.03[b]**	**0.35 ± 0.03[b]**	**0.48 ± 0.05[a]**	**0.52 ± 0.05[a]**
Cu	**5.26**	**0.002**	**1.78 ± 0.24[a]**	**1.02 ± 0.19[b]**	**1.21 ± 0.24[b]**	**1.80 ± 0.30[a]**
Fe	1.77	0.158	17.26 ± 2.75	14.99 ± 1.32	11.95 ± 1.24	17.29 ± 1.77
La	–	–	<1.00	<1.00	<1.00	<1.00
Mg	**37.93**	**<0.001**	**1041.58 ± 40.7[c]**	**1270.53 ± 14.4[b]**	**1447.23 ± 18.2[a]**	**1240.05 ± 21.6[b]**
Mn	**34.71**	**<0.001**	**0.67 ± 0.10[bc]**	**2.75 ± 0.31[a]**	**1.37 ± 0.12[b]**	**0.75 ± 0.06[c]**
Ni	**4.82**	**0.003**	**0.55 ± 0.15[b]**	**0.59 ± 0.23[a]**	**0.46 ± 0.05[b]**	**0.42 ± 0.05[b]**
Pb	–	–	<0.77	<0.77	<0.77	<0.77
Rb	**23.78**	**<0.001**	**5.51 ± 0.39[b]**	**3.47 ± 0.17[c]**	**3.22 ± 0.22[c]**	**6.57 ± 0.29[a]**
Se	**52.34**	**<0.001**	**1.10 ± 0.02[b]**	**2.74 ± 0.24[a]**	**1.18 ± 0.03[b]**	**1.08 ± 0.01[b]**
Sr	**23.56**	**<0.001**	**1.86 ± 0.14[c]**	**6.75 ± 0.87[a]**	**4.29 ± 0.41[b]**	**2.06 ± 0.50[c]**
Tl	–	–	<0.55	<0.55	<0.55	<0.55
U	–	–	<3.80	<3.80	<3.80	<3.80
V	–	–	<0.15	<0.15	<0.15	<0.15
Zn	**115.40**	**<0.001**	**22.00 ± 1.96[b]**	**24.53 ± 0.74[b]**	**43.53 ± 1.44[a]**	**14.34 ± 0.78[c]**

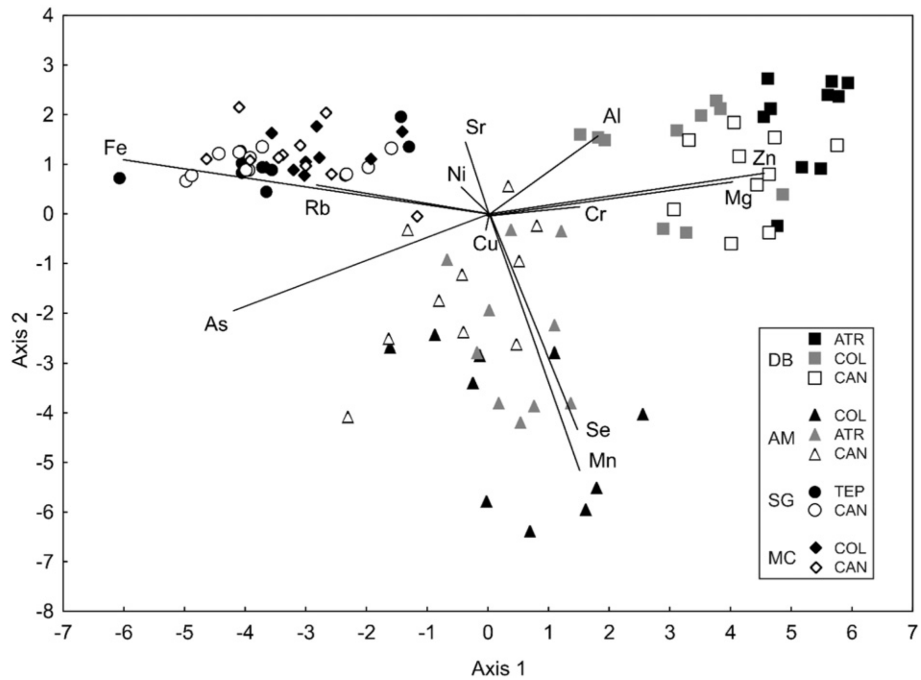

Fig. 5. Biplot of linear discriminant analysis. See sites codes in Fig. 1. DB = *Diapterus brevirostris*; MC = *Mugil curema*; AM = *Achirus mazatlanus*; AG = *Sciades guatemalensis*.

concentrations. Therefore, the main conclusion is that there are different metal signatures for each species, a result found also by other authors (Kalay et al., 1999; Andres et al., 2000; Canli and Atli, 2003; Merciai et al., 2014) which can be the result of differences in their ecology, swimming behaviors, diets, and metabolic activities (Canli and Atli, 2003).

In general, the metals levels found in fishes in the Barra de Navidad lagoon were lower than what has been measured in fishes elsewhere. Cadmium, chromium, copper, iron, manganese, nickel, lead and zinc levels found in this current study for *Mugil curema* were lower than or similar to those reported by Marcovecchio (2004) for *Mugil liza* from La Plata River's estuary, by Frías-Espericueta et al. (2010) for *Mugil cephalus* from Mazatlán port; by Fernandes et al. (2008) for *Liza saliens* from a coastal lagoon in Portugal and by Dural et al. (2007) for *Mugil cephalus* from a coastal lagoon in Turkey. Values in our samples were also lower than or comparable to those reported for species of Mugilidae collected in non-estuarine habitats by other authors (Mansour and Sidky, 2002; Canli and Atli, 2003; Uluozlu et al., 2007; Ebrahimzadeh et al., 2011). *Diapterus brevirostris* from the Barra de Navidad lagoon had concentrations of arsenic, cadmium, copper, iron, lead, selenium and zinc which were lower than those found by other authors in species of Gerreidae (Frías-Espericueta et al., 2010; Spanopoulos-Zarco et al., 2015). Values for aluminum, arsenic, chromium, copper, iron, manganese, nickel, lead, selenium, strontium and zinc found in *Achirus mazatlanus* muscle from the lagoon in the current study were lower than those found for species of Achiridae by Rocha et al. (2014). Concentrations of cadmium, cobalt, chromium, lead, vanadium and zinc found in muscle tissue of *Sciades guatemalensis* from our study were lower than those found in species of the family Ariidae in other estuarine and coastal habitats (Vázquez et al., 2008; Azevedo et al., 2012; Angeli et al., 2013; Rodriguez-Amador et al., 2014), while arsenic, copper and nickel concentrations in fish from our study were higher when compared to those of other studies (Azevedo et al., 2012 for Cu; Angeli et al., 2013 for As, Cu and Ni). However, levels of arsenic, chromium and selenium in all species included in our study were higher than the

Table 9
Standardized coefficients for first four axes of LDA based on log-transformed metal concentrations. Bold indicates coefficients with a high contribution to each of the three roots with significant values ($p < 0.001$) of Wilks' Lambda.

	Axis 1	Axis 2	Axis 3	Axis 4
Al	0.307	0.276	**0.484**	−0.388
As	**−0.707**	−0.328	**0.431**	−0.046
Cr	0.238	0.033	**−0.656**	0.239
Cu	−0.014	0.042	−0.212	0.182
Fe	**−1.010**	0.189	0.045	0.028
Mg	**0.675**	0.124	**−0.784**	−0.244
Mn	0.252	**−0.852**	−0.324	1.211
Ni	−0.073	0.095	−0.041	−0.083
Rb	**−0.476**	0.091	0.264	0.586
Se	0.242	**−0.715**	−0.119	−0.165
Sr	−0.068	0.250	0.306	−0.891
Zn	**0.777**	0.135	**0.651**	0.391
Eigenvalue	11.014	4.035	2.094	0.321
Cumulative proportion	0.614	0.839	0.956	0.974
Wilks' Lambda	0.003	0.031	0.158	0.489
Chi-squared	523.2	304.5	162.2	62.8
df	108	88	70	54
p	<0.001	<0.001	<0.001	0.191

Table 10
Matrix of Mahalanobis distances among groups centroids (upper half) and probabilities of F values yielded by Hotelling's test (lower half). Gray cells indicate comparisons among sites within each species. Bold indicates significant Mahalanobis distances. Only two pairs of sites within a species have significant Mahalanobis distances (AM-COL vs AM-CAN & DB-ATR vs DB-COL). Groups are identified by species codes (see Table 8) and sites codes.

	SG–TEP	SG–CAN	AM–COL	AM–ATR	AM–CAN	DB–ATR	DB–COL	DB–CAN	MC–COL	MC–CAN
SG–TEP	–	3.96	**47.92**	**35.10**	**22.77**	**82.92**	**48.02**	**64.79**	**29.95**	**14.73**
SG–CAN	0.135	–	**47.34**	**33.35**	**21.51**	**82.39**	**48.69**	**67.67**	**23.80**	**11.36**
AM–COL	<0.001	<0.001	–	4.60	**9.20**	**60.28**	**37.40**	**40.30**	**50.74**	**45.54**
AM–ATR	<0.001	<0.001	0.087	–	2.80	**41.81**	**22.77**	**26.99**	**33.11**	**28.61**
AM–CAN	<0.001	<0.001	<0.001	0.434	–	**45.63**	**21.98**	**30.20**	**22.52**	**17.34**
DB–ATR	<0.001	<0.001	<0.001	<0.001	<0.001	–	**8.63**	5.42	**76.13**	**75.16**
DB–COL	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	–	4.43	**48.11**	**43.20**
DB–CAN	<0.001	<0.001	<0.001	<0.001	<0.001	0.037	0.103	–	**61.58**	**59.97**
MC–COL	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–	4.03
MC–CAN	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.186	–

maximum tolerable limits for human consumption reported by Rocha et al. (2014) based on Brazilian regulations and US EPA guidelines (US EPA, 2000).

Although seasonal effects of wastewater discharges on fish δ^{15}N, δ^{13}C and metals were not examined in the current study, it is possible that this occurs in Barra de Navidad lagoon. Sampling occurred after the wet season (June–October) and when higher sediment loads to the lagoon would be expected. However, there are similar salinities in the lagoon over time due to its constant exchanges with the ocean and the sources of wastewater remain constant over time (González-Sansón et al., 2014). The previous, above-mentioned studies did not examine how wastewater discharges affect the δ^{15}N and δ^{13}C values of fishes over seasons, however seasonal variability has been observed in lagoon fishes (e.g. Vizzini and Mazzola, 2003). As such, this warrants investigation in future studies on the chemical composition of lagoon fishes impacted by municipal wastewater discharges.

It is clear from this study that stable isotopes can be a useful tool for separating fish exposures in sewage contaminated areas. Although exposure levels to sewage and nutrients were not sufficient to alter fish sizes or organ weights in most cases, there were still detectable differences in THg and stable isotopes at sewage-impacted sites, showing the utility of these tools to delineate such exposures. The study also provides the first information on several fish species for coastal areas of Mexico, and can serve as a baseline for future studies.

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