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Historical Zooplankton Composition Indicates Eutrophication Stages in a Neotropical Aquatic System: The Case of Lake Amatitlán, Central America

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Abstract: This paper presents a study of freshwater zooplankton biodiversity, deemed as a reliable indicator of water quality. The Guatemalan Lake Amatitlán, currently used as a water source, has shown signs of progressive eutrophication, with perceptible variations of the local zooplankton diversity. Biotic and abiotic parameters were determined at four sites of Lake Amatitlán (Este Centro, Oeste Centro, Bahía Playa de Oro, and Michatoya) in 2016 and 2017. The local composition, the species richness and abundance of zooplankton, and the system environmental parameters were analyzed during both years surveyed. Biological data suggesting eutrophication of this tropical system were obtained, including a high rotifer abundance (11 species: the rotifers *Brachionus havanaensis* (109 ind L^{-1}) and *Keratella americana* (304 ind L^{-1}) were the most abundant species in this lake). The presumably endemic diaptomid copepod species, *Mastigodiaptomus amatitlanensis,* was absent in our samples, but we report the unprecedented occurrence of two Asian cyclopoid copepods (i.e., *Thermocyclops crassus* and *Mesocyclops thermocyclopoides*) for Lake Amatitlán and Guatemala. The presence of larger zooplankters like adults and immature copepods (i.e., *Arctodiaptomus dorsalis*) and cladocerans (*Ceriodaphnia* sp.) at site "Este Centro" indicates a relatively healthy zooplankton community and represents a focal point for managing the conservation of this lake.

Keywords: conservation; eutrophication; exotic species; tropical lakes; zooplankton

1. Introduction

The knowledge of zooplankton in the Neotropical region is growing with fragmented studies. Therefore, it is likely that the species richness of zooplanktonic taxa is underestimated because of the presumably high diversity and scarcity of zooplankton taxonomists [1–3]. In addition, the progressive destruction of aquatic habitat and the progressive spread of exotic species threaten native biodiversity, ecosystem health, and environmental services.

The zooplankton community and abundance are closely linked to the trophic state of the water system; for this reason, its diversity has been deemed as an indicator of water quality [4]. In eutrophicated systems (at tropical and temperate latitudes), the dominance of microzooplankton is common, compared with larger organisms, owing to the increased availability of food and water conditions [5,6].

For four decades, the Guatemalan Lake Amatitlán has shown signs of progressive eutrophication related to anthropic factors (i.e., peripheral population growth and urbanization, intensive use of water for agricultural irrigation), thus promoting the advancement towards eutrophication, related to the input of nearly 50% of the untreated residual urban and industrial waters from Guatemala City [7–10]. Because of this, some actions have been proposed to address this problem, either from the governmental level (i.e., Autoridad para el Manejo Sustentable de la cuenca del lago Amatitlán, AMSA 1996) or from descriptive studies of the lake involving the lake zooplankton biodiversity, like those by Basterrechea-Díaz (1997) [7] and Brandorff (2012) [11]; however, studies related with tropical epicontinental waterbodies have been more focused on environmental factors rather than biological community attributes or general limnology [12,13]; thus, the zooplankton biodiversity in Guatemala remains largely unknown [14], with only a few studies in Guatemalan lakes [15,16]. Most studies in Lake Amatitlán and Guatemala are more focused on current data instead of historical analysis.

Based on the analysis of both, historical and current data of zooplankton biodiversity and environmental conditions of Lake Amatitlán, we present information on the zooplankton distribution, species richness, abundance, and its relation with successive changes of its trophic state.

2. Materials and Methods

2.1. Study Sites and Sampling Methods

Lake Amatitlán is the fourth largest lake in Guatemala, Central America, and one of the most emblematic waterbodies of this country. This lake is a warm monomictic waterbody in the highland of Guatemala, located at an altitude of 1186 m above sea level (m.a.s.l.), with an area of 15.2 km^2 and 11 km length and a maximum depth of 23 m. Its formation originated from volcanic activity of Pacaya, Fuego, and Agua in the late Quaternary [10,14,17].

Four sampled sites were considered: Este Centro (EC), Oeste Centro (OC), Bahía Playa de Oro (BPO), and Michatoya (MICH) to analyze the zooplankton species that inhabit the eastern and western regions of Lake Amatitlán (Figure 1). The latter two sites (BPO and MICH) are in the runoff of Villalobos and Michatoya rivers, respectively [14]. Water samples for biotic and abiotic variables were collected for 2016 and 2017 in the rainy (May–October) and dry seasons (November–April).

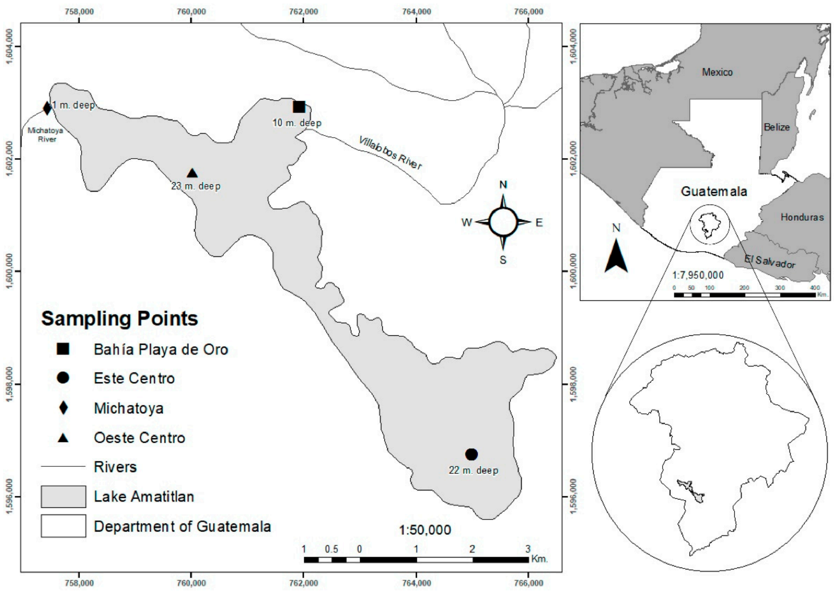

Figure 1. Location of Lake Amatitlán and sampling points for the biotic collection methodology. Water-filtered, vertical, and horizontal trawls as defined by Cervantes-Martínez & Gutiérrez-Aguirre (2015) [2].

2.1.1. Species Richness

Zooplankton samples (*n* = 8) were collected by vertical and horizontal trawls with a 45 µm plankton net between 1 and 22 m depth to ensure representative samples to evaluate the species richness in the lake, as it is well known that zooplankton tends to have vertical and horizontal migrations [2].

2.1.2. Species Abundance and Abiotic Variables

To estimate the zooplankton abundance, a known volume of water between 30 and 100 L was filtered through a 45 µm zooplankton net. The water was determined with a 2.1 L^{-1} capacity Van Dorn bottle [2,18]. Species abundance was determined by the account of two main groups: Rotifera and Copepoda, present in three aliquots of 1 mL each from the filtered samples, then the data were standardized as individuals per liter (ind L^{-1}) in each sampled site [19].

Abiotic variables were measured in situ monthly for both years of study and in all the water columns, with the multiparametric proves WTW Cond 197i, WTW Oxi 1970i, and HACH HQ for water temperature (°C), pH, oxygen concentration O_2 (mg L^{-1}), total dissolved solids (mg L^{-1}), and conductivity (µS cm^{-1}). With the actual environmental, richness, and zooplankton abundance data, a description of the trophic state of Lake Amatitlán was proposed.

2.2. Historical and Actual Records of Zooplankton and Environmental Parameters Analysis

Specific classification of Rotifera, Cladocera, and Copepoda of recently collected samples (collected in 2016 and 2017) was done according to Koste (1978) [20], Fontaneto & De Smet (2015) [21], Elías-Gutiérrez et al., (2008) [22], and Suárez-Morales et al., (2020) [23].

The presence/absence of the current zooplankton inventory was compared with previous surveys by Juday (1915) [24], Basterrechea-Díaz (1997) [7], and the record of copepods from the previous surveys of Wilson (1941) [25] and Brandorff (2012) [11], in order to analyze the historical composition of zooplankton of Amatitlán lake.

Historical environmental data recorded by Juday (1915) [24], Brezonik & Fox (1974) [26], Basterrechea-Díaz (1997) [7], and Ellenberg (2014) [27] were compared with the current data surveyed in this study.

3. Results

3.1. Species Richness

A total of 15 species of zooplankters including rotifers and crustaceans were found in the lake for 2016–2017 (Table 1); rotifers showed the highest species richness (80% of zooplankton species recorded), while copepods represented 20% of all zooplankton species in the lake.

We provide the first record of two cyclopoid exotic species (*Mesocyclops thermocyclopoides* and *Thermocyclops crassus*) for Lake Amatitlán and Guatemala. The endemic calanoid copepod, *Mastigodiaptomus amatitlanensis*, was absent in our current survey and the record of *Arctodiaptomus dorsalis* in Lake Amatitlán was confirmed here. Cladoceran crustaceans were very scarce in our samples; only a single specimen of *Ceriodaphnia* sp. was observed. The Brachionidae was the family with the highest species richness among rotifers in 2016 and 2017 (Table 1).

Nowadays, the east region (site EC) of Lake Amatitlán had the highest species richness in the lake (14 species), compared with the western region (9 species including the exotic *T. crassus* at OC). The largest zooplankters of the lake, including the cladoceran *Ceriodaphnia*, (~2 mm) [21], the calanoid copepod *A. dorsalis*, and the cyclopoid copepod *M. thermocyclopoides*, occurred in eastern region.

Table 1. Current and historical records of zooplankton species richness in Lake Amatitlán. Currently recorded species are shown in columns (1) EC, (2) OC, (3) BPO, and (4) MICH. Historical records are shown in columns 5–8, following data by Brandorff (2012) [11]; Basterrechea-Díaz (1997) [7]; Wilson (1941) [25]; and Juday (1915) [24], respectively. Presence (x), absence (-), new records (*).

Species	Current Data				Historical Data			
	1	2	3	4	5	6	7	8
Phylum: Rotifera Monogononta: Ploimida Family: Epiphanidae Harring, 1913								
Epiphanes macroura Barrois & Daday, 1894 *	x	x	-	-	-	-	-	-
Family: Brachionidae Ehrenberg, 1838	-	-	-	-	-	-	-	-
Anuraeopsis fissa (Gosse, 1851) *	x	-	-	-	-	-	-	-
Brachionus angularis (Gosse, 1851) *	x	x	x	x	-	-	-	-
B. calyciflorus Pallas, 1766 *	x	x	x	x	-	-	-	-
B. plicatilis Müeller, 1786 *	x	-	-	x	-	-	-	-
B. havanaensis Rousselet, 1911 *	x	x	x	x	-	-	-	-
Keratella sp.	-	-	-	-	-	x	-	-
K. americana Carlin, 1943 *	x	x	x	x	-	-	-	-
K. cochleraris (Gosse, 1851)	-	-	-	-	-	-	-	x
Family: Trichocercidae Harring, 1913								
Trichocerca cf. *longiseta* (Schrank, 1802) *	-	x	x	x	-	-	-	-
T. pusilla (Lauterborn, 1898) *	-	x	x	x	-	-	-	-
Family: Asplanchnidae Eckstein, 1883								
Asplanchna sieboldi (Leydig, 1854) *	x	x	x	-	-	-	-	-
Flosculariaceae: Family: Trochosphaeridae Harring, 1913								
Filinia longiseta (Ehrenberg, 1834)	x	x	x	x	-	-	-	x
F. terminalis (Plate, 1886) *	x	x	x	-	-	-	-	-
Subclass: Bdelloidea *	x	-	-	-	-	-	-	-
Superclass: Crustacea Brachiopoda: Cladocera: Anomopoda								
Family: Daphniidae Straus, 1820								
Daphnia sp.	-	-	-	-	-	x	-	-
D. hyalina Leydig, 1860	-	-	-	-	-	-	-	x
Ceriodaphnia sp.	x	-	-	-	-	x	-	x
C. lacustris Birge, 1893	-	-	-	-	-	-	-	x
C. pulchella Sars, 1862	-	-	-	-	-	-	-	x
Family: Bosminidae Sars, 1865								
Bosmina sp.	-	-	-	-	-	x	-	-
Bosmina longirostris O. F. Müeller, 1776	-	-	-	-	-	-	-	x
Family: Chydoridae Stebbing, 1902								
Chydorus sphaericus (O.F. Müeller, 1785)	-	-	-	-	-	-	-	x
Copepoda: Calanoida Family: Diaptomidae G.O. Sars, 1932								
Subfamily: Diaptominae Kiefer, 1932								
Arctodiaptomus dorsalis (Marsh, 1907)	x	-	-	-	x	-	-	-
Mastigodiaptomus albuquerquensis (Herrick, 1895)	-	-	-	-	-	-	-	x
M. amatitlanensis (Wilson, 1941)	-	-	-	-	-	-	x	-
Copepoda: Cyclopoida Family: Cyclopidae Kiefer, 1927								
Subfamily: Cyclopinae Kiefer, 1927								
Thermocyclops crassus (Fischer, 1853) *	-	x	-	-	-	-	-	-
Mesocyclops thermocyclopoides Harada, 1931 *	x	-	-	-	-	-	-	-
Subfamily: Eucyclopinae Kiefer, 1927								
Eucyclops serrulatus (Fischer, 1851)	-	-	-	-	-	-	-	x
Nauplii	x	x	x	x	-	x	-	x
Juvenile Cyclopoid	x	x	x	x	-	-	-	-
Juvenile Calanoid	x	x	x	x	-	-	-	-

Our revision of the zooplankton community (Table 1) indicates that the historical data presented a great microcrustacean richness with the record of eight cladoceran species (*Dapnia* sp., *D. hyalina, Ceriodaphnia* sp. *C. lacustris, C. pulchella, Bosmina* sp., *B. longirostris,* and *Chydorus sphaericus*) and the three calanoid copepods: *A. dorsalis*, *Mastigodiaptomus albuquerquensis*, and the endemic *M. amatitlanensis*. The historical record of rotifers had the lowest species richness including three monogonont species. In our survey, the rotifer species richness increased significantly with 12 species not hitherto reported from the lake, including the record of organisms from the Subclass Bdelloidea.

3.2. Species Abundance

In this study, the total rotifer abundance was 522.7 ind L^{-1}. Rotifers represent the most abundant group in the lake; their numerical abundance is considerably higher than that recorded for copepods, including immature stages (7.1 ind L^{-1}). Cladocerans were almost absent from our samples.

Species with the highest abundance at all sites were the rotifers *B. havanaensis* (109 ind L^{-1}) and *K. americana* (304 ind L^{-1}), with a considerably lower abundance in the eastern area (9.3 and 121.8 ind L^{-1}, respectively). Species of the family Brachionidae were the most abundant mainly in the western region (sites OC, BPO, and MICH), whereas the lowest abundance of rotifers occurred in the eastern region (site EC) (see Table 2).

Table 2. Abundance (ind L^{-1}) calculated from zooplankton samples for all the studied points of Lake Amatitlán in 2017.

Species	Abundance (ind L^{-1})			
	EC	OC	BPO	MICH
Brachionusangularis	0.00	0.00	71.56	1.87
Brachionuscalyciflorus	0.70	0.70	85.56	4.67
Brachionusplicatilis	0.00	0.47	0.00	0.00
Trichocerca cf. *longiseta*	0.00	0.00	14.00	12.60
Trichocercapusilla	0.00	8.40	13.22	11.20
Asplanchnasieboldi	1.40	1.17	2.33	0.93
Filinialongiseta	0.00	1.40	28.00	13.07
Filiniaterminalis	8.17	49.23	35.00	60.20
Brachionushavanaensis	9.33	153.53	108.89	165.20
Keratellaamericana	121.80	432.60	265.22	408.33
Nauplii	3.50	2.57	3.11	1.40
Juvenile Cyclopoid	5.83	1.63	1.56	0.93
Juvenile Calanoid	2.80	0.47	3.89	0.47
M. thermocyclopoides	0.23	0.00	0.00	0.00

The local copepod abundance was represented mainly by nauplii and juvenile stages of Calanoida and Cyclopoida (average = 2.6, 2.5 and 1.9 ind L^{-1}, respectively), values resembling those recorded for the Rotifera like *B. plicatilis* (1.1 ind L^{-1}) and *A. sieboldi* (2.3 ind L^{-1}) in all the study sites, compared with adult copepods, where the abundance of the adult *M. thermocyclopoides* present only in EC was 0.23 ind L^{-1}.

3.3. Environmental Variables

Environmental variables values in both analyzed years, in general, presented basic pH values (>8 ± 0.33), dissolved oxygen showed an average of 4.76 ± 5.21 and 4.65 ± 4.92 mg L^{-1}, whereas temperature averaged 24 ± 1.31 °C, conductivity presented average values of 655.95 ± 59.52 and 678.23 ± 68.29 µS cm^{-1}, and finally TDS showed average values of 339.99 ± 47.35 and 341.43 ± 30.30 mg L^{-1}, respectively (Table 3).

Table 3. Historical and current environmental mean data of the water column recorded by previous surveys and this study. Juday (1915) [24], Brezonik & Fox (1974) [26], Basterrechea-Díaz (1997) [7], Ellenberg (2014) [27]. ND: no data available.

Environmental Variables	1910 [24]	1969 [26]	1985–1995 [7]	2008–2013 [27]	2016 ***	2017 ***
pH	ND	7.70	7.75	8.69	8.26	8.33
Water temperature (°C)	19.86	ND	22.75	25.00	24.46	24.23
Conductivity (µS cm^{-1})	ND	830	802	682	655.95	678.23
TDS (mg L^{-1})	ND	ND	610	ND	339.99	341.43
Dissolved oxygen O_2 (mg L^{-1})	4.74 *	8.40 **	4.20	8.90	4.76	4.65

* Data originally recorded in cubic centimeters per liter of water. ** Original data recorded at surface. *** Data recorded in this study.

The historical data presented in Table 3 show pH with slightly neutral values in 1969 to 1985–1995, whereas in the first two decades of the XXI century, the pH increased to reach clearly basic values, over 8. The water temperature changed along the time, 19.86 °C in 1910 to 24.23 °C in 2017. Conductivity and total dissolved solids decreased on average by 18.29 and 44.10%, respectively.

4. Discussion

The environmental parameters surveyed in this study can show the progressive eutrophication on Lake Amatitlán, according to the historical data recorded by authors like Juday (1915) [24], Brezonik & Fox (1974) [26] Basterrechea-Díaz (1997) [7], and Ellenberg (2014) [27]. The historical change in environmental and biological variables could reveal strong evidence of the current eutrophication of this lake. For instance, the observed changes of pH values, that is, an average of 8.26 and 8.33 in 2016–2017, differ in contrast from the values recorded in 1969 (7.70) [26], 1985–1995 (7.75) [7], and 2008 (9.3) [17].

The basic pH and the high concentration of dissolved oxygen at the surface promoted an increase of microzooplankters, like rotifers (especially *B. havanaensis* and *K. americana*), and a decrease of larger species like cladocerans and adult copepods, indicators of the system trophic state per se. Similar conditions have been recorded in American eutrophicated subtropical and tropical water bodies [4,28,29] as well as in other water bodies (i.e., temperate coastal water bodies) in which the replacement of larger copepod with smaller ones has been reported to the result from the eutrophication process [6].

Recently, phytoplankton blooming has been described as a consequence of this eutrophication progress in Lake Amatitlán, presenting a high concentration mainly in *Microcystis* sp. and *Dolichospermum* sp. cyanobacteria preceded by the diatom algae *Niszcha* sp. at the surface of the lake [9], which in turn allows herbivorous zooplankters like brachionid rotifers to become dominant organisms in eutrophicated epicontinental waterbodies [20].

In earlier studies on Lake Amatitlán, the zooplankton community was largely dominated by cladocerans and copepods. In 1915 [24], zooplankton had a widely different composition compared with our results: rotifers were then the less abundant zooplankton group in the lake (0.3 ind L^{-1}), preceded by copepods (11.6 ind L^{-1}) and cladocerans, the most abundant zooplankton group at that time (14.4 ind L^{-1}). The system trophic state is also related to the zooplankters body size; that is, a stronger level of eutrophication is frequently expressed by a greater abundance and species richness of microzooplankters like small rotifers [4,6,28,29]. A possible explanation of the local absence or scariness of larger zooplankters (i.e., *Ceriodaphnia* sp., adult cyclopoid and calanoid copepods, including *M. amatitlanensis*) could result from the competition for available food [5], eventually explaining the strong dominance of small brachionid herbivorous rotifers like *B. havanaensis* and *K. americana*.

The presence and high abundance of these latter species, together with another species of *Brachionus* and *Keratella* at the east region of Lake Amatitlán, suggest that eutrophic conditions that make food available for these microphagous species [30].

In the case of *A. dorsalis*, this species is widespread in America [31] and has been recorded as an invasive exotic copepod in Asiatic waterbodies [32,33]. The environmental conditions of Lake Amatitlán seem to be adequate for the development of this species because it shows a selective feeding on phytoplankton; thus, it frequently inhabits moderately to strongly eutrophicate environments [31,32], like Amatitlán lake.

It is well known that many diaptomid copepods tend to have restricted distributional patterns and endemic distributions in neotropical lakes [34]. Then, the local absence of the endemic copepod *M. amatitlanensis* in this study could be another indicator of the progressive eutrophication of Lake Amatitlán, because, since its description by Wilson (1941) [25], this species has not been recorded in other regional studies (i.e., Elías-Gutiérrez et al., 2008 [35]; Brandorff, 2012 [11]; and Gutiérrez-Aguirre, et al., 2020 [36]). It is probable that *M. amatitlanensis* occurs in other lakes of Guatemala (or Central America) and it is expected to be collected from adjacent systems. It is also probable that this species dwells at higher depths not easily reached by standard nets.

Our results showed a clear zonification; the eastern region (site EC) diverges from the other sites because of the absence of adjacent rivers (see Figure 1), its distance from the other sampling points (the closest site is OC, 7.04 km away), and its separation from other sites owing to a train riel that divides the lake in two [14]. Therefore, the EC area has the best conservation status of the lake, precisely where we found the greatest species richness and the larger zooplankters, with the copepods *T. crassus* (average body length of 0.56–0.93 mm) [37], *M. thermocyclopoides* (0.78–0.89 mm) [38], and *A. dorsalis* (0.77–1.13 mm) [31] among them. Thus, it is convenient to consider EC as a potential conservation site as it has better environmental conditions for the conservation and preservation of zooplankton biodiversity.

On the other hand, we report the presence of two exotic cyclopoid copepod species for the Central American Lake Amatitlán and Guatemala country, *M. thermocyclopoides* and *T. crassus. M. thermocyclopoides* is a native species from Taiwan and is well spread in Asia and Africa, and commonly widespread at tropical latitudes. This species has been recorded in lakes from South Mexico in epicontinental waterbodies from Chiapas state, Mexico, considering that their introduction may be related to anthropic factors (i.e., agriculture and aquaculture) [37,38]. This is the second record of the invasion of this species in Central American countries, as it has been recorded before in Costa Rican water bodies by Collado et al. (1894) [39], and the ecological potential of *Mesocyclops* use as biocontrol of vector mosquitoes like *Aedes aegypti* is well known [40–42]. Therefore, its finding in Guatemalan lakes represents a source for mass culture of this copepod to be used as biocontrol.

Thermocyclops crassus is commonly spread at tropical latitudes in Africa, Australia, and Asia; it was also recorded in Laurentian great lakes in the United States of America [43]; recorded for the first time in tropical lakes from Tabasco state, Mexico [37]; as well as in small ponds of San José Province in Costa Rica [39]. Being a thermophilic species, *T. crassus* has a narrow temperature tolerance [44], so it may be a local indicator of the temperature changes in the lake along time.

Finally, the physical, chemical, and biological conditions of the lake have clearly changed over time, from being a lake with oligotrophic characteristics to one with hypertrophic conditions in a relatively short period of time (100 years, approximately), allowing us to follow and describe the stages and speed of the eutrophication process of a large neotropical lake.

5. Conclusions

The historical analysis of zooplankton composition in the lake presented in this study reinforces the knowledge of its eutrophic state, suggesting a useful role of the zooplankton as a bioindicator and making possible the visualization of the changes in its composition over time, showing the progressive trophic state towards eutrophic or hypereutrophic conditions.

It is likely that the absence of the endemic species *M. amatitlanensis* is a warning sign regarding the accelerated loss of biodiversity and reinforces the idea that zooplankton is a great tool as a bioindicator of the health status for continental aquatic ecosystems, in both tropical and temperate latitudes.

Further studies analyzing bottom sediments to search resting eggs of zooplankton in Lake Amatitlán and around it can answer the question of the absence of *M. amatitlanensis*, where this type of knowledge is also scarce in inland aquatic systems of the region.

Finally, is convenient to consider the isolated site EC as a focal point for conservation as it presents better environmental conditions for the conservation and preservation of zooplankton biodiversity, owing to the record of the largest zooplankters found in this site.

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Data Availability Statement: Data presented in this study are available on request from the corresponding author.

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Baseline

Total mercury content in the California ribbed sea mussel *Mytilus californianus* from the west coast of Baja California, México: Levels of contamination and human health risk

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ABSTRACT

We analyzed spatial and temporal variations in total mercury concentration (THg) in *Mytilus californianus* from the west coast of Baja California, México, and assessed the potential risk for human health. The sites from the northern zone showed the highest levels of THg over the entire three years of study, however, no significant differences among years were found. The highest level of THg (0.110 μg/g d.w.) was recorded in 2010 at Bajamar (SS2), and the lowest (0.011 μg/g d.w.) in 2007 and 2008 at Eréndira (SS4) and Los Ojitos (SS7), respectively. The estimated daily intake (EDI) values for adults through mussel consumption were lower than the oral reference dose (RfDo) and the acceptable daily intake (ADI) values established by the USEPA and the FAO/WHO, respectively. The target hazard quotient (THQ) values were <1.0, indicating that mercury concentrations in *M. californianus* are not likely to pose a risk for human health.

1. Introduction

Mercury (Hg) is one of the most toxic chemical elements and is considered an environmental pollutant that represents a permanent contamination risk to aquatic ecosystems worldwide (Blackwell et al., 2013). This element can be found in three primary forms: elemental (Hg^0), inorganic (Hg^{2+}) and organic (methylmercury or MeHg), all of which show a large variety of properties that affect their distribution, biomagnification and toxicity (Leermakers et al., 2005). Although the presence of mercury in the environment is due to natural processes, anthropogenic activities have disturbed its global cycle (Ruelas-Inzunza et al., 2013). Among the major anthropogenic sources of Hg in aquatic systems are: the erosion of contaminated surfaces (e.g. mercury contaminated soil), urban and industrial discharges, agriculture, mining, and combustion (e.g. coal combustion) (Wang et al., 2004). Through all these processes Hg enters into the aquatic environment, and once there can be accumulated by aquatic biota and biomagnified as it moves through the food web (Delgado-Alvarez et al., 2017). This results in extremely high concentrations in the tissues of large carnivorous fishes (e.g. tuna and shark), which pose a potential health risk to consumers (Man et al., 2014; Ratcliffe et al., 1996; Tao et al., 2016). The main effects of Hg on human health from the consumption of contaminated seafood include renal dysfunction, and neurological and gastrointestinal disorders (Chan and Egeland, 2004; Genchi et al., 2017).

Previous studies on heavy metals in the west coast of Baja California, México, have indicated that this zone is affected by the improper disposal of domestic and industrial effluents and agricultural runoff, among other anthropogenic sources (Carreón-Martínez et al., 2002; Gutiérrez-Galindo et al., 2008). Numerous studies have evaluated the levels of pollution resulting from these sources in the marine environment in this region using approaches such as the analyses of sediments, water, algae and marine organisms (Gutiérrez-Galindo and Muñoz-

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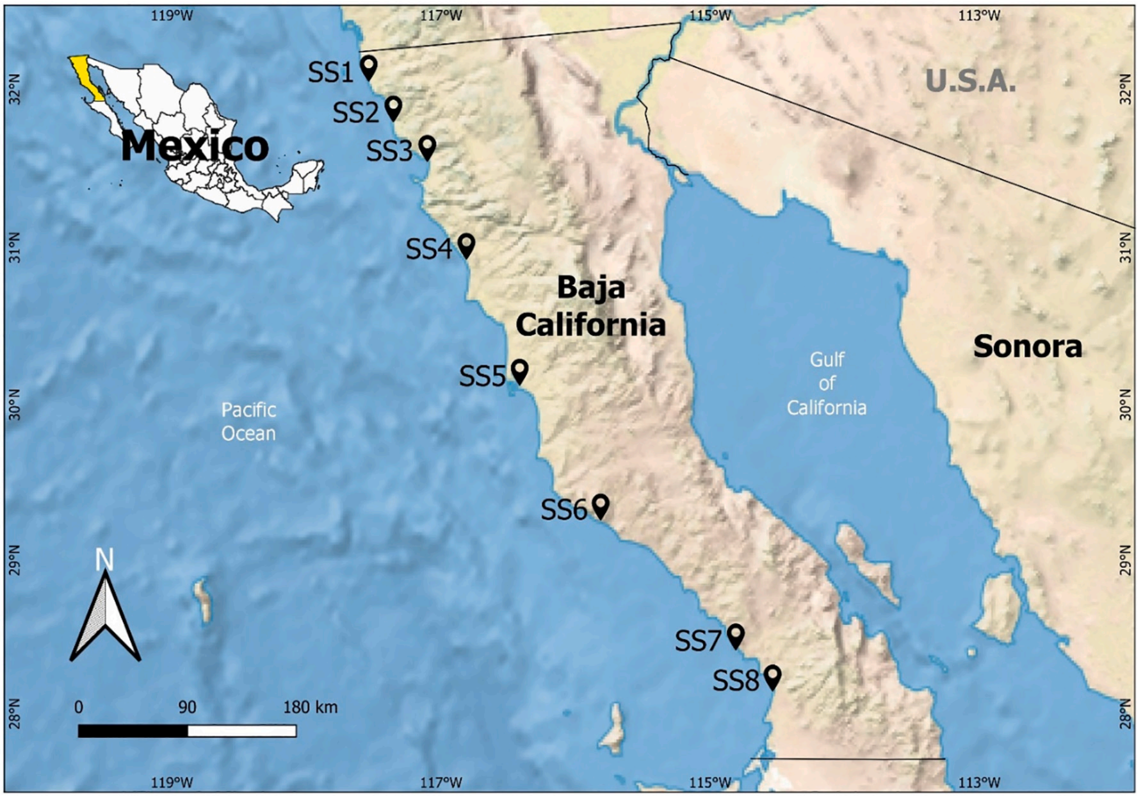

Fig. 1. Study area and location of the sampling sites of the mussels, *Mytilus californianus*.

Barbosa, 2001; Martin et al., 1988; Muñoz-Barbosa et al., 2000; Sañudo-Wilhelmy and Flegal, 1991; Segovia-Zavala et al., 2004; Villaescusa-Celaya et al., 1997, 2000). In particular, mussels from the *Mytilus* genus have been shown to be very good bioindicators of heavy metals due to their ability to accumulate trace metals and other substances (Alfonso et al., 2005; Apeti et al., 2010), which can provide data on contamination levels that can be temporally and spatially integrated (Bat, 2012; Rainbow, 1995). However, few studies have focused on evaluating mercury levels in mussels from this region (e.g. Gutiérrez-Galindo and Muñoz-Barbosa, 2003).

The California ribbed sea mussel, *M. californianus*, is an abundant species of open-coast rocky intertidal communities along much of the west coast of North America (Blanchette et al., 2007; Logan et al., 2012). These mussels are easy to access and manipulate being available year-round in the region. In addition, this species is widely harvested for human consumption and often used in aquaculture systems (Pfister et al., 2016). As a consequence, these mussels are readily available for human consumption along the west coast of the Baja California region. With this in mind, the aim of this study was to determine the spatial and temporal variations in total Hg concentrations (THg) in *M. californianus* from the Pacific coast of Baja California during November 2007, June 2008 and June 2010, and to assess the potential risks to human health from the consumption of these organisms.

2. Materials and methods

2.1. Sampling and sample preparation

During November 2007 (late autumn), June 2008 and June 2010 (late spring), 45 *M. californianus* individuals were collected at each of 8 sites located between the México-USA border (Tijuana-San Diego) and Sebastián Vizcaíno Bay, Baja California (B.C.) (Fig. 1). Sampling sites were selected within the coastal belt, in the mid-intertidal rock zone. In order to minimize any variations in Hg concentrations due to age, size and filtration rate, individuals of a similar size (40–60 mm) were collected (Coleman, 1980; Muñoz-Barbosa and Huerta-Diaz, 2013). Similarly, in order to avoid variations in Hg concentrations due to differences in air exposure, individuals were sampled from the same bed and tide level (Boyden, 1977; Muñoz-Barbosa et al., 2000). Immediately after collection the mussels were transferred to trace-metal-free polyethylene bags, stored in ice chests and transported to the laboratory where they were frozen at −20 °C until analysis.

Prior to analysis, the samples (made up of 45 individuals each) were defrosted, rinsed with deionized water to remove the sand and epibiota, and split into three sub-samples of 15 individuals each (Stephenson et al., 1979; Stephenson and Leonard, 1994). The mussels were dissected to separate gonads. The gonads were removed and discarded to minimize the seasonal variation in tissue weight due to the the reproductive cycle (Martin et al., 1988; Ouellette, 1981). The shell dimensions (length, height, width) and wet weight (w.w.) of the tissue of each individual within the sub-samples were then measured.

The accumulation of heavy metals in mussels may be influenced by their physiological status (e.g. growth, weight loss) which is itself associated with food availability and the physico-chemical characteristics of the surrounding water (Benedicto et al., 2011; Rouane-Hacene et al., 2015). In this context, the condition index (CI) is an excellent indicator of physiological status in bivalves (Jędruch et al., 2019). We calculated the CI as the ratio between the dry weight (d.w.) of tissue and the shell dimensions (CI = tissue dry weight / (shell length × shell width × shell height)) (Lobel et al., 1991) (Fig. 2e). Finally, the tissue from each of the 15-individual subsamples was homogenized using a Vitris 45 homogenizer equipped with titanium blades.

2.2. Mercury concentrations in the mussels

2.2.1. Digestion

An aliquot of 5 g w.w. of the homogenized tissue from each subsample was placed in 25 ml glass beakers and 10 ml of concentrated

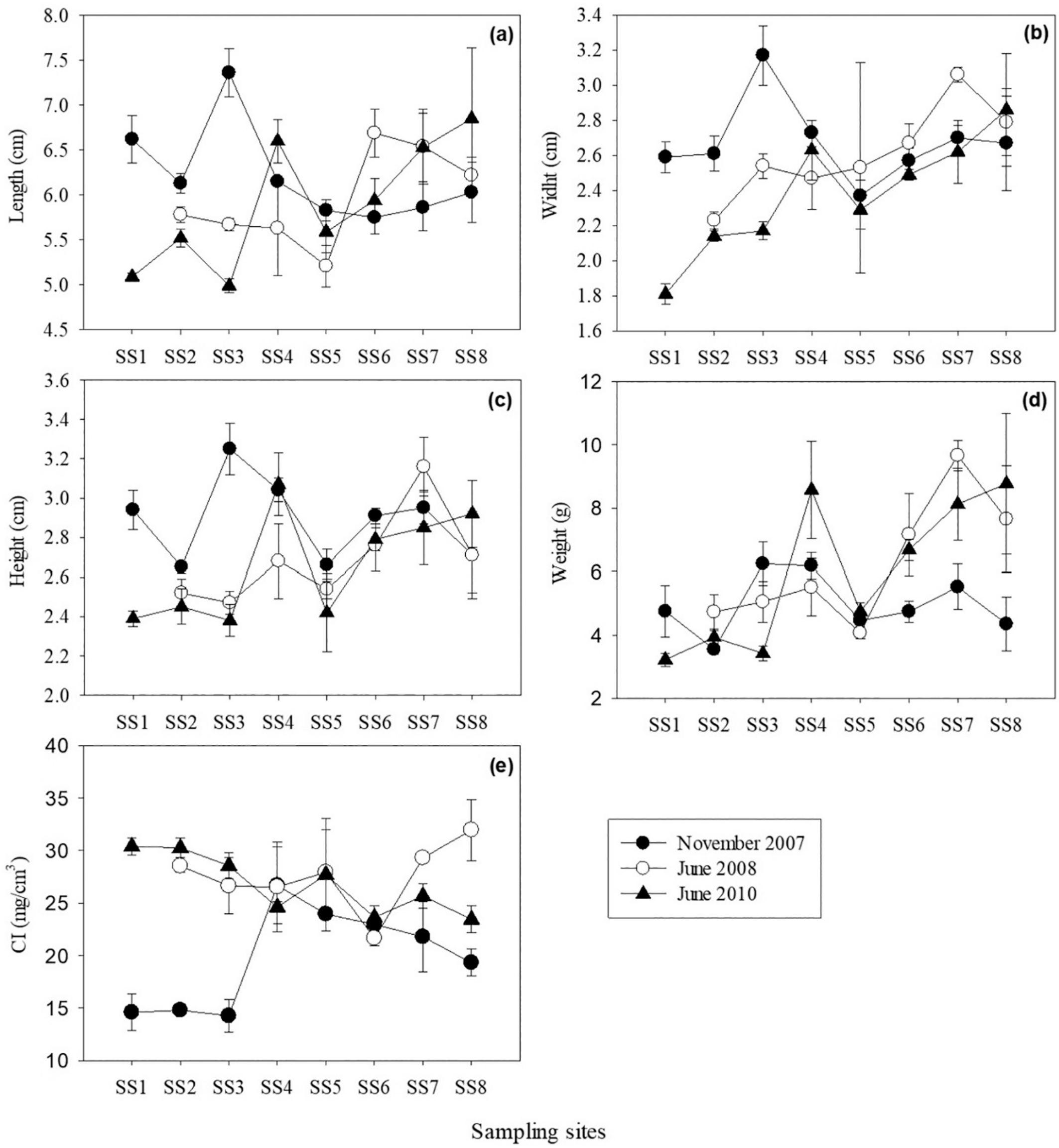

Fig. 2. Spatial variations in length (a), width (b), height (c), weight (d) and condition index (e) of *M. californianus* mussels during the study period. The symbols (solid circles, open circles and filled triangles) represent the mean values, and the vertical bars the standard deviation.

HNO_3 (trace metal grade) were added. The beakers were then covered with a watch glass and left overnight at room temperature. Subsequently, the samples were heated to 50 °C for 2 h and then refluxed at 100–150 °C for 5 h. After cooling, 15 ml of deionized water and 5 ml of 1% $K_3Cr_2O_7$ were added. Finally, the digested material was transferred to an acid-cleaned 50 ml polyethylene vial, diluted to 40 ml with deionizer water and left to stabilize for at least 20 min.

2.2.2. THg analysis

Mercury concentrations were measured by cold-vapor atomic absorption spectroscopy utilizing a Varian model SpectrAA 220 spectrometer equipped with a VGA-77 vapor generator. To confirm the accuracy and precision of the analytical procedures, one blank and one sample of reference material were analyzed for every six mussel samples. Standard Oyster Tissue SRM-1566b (National Institute of Standard and Technology) with a certified mean THg concentration of 0.0371 ± 0.0013 μg/g d.w. (mean ± standard deviation) was used as the reference material. The SRM-1566b concentration measured was 0.0347 ± 0.0061 μg/g d.w., which represents a recovery percentage of 93.53%. The detection limit (0.010 μg/g d.w.) was estimated as three times the standard deviation of ten blanks.

2.3. Health risk assessment

The potential health risk to mussel consumers was evaluated by calculating the estimated daily intake (EDI), target hazard quotient (THQ) and maximum safe mussel consumption (MSMC). The EDI and THQ were estimated according to Zhelyazkov et al. (2018). EDI can be calculated using the following formula:

$$\text{EDI }(\mu g\text{ Hg/kg body weight/day}) = (\text{FIR} \times \text{C})/\text{BWa}$$

where FIR is the daily mussel uptake rate (kg/person), C is the average THg in mussel tissue (μg/kg w.w.), and BWa is the average body weight (kg). In this risk assessment the calculations were done assuming a body weight of 70 kg for Mexican adults (both genders) and using two sets of data for the shell mollusk consumption rate in México: 0.93 kg/capita/year (or 0.002 μg/capita/day) in 2013 and 0.58 kg/capita/year (or 0.001 μg/capita/day) in 2017 (FAO, 2017 and CONAPESCA, 2017, respectively). The oral reference dose (RfDo) and the acceptable daily intake (ADI) for Hg were taken as the standards against which the EDI values obtained were compared, and used to determine whether health guidance values were exceeded. In addition, we compared our estimates of the potential risk with the risk indexes for MeHg due to the fact that this is the most toxic chemical form for human and the most predominant in fish and shellfish (Jędruch et al., 2019; Jović and Stanković, 2014; Polak-Juszczak, 2015). RfDo, ADI and provisional tolerable weekly intake (PTWI) values for MeHg are: RfDo, 0.1 μg/kg/day (equal to 1×10^{-4} mg/kg/day) (US EPA, 2015); ADI, 0.23 μg/kg/day and PTWI, 1.6 μg/kg/week (FAO/WHO, 2004; Tao et al., 2016).

THQ indicates the risk level resulting from exposure to a pollutant, e.g. the consumption of seafoods (mussels) contaminated with Hg (Storelli, 2008) and was developed by the United States Environmental Protection Agency (US EPA, 1989). It is expressed by the following equation:

$$THQ = (EF \times ED \times FIR \times C)/(RfDo \times BWa \times AT)$$

where EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years), FIR is the food ingestion rate (kg/day), C is the concentration of a metal in mussels (mg/kg, w.w.), RfDo is the Hg oral reference dose (mg/kg/day), BWa is the average body weight (kg, both genders) and AT is the average exposure time for non-carcinogens (365 days/year x ED). A THQ value <1 indicates that there are no negative health effects for the exposed human population. In contrast, a THQ value >1 means that the pollutant is likely to have some deleterious effects on human health.

Although mussels are excellent sources of nutrients for humans, the Hg content in the edible part can cause health problems. In this case, the quantity of mussels that may be consumed safely must be such that the mercury levels consumed do not exceed the PTWI (provisional tolerable weekly intake). The MSMC per week was calculated according to the following formula:

$$MSMC\ (g/week) = (PTWI \times BWa)/C$$

where PTWI is the provisional tolerable weekly intake established by the FAO/WHO (2004), C is the average THg reported in mussel tissue (μg/g, w.w.) and BWa is the average body weight (kg). In this case, average body weights of 70, 60 and 16 kg were assumed for adult men and women, and children respectively, as in Romo-Piñera et al. (2018).

2.4. Coastal upwelling index

In order to understand how coastal upwelling affects the THg in mussels, Coastal Upwelling Index (CUI) values (m^3/s/100 m of coastline) for the study area were obtained from the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration (NOAA) through the Columbia Basin Research group at the University of Washington. (http://www.cbr.washington.edu/dart/query/upwell_graph_text). For this study, the San Diego, California index (33° N 119° W) was chosen for SS1 (Rosarito) and SS2 (Bajamar), for SS3 (Ensenada) and SS4 (Eréndira) the index was interpolated between San Diego and Guadalupe, Baja California (30° N 119° W), the Guadalupe index was chosen for SS5 (San Quintín) and SS6 (Punta Baja), and for SS7 (Los Ojitos) and SS8 (Playa Esmeralda) the index was interpolated between Guadalupe and the Cedros, Baja California (27° N 16° W).

2.5. Statistical analyses

Differences between the biometric parameters (length, width, height, weight, CI) and THg among sites for each year were analyzed by means of a one-way analysis of variance coupled with a post hoc (Tukey) test. Normality and homogeneity of variances were tested using the Shapiro-Wilk and Bartlett's tests, respectively. THg data were normalized relative to the CI of the mussels and are represented as THg_{NORM} (μg Hg/cm^3 d.w.). This normalization decreases the variability of the data by reducing the effects of external factors such as temperature, salinity, depth of the water column and the nutritional state of the mussels (Gutiérrez-Galindo and Muñoz-Barbosa, 2001; Lares et al., 2002; Lares and Orians, 1997; Segovia-Zavala et al., 2004). Pearson's correlation coefficients were estimated in order to assess associations between the biometric parameters, THg and THg_{NORM}. For this test any variables that did not pass the test of normality were log-transformed in order to meet this prerequisite. Furthermore, for each year Spearman's correlation coefficients were calculated to assess associations between the CUI and CI values. All tests were performed using R (R Core Team, 2021) with $\alpha \leq 0.05$.

Table 1
Pearson's correlation coefficients between the biometric data, the condition index (CI), normalized mercury (THg_{NORM}) and total mercury concentration (THg) in *M. californianus*.

	THg	THg_{NORM}[b]	Length	Width	Height	Weight
2007						
THg_{NORM}[b]	0.84**					
Length[a]	0.26	−0.07				
Width	−0.18	−0.29	0.80*			
Height	−0.35	−0.37	0.66	0.78*		
Weight	−0.65	−0.69	0.45	0.66	0.91*	
CI	−0.80*	−0.42	−0.46	−0.03	0.23	0.41
2008						
THg_{NORM}[b]	0.96**					
Length	−0.05	−0.16				
Width	−0.63	−0.67	0.64			
Height	−0.62	−0.66	0.72	0.85*		
Weight	−0.51	−0.55	0.87*	0.89**	0.92**	
CI	−0.68	−0.50	0.19	0.62	0.64	0.60
2010						
THg_{NORM}[b]	0.99**					
Length	−0.75*	−0.76*				
Width	−0.82*	−0.85**	0.93**			
Height	−0.74*	−0.76*	0.93**	0.87**		
Weight	−0.81*	−0.83**	0.98**	0.94**	0.97**	
CI	0.77*	0.83*	−0.83*	−0.91**	−0.87**	−0.90**

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.
[a] Variable log-transformed for analysis.
[b] Data were normalized in relation to the condition index of the mussels.

3. Results and discussion

3.1. Biometric data and condition index

The biometric data and CI of *M. californianus* varied significantly over the three years of the investigation, particularly in sampling sites from the northern zone of the study area (Fig. 2a-e). For example, significant differences ($p < 0.05$) were found between the length and CI values in 2007 (late autumn) and 2008 and 2010 (late spring) values. Mean CI values were significantly higher ($p < 0.05$) in the late spring (June 2008 and 2010; 28.78 ± 5.19 and 26.78 ± 2.83 mg/cm^3, respectively) than in the late autumn (November 2007; 18.57 ± 4.83 mg/cm^3). This agrees with the variability in the stored energetic reserves found in marine bivalves in accordance with reproductive cycles, development and growth (Cossa et al., 1980; Okumuş and Stirling, 1998; Rouane-Hacene et al., 2015). A study carried out by Okumuş and Stirling (1998) with *M. edulis* mussels from the west coast of Scotland, reported minimum CI values in early spring (March–April) and higher levels in late spring and early summer (May–July). Furthermore, these authors observed that the minimum CI values in April followed by a rapid increase in May, correlated with the main period of spawning and recovery. In addition, the environmental conditions in spring and summer are more favorable than in winter for the physiological development and growth of mussels due to the increased quantity and quality of food available (e.g. high concentrations of organic matter, including bacteria, phyto- and zooplankton) (Rouane-Hacene et al., 2015). In our case, the breeding season of *M. californianus* along the west coast of Baja California is from winter to summer, with major reproductive activity during spring and a non-reproductive period during the rest of the year (Curiel-Ramírez and Cáceres-Martínez, 2004).

We also observed a similar trend between the CUI and CI values, whereby lower CUI values in the late autumn of 2007 (39.92 ± 10.27 m^3/s/100 m) and higher CUI values in the late spring of 2008 (140.40 ± 25.32 m^3/s/100 m) and the late spring of 2010 (142.84 ± 32.10 m^3/s/100 m), corresponded with lower and higher CI values, during these same periods. Upwelling, which occurs throughout the year off the Baja California coast and is more intense during spring and summer (Alvarez-Borrego and Alvarez-Borrego, 1982; Barton and Argote, 1980; Segovia-Zavala et al., 2003), is an important mechanism transporting metals (Bruland, 1980) and nutrients from deep to coastal surface waters (Lares et al., 2002; Segovia-Zavala et al., 1998). The availability of nutrients generated by this phenomenon is generally associated with increases in phytoplankton biomass. Thus, the high availability of food during summer and the ability of mussels to filter large volumes of water (between 0.2 and 5 L/h) to satisfy their nutritional and respiratory requirements (Rouane-Hacene et al., 2015) could explain the higher CI values. In addition, the Spearman rank correlation analyses performed for the CI and CUI values for each year gave positive ($r = 0.097$) and negative ($r = -0.826$) correlations in 2007 (late autumn) and 2008 (late spring), respectively. However, in 2010 (late spring), the analysis gave a strong positive ($r = 0.683$) correlation, indicating that the CI of mussels is associated with the upwelling process (intensity).

Pearson's correlation analysis showed significant negative correlations between THg and biometric data over the whole three years of study (Table 1). According to Gutiérrez-Galindo and Muñoz-Barbosa (2003), an inverse relationship between metal concentrations and size can be attributed to the dilution of the former by the organism's growth. Nevertheless, studies have shown that heavy metals can negatively affect the shell growth rate of mussels (Manley et al., 1984). Strömgren (1982) reported that the growth rate in *M. edulis* was significantly reduced by about 50% due to Hg (0.3 μg/L). In our study, the CI was inversely correlated with THg in 2007 and 2008, which means that physiological changes only had a small effect on the THg in the tissues of the mussels (Mourgaud et al., 2002). In contrast, in 2010 we found a significant positive correlation ($r = 0.77$, $p < 0.05$) between THg and CI, indicating that the mussels were ingesting large amounts of organic matter and adsorbing high concentrations of metals, and thus contributing to an increase in both CI and accumulated metal (Rebelo et al., 2005). This response could be a result of the upwelling process, particularly in 2010 when this was more intense in the study region.

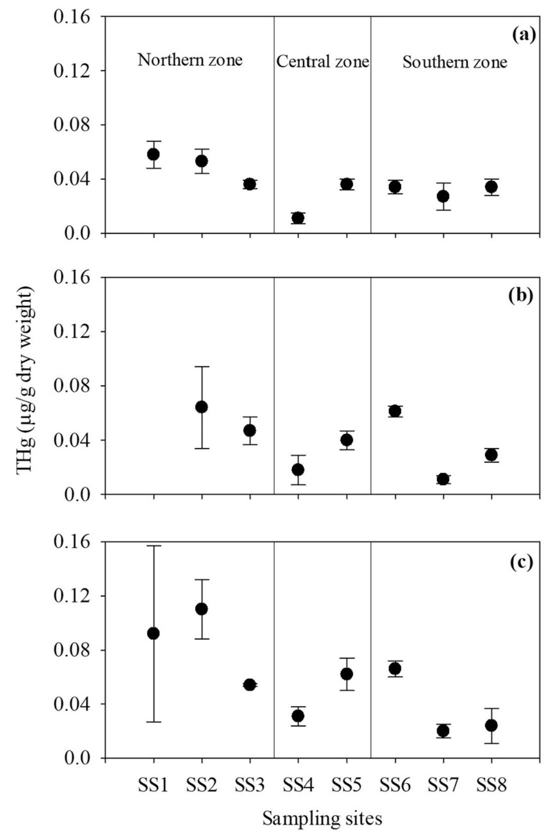

Fig. 3. Spatial distribution of THg in the soft tissues of *M. californianus* from the west coast of Baja California in (a) November 2007, (b) June 2008 and (c) June 2010. The solid circles represent the mean values, and the vertical bars the standard deviation.

3.2. THg content

In spite of the low spatial variability, over the three years of our study we found that the THg at sites from the northern region were significantly higher ($p < 0.01$) than that from sites in the southern region. In November 2007, THg levels in *M. californianus* at SS1 and SS2 were significantly higher ($p < 0.05$) than at those located in the southern zone (Fig. 3a). In June 2008 and June 2010, Hg concentrations at SS1, SS2 and SS3 were significantly higher ($p < 0.05$) than those observed at SS7 and SS8 (Fig. 3b and c). The higher concentrations of heavy metals in *M. californianus* (Hg, Ag and Pb) in the north compared to those found in this species in the south of Baja California has been associated with the larger human settlements, more developed socio-economic activities and higher waste production in the former (México-US border) (Gutiérrez-Galindo and Muñoz-Barbosa, 2003).

This suggests that the significant differences in the magnitudes of anthropogenic activities between the northern and southern regions could be a key factor driving the north-south gradients of the concentrations of THg, with the highest values at sites from the northern zone, and the lowest at sites from the southern zone.

In addition, the SS5 site (San Quintin Valley) must be considered a special case. A previous study reported Hg concentrations in *M. californianus* two orders of magnitude higher than those we registered. In this study the mollusks were collected during the months of April (1.44 μg/g d.w.), July (1.89 μg/g d.w.) and September (0.030 μg/g d.w.) (Gutiérrez-Galindo and Flores-Muñoz, 1986). These authors associated the Hg levels they reported with the intense upwelling phenomena experienced in this region, which bring water rich in nutrients and trace metals to the surface and thus constitute the main source of Hg in these coastal areas (Gardner, 1975; Gworek et al., 2016). In contrast, for this same site and using *M. californianus* as a bioindicator, Lares et al. (2002) recorded higher Hg concentrations from January to April than from May to October. These authors found that not only Hg, but also Al and Mn, were highly correlated with pluvial precipitation, suggesting that part of the variability in Hg concentrations could have a terrigenous origin. The most important economic activity in SS5 is agriculture, which has rapidly increased over the last decades. Associated with the growth of this activity, the extensive use of fertilizers, pesticides and fungicides containing heavy metals has also increased (Green-Ruiz and Páez-Osuna, 2003; Wuana and Okieimen, 2011). Hence, we suggest that this factor represents a possible source of Hg and must not be discarded.

The Pacific coast of Baja California is dominated by the California Current System (CCS) which flows southward along the coast of Baja California during winter and summer (Durazo, 2015). Thus, considering the local hydrodynamic, the input of metals at San Quintin due to agricultural practices may also be transported towards SS6. Furthermore, Pearson's correlation analysis showed a significant positive correlation ($p < 0.05$) between THg and THg_{NORM} during the three years of study (Table 1), suggesting that environmental Hg levels are well reflected in *M. californianus*. In this context, the relatively high concentrations of Hg in SS5 and at SS6 over the study period could be partially associated with an anthropogenic source (e.g. agricultural runoff) and the local hydrodynamic, respectively.

In general, our results were similar to those reported by Jara-Marini et al. (2008) in *Mytella strigata* from the southeast coast of the Gulf of California, and Lares et al. (2002) in *M. californianus* from San Quintín Bay, both in México (Table 3). However, studies carried out by Gutiérrez-Galindo and Flores-Muñoz (1986) and Gutiérrez-Galindo and Muñoz-Barbosa (2003) with *M. californianus* in the same study area reported much higher (an order of magnitude) Hg concentrations than those we registered. Our observations thus suggest that there has been a reduction in the amount of Hg in the region. We compared our data with the Background Assessment Concentrations (BACs) established by the Oslo and Paris convention (OSPAR). This convention has developed Background Concentrations (BCs) for trace metals (Hg, Cd and Pb) in biota (mussels and oysters). A BC is the concentration of a contaminant at 'pristine' and 'remote' sites based on contemporary or historical data (Moffat et al., 2004). Observed concentrations are said to be 'near background' if the mean concentration is statistically significantly below the corresponding BAC (Webster et al., 2009). BACs were estimated from data collected under the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP) and used them as reference to evaluate whether the Hg concentrations we registered in the mussels could be considered as background or close to it. From this comparison, the THg levels in *M. californianus* in our study were found to be below the BAC value (0.090 μg/ g d.w.) at all sites, except for mussels collected in SS1 (0.092 μg/g d.w.) and SS2 (0.110 μg/g d.w.) in 2010. This suggests that most of our study area (especially the central and southern regions) could be considered as a non-impacted zone by Hg.

Comparing with other studies undertaken in different regions around the world (Table 3), the mean THg in *M. californianus* reported here was similar to that found in *M. edulis* in Álftafjörðurs, Iceland (Hixson, 2019) and slightly lower than that registered for *M. galloprovincialis* along the coastline of Libya (Galgani et al., 2014), in the Ulsan and Onsan Bays, Korea (Kim and Choi, 2017) and at Portonovo, Italy (Fattorini et al., 2008). However, studies carried out with mussels in Algeria (Benzaoui et al., 2015), Morocco (Maanan, 2008), Turkey (Kayhan, 2007), Croatia (Kljakovic-Gašpić et al., 2010), France (Briant et al., 2017), Italy (Di Leo et al., 2010; Spada et al., 2013), Montenegro (Jović and Stanković, 2014), Spain (Besada et al., 2011; Vázquez-Luis et al., 2016), Poland (Jędruch et al., 2019), and USA (Edwards et al., 2014; Hunt and Slone, 2010), among others, reported a mean Hg level higher than that detected by us. According to international criteria defined by NOAA and available from the National Status and Trend 'Mussel Watch' program (Kimbrough et al., 2008), the sites surveyed in our study would be classified as low polluted as regards the THg levels measured.

Table 2
Mean THg (μg/g w.w.), standard deviation (±s.d.) and conversion factor (*i*) in the soft tissues of *M. californianus* collected from the west coast of Baja California.

Sampling sites	*i*[a]	November 2007	*i*[a]	June 2008	*i*[a]	June 2010
SS1	6.33	$9.16 \times 10^{-3} \pm 0.001$	n.a.	n.a.	4.62	$1.99 \times 10^{-2} \pm 0.001$
SS2	5.92	$8.96 \times 10^{-3} \pm 0.001$	5.16	$12.42 \times 10^{-3} \pm 0.006$	5.07	$2.17 \times 10^{-2} \pm 0.004$
SS3	6.24	$5.78 \times 10^{-3} \pm 0.001$	5.79	$8.11 \times 10^{-3} \pm 0.001$	4.62	$1.17 \times 10^{-2} \pm 0.000$
SS4	4.12	$2.67 \times 10^{-3} \pm 0.001$	5.13	$3.51 \times 10^{-3} \pm 0.003$	5.59	$5.54 \times 10^{-3} \pm 0.001$
SS5	5.28	$6.82 \times 10^{-3} \pm 0.000$	5.89	$6.79 \times 10^{-3} \pm 0.001$	4.88	$1.27 \times 10^{-2} \pm 0.000$
SS6	4.97	$6.85 \times 10^{-3} \pm 0.001$	6.67	$9.14 \times 10^{-3} \pm 0.000$	6.35	$1.04 \times 10^{-2} \pm 0.000$
SS7	5.40	$5.01 \times 10^{-3} \pm 0.001$	5.12	$2.15 \times 10^{-3} \pm 0.001$	5.65	$3.54 \times 10^{-3} \pm 0.000$
SS8	5.19	$6.55 \times 10^{-3} \pm 0.001$	5.14	$5.65 \times 10^{-3} \pm 0.001$	5.81	$4.13 \times 10^{-3} \pm 0.002$
Minimum		$2.67 \times 10^{-3} \pm 0.001$		$2.15 \times 10^{-3} \pm 0.001$		$3.54 \times 10^{-3} \pm 0.000$
Maximum		$9.16 \times 10^{-3} \pm 0.001$		$12.4 \times 10^{-3} \pm 0.006$		$2.17 \times 10^{-2} \pm 0.004$
Mean	5.53	0.0065 ± 0.002	5.73	0.0068 ± 0.003	4.64	0.0112 ± 0.006

n.a. = not available.
[a] *i* = conversion factor (dry weight concentration = wet weight concentration * *i*).

3.3. Human health risk assessment

The health risk assessment indexes, EDI and THQ, were also calculated based on two sets of data taken from FAO and CONAPESCA (Table 4). Our results showed that according to the consumption rate of mollusks by adult Mexicans, the highest annual mean EDI value was 0.0004 μg/kg/day in 2010 following the estimated consumption rate given by the FAO (2017) and the lowest value was 0.0002 μg/kg/day in 2007 and 2008 according to both consumption rate data, FAO (2017) and CONAPESCA (2017). In addition, the EDI values estimated for all sampling sites over the three years were well below the RfDo (0.1 μg/kg/ day) and ADI (0.23 μg/kg/day) values. Nevertheless, the highest EDI values were observed in 2010 at SS1, SS2 and SS3 from the northern zone of the study area. Furthermore, all THQ values were lower than 1: the highest annual mean THQ value recorded was 0.0041 in 2010 (data from FAO (2017)) and the lowest annual mean THQ value was 0.0015 in 2007 (data from CONAPESCA (2017)). These results indicate that health risks for people from the intake of Hg through mussel consumption are not significant. Comparing with the literature, we note that the EDI values observed in this study were lower than those observed by Jędruch et al. (2019). These authors estimated an average EDI value (0.0014 μg/ kg/day) based on the consumption rate of mussels (*Mytilus trossulus*) in the European Union. In a further investigation, Zhelyazkov et al. (2018)

Table 3
Mean values and range of THg levels (μg/g d.w.) in marine mussels around the world (A) and in México (B).

Species	Study area	THg	Reference
A			
Brachidonte exustus	Todos os Santos Bay, Brazil	0.147 (0.03–0.15)	de Souza et al. (2011)
Mytella guyanensis	Todos os Santos Bay, Brazil	0.063 (0.03–0.35)	de Souza et al. (2011)
Mytilus galloprovincialis	Ulsan and Onsan Bays, Korea	0.051 (0.009–0.114)	Kim and Choi (2017)
	El Jadida coast, Morocco	1.160 (0.02–2.3)	Maanan (2008)
	Oran Bay, Algeria	0.895 (0.24–2.27)	Benzaoui et al. (2015)
	Libya coast, Libya	0.053 (0.045–0.069)	Galgani et al. (2014)
	Bosporus, Turkey	1.724 (0.14–2.86)	Kayhan (2007)
	Trieste Gulf, Slovenia	0.122 (0.07–0.237)	Ramšak et al. (2012)
	Normandy coast, France	0.120 (0.07–0.17)	Séguin et al. (2016)
	Mediterranean Sea, France	0.293 (0.11–0.7)	Briant et al. (2017)
	Adriatic Croatian coast, Croatia	1.050 (0.12–10.3)	Kljakovic-Gašpić et al. (2010)
	Mar Piccolo, Italy	0.361 (0.236–0.559)	Di Leo et al. (2010)
	Portonovo, Italy	0.052 (0.022–0.19)	Fattorini et al. (2008)
	Apulia coast, Italy	0.272 (0.1–0.81)	Spada et al. (2013)
	Boka Kotorska Bay, Montenegro	0.501 (0.14–2.651)	Jović and Stanković (2014)
	Varna Bay, Bulgaria	0.085 (0.055–0.115)	Zhelyazkov et al. (2018)
	Biscay Bay, Spain	0.220 (0.02–0.42)	Solaun et al. (2013)
	Balearic Islands, Spain	0.204 (0.158–0.256)	Deudero et al. (2009)
	Bilbao Estuary, Spain	0.106 (0.04–0.2)	Bartolomé et al. (2010)
	Galician coast, Spain	0.108 (0.037–0.444)	Besada et al. (2011)
	Asturias, Spain	0.252 (0.054–0.536)	Besada et al. (2011)
	Cantabria, Spain	0.235 (0.145–0.428)	Besada et al. (2011)
	Basque country, Spain	0.18 (0.135–0.225)	Besada et al. (2011)
	Adriatic Sea	0.089 (0.05–0.13)	Bajt et al. (2019)
Mytilus edulis	Unalaska Islands, USA	0.11 (0.05–0.22)	Savoy et al. (2017)
	Massachusetts Bay, USA	0.34 (0.13–0.55)	Hunt and Slone (2010)
	Cape Cop Bay, USA	0.24 (0.06–0.42)	Hunt and Slone (2010)
	Casco Bay, USA	0.125 (0.11–0.14)	Hunt and Slone (2010)
	Gulfwatch, USA	0.22 (0.09–0.44)	Elskus et al. (2020)
	Mussel Watch, USA	0.23 (0.09–0.33)	Elskus et al. (2020)
	Gulfwatch, Canada	0.17 (0.09–0.38)	Elskus et al. (2020)
	Skutulsfjörður, Iceland	0.073 (0.063–0.089)	Hixson (2019)
	Álftafjörðurs, Iceland	0.041 (0.032–0.051)	Hixson (2019)
	The Icelandic coastline	0.061 (0.041–0.081)	Sturludottir et al. (2013)
	English Channel, France	0.210 (0.05–0.66)	Briant et al. (2017)
	Atlantic coast, France	0.156 (0.12–0.22)	Briant et al. (2017)
Mytilus spp.	California Coast, USA	0.143 (0.02–0.99)	Edwards et al. (2014)
Mytilus trossulus	Puck Bay, Poland	0.145 (0.045–0.671)	Jędruch et al. (2019)
	Adak Island, Alaska, USA	0.086 (0.043–0.129)	Burger and Gochfeld (2006)
Pinna nobilis	Andratx, Spain	0.3 (0.25–0.35)	Vázquez-Luis et al. (2016)
	Magaluf, Spain	0.8 (0.56–1.09)	Vázquez-Luis et al. (2016)
	Santa Maria Bay, Spain	0.96 (0.82–1.16)	Vázquez-Luis et al. (2016)
Perna viridis	Sucre, Venezuela	0.61 (0.084–1.129)	Rojas et al. (2009)
B			
Mytella strigata	Urías Lagoon, México	0.067 (0.034–0.145)	Jara-Marini et al. (2008)
Mytilus californianus	San Quintín Bay, México	0.078 (0.048–0.107)	Lares et al. (2002)
	Baja California coast, México	0.485 (0.27–0.95)	Gutiérrez-Galindo and Flores-Muñoz (1986)
	Baja California coast, México	0.192 (0.053–0.331)	Gutiérrez-Galindo and Muñoz-Barbosa (2003)
	Baja California, México	0.046 (0.011–0.11)	This study

reported an EDI (0.0001 μg/kg/day) and THQ (0.0006) for *Mytilus galloprovincialis*, lower than those obtained by us. However, THQ values much higher than those reported in our study were registered by Jović and Stanković (2014) who calculated the THQs for average and high level mussel consumers (*Mytilus galloprovincialis*) in Albania (0.049–0.099), Montenegro (0.072–0.144), Slovenia (0.023–0.045), Croatia (0.161–0.321) and Italy (0.048–0.096).

In order to establish a safe human consumption limit for the mussels collected in our study area, we estimated the MSMC using the highest average THg (0.0217 μg/g w.w.) recorded in our study (Table 2). The MSMC of *M. californianus* that could be consumed per week was 5161 g/week for an adult man (~70 kg), 4424 g/week for an adult woman (~60 kg) and 1180 g/week for a child (~16 kg). Also, considering the average *M. californianus* weight without the shell (5.72 ± 0.67 g), an adult man could consume approximately 902 mussels per week (~129 mussels per day) without risk, whereas an adult woman could consume 773 mussels per week (~110 mussels per day), and a child 206 mussels per week (~29 mussels per day). Another index, the Tolerable Weekly Intake (TWI) has been established by the European Food Safety Authority (EFSA). The TWI for MeHg is 1.3 μg/kg/week (EFSA, 2012; Jędruch et al., 2019), and we recalculated the MSMC accordingly. In this new scenario, the MSMC values per week were about 4194 g (~733 mussels per week or 105 per day) for an adult man, 3594 g (~628 mussels per week or 90 per day) for an adult woman and 959 g (or 167 mussels per week or 24 per day) for a child. Notwithstanding this, in general we suggest that adult men and women, and especially children, as well as pregnant and lactating women, should pay particular attention to the amount of the mussels in their diet as well as other seafoods that originate from the same region. This is especially relevant for populations with a high seafood consumption (e.g. coastal and fishing communities).

4. Conclusions

Our study showed that the condition index (CI) values were lower in 2007 than in 2008 and 2010, and are likely to be associated with seasonal cycles and the intensity of coastal upwelling in this region. We did not find significant differences in THg (in mussel tissue) among the three years, whereas we did detect spatial variations, showing the highest Hg

Table 4
Estimated daily intake (EDI, µg/kg/day) and target hazard quotient (THQ) values for the consumption of mussels by adults per sampling site per year, calculated using the shell mollusk consumption data for Mexican people from two sources (i.e., FAO, 2017; CONAPESCA, 2017).

	Sampling sites	EDI			THQ		
		2007	2008	2010	2007	2008	2010
FAO, 2017	SS1	0.0003	n.a.	0.0007	0.0033	n.a.	0.0069
	SS2	0.0003	0.0004	0.0008	0.0033	0.0044	0.0080
	SS3	0.0002	0.0003	0.0004	0.0032	0.0029	0.0044
	SS4	0.0001	0.0001	0.0002	0.0011	0.0015	0.0022
	SS5	0.0003	0.0003	0.0005	0.0025	0.0025	0.0047
	SS6	0.0003	0.0003	0.0004	0.0025	0.0033	0.0036
	SS7	0.0002	0.0001	0.0001	0.0018	0.0007	0.0015
	SS8	0.0003	0.0002	0.0001	0.0025	0.0022	0.0015
	Mean	0.0002	0.0002	0.0004	0.0024	0.0025	0.0041
CONAPESCA, 2017	SS1	0.0002	n.a.	0.0004	0.0020	n.a.	0.0043
	SS2	0.0002	0.0003	0.0005	0.0020	0.0027	0.0050
	SS3	0.0001	0.0002	0.0003	0.0014	0.0018	0.0027
	SS4	0.0001	0.0001	0.0001	0.0007	0.0009	0.0014
	SS5	0.0002	0.0002	0.0003	0.0016	0.0016	0.0030
	SS6	0.0002	0.0002	0.0002	0.0016	0.0020	0.0023
	SS7	0.0001	0.0000	0.0001	0.0011	0.0005	0.0009
	SS8	0.0002	0.0001	0.0001	0.0016	0.0014	0.0009
	Mean	0.0002	0.0002	0.0003	0.0015	0.0016	0.0026

n.a. = not available.

levels were found at sites from the northern Baja California region and may be associated with anthropogenic inputs (e.g. sewage wastewater). The THg concentrations for *M. californianus* recorded in our study were lower than the concentrations of this metal found in other mussels collected from polluted areas around the world. The EDI values for adults consuming mussels were lower than published RfDo and PTWI values. The THQ was lower than 1, leading us to consider that the consumption of mussels does not pose any risk for the health of the adult population. These results thus indicate that, at least for the time being, the concentration of mercury in wild mussels is not a matter of concern. Nevertheless, in view of the increasing urbanization, industrialization and agriculture along the north and central Pacific coast of Baja California, México the THg in *M. californianus* should be frequently monitored to determine levels and trends with the aim of preventing deleterious effects on both the biota and human health from the consumption of contaminated seafood.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Russell Giovanni Uc-Peraza: Writing – original draft, Methodology, Conceptualization, Investigation. **Efraín Abraham Gutiérrez-Galindo**: Writing – review & edition, Funding acquisition. **Victor Hugo Delgado-Blas**: Writing – review & edition, Investigation. **Albino Muñoz-Barbosa**: Methodology, Writing – review & edition, Investigation. All authors analyzed the data, edited the manuscript, and gave approval for publication.

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