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Organotin contamination in seafood from the Yucatán Peninsula, Mexico: Is there a potential risk for the health of consumers?

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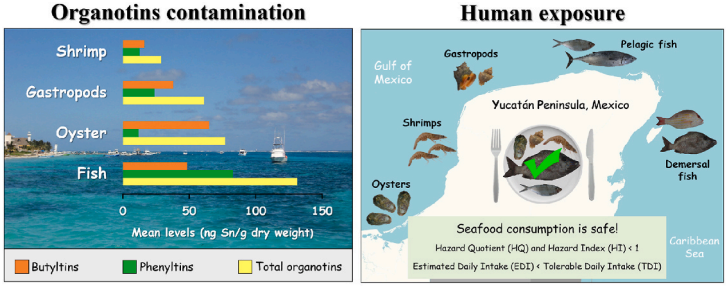
HIGHLIGHTS

- Seafood from the Yucatán Peninsula is contaminated by organotins (OTs).
- MPhT followed by TBT were the predominant OTs in fish muscle, and crustaceans and mollusks tissues.
- Fish was the main contributor to human OTs exposure.
- TBT > DBT ≫ TPhTs were the main contributors to the toxicity assessed.
- Seafood consumption does not seem to pose any risk to consumers in the study area.

GRAPHICAL ABSTRACT



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ABSTRACT

Since seafood is considered an important source of organotin compounds (OTCs), the present study assessed the potential risk to human health of ingesting butyltins (BTs) and phenyltins (PhTs) along with this type of food. Seafood samples were collected at five fishing sites in the Yucatán Peninsula (Mexico) during February and March 2018. In general, organotins were detected in all samples, suggesting a widespread occurrence of these compounds in the investigated region. The average concentration of total organotins in the muscle of demersal fish (*Lutjanus synagris*, *Lutjanus campechanus*, *Calamus pennatula*, *Haemulon plumierii*, *Rhomboplites aurorubens*), pelagic fish (*Euthynnus alletteratus*, and *Opisthonema oglinum*), gastropods (*Melongena bispinosa* and *Strombus pugilis*), oyster (*Crassostrea virginica*) and shrimp (*Penaeus duorarum*) was 146.7 ± 76.2, 93.1 ± 92.6, 61.0 ± 53.0, 76.7 ± 2.6, and 28.8 ± 2.7 ng Sn g^{-1} dry weight, respectively. Overall, MPhT among PhTs was the dominant compound in fish, while TBT among BTs was the dominant compound in shellfish. Regarding the toxic OTCs, TBT followed by DBT were the predominant compounds in all seafood species, while TPhT was below the quantification limit in most samples. The estimated daily intake values were lower than the tolerable daily intake (TDI) for the sum of organotins established by the European Food Safety Authority (EFSA). Furthermore, the hazard

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quotients (HQ) and hazard indices (HI) values were all lower than 1, suggesting that daily exposure to these levels of organotins is unlikely to cause any harm to the human health of seafood consumers at the Yucatán Peninsula. Thus, consumers may not be at risk through the inclusion of these investigated seafood species in their normal diet. However, due to the increasing coastal urbanization, maritime activities, and the likely illegal use of tin-based paints in Mexico, additional monitoring is needed to assess organotin levels in other regions along the Mexican coastal zone and using other seafood species.

1. Introduction

Seafood constitutes an important source of proteins, minerals, vitamins, and polyunsaturated fatty acids (PUFAs), and its consumption provides nutritional benefits for human health (Marquès et al., 2021). However, these marine products can also contain a variety of environmental contaminants (Storelli, 2008). Seafood consumption is considered the most important pathway of human exposure to organotin compounds (OTCs) (Chung et al., 2020; Rosenberg, 2013), and might lead to a potential risk to the health of consumers (Ho and Leung, 2014; Sham et al., 2020). In this regard, some studies indicated that OTCs are probably able to induce adverse health effects on humans, including reproductive and developmental abnormalities, immunosuppression, possible carcinogenic activity (Antizar-Ladislao, 2008; Ho and Leung, 2014), and obesity (Grün and Blumberg, 2006; Heindel and Blumberg, 2019; Ren et al., 2020). In addition, OTCs cannot be degraded or destroyed after cooking (Chung and Wu, 2017; Willemsen et al., 2004), which means that these compounds may be completely available to seafood consumers.

OTCs contamination in seafood is primarily caused by the legacy and, in some cases, current illegal use of tri-substituted organotins, tributyltin (TBT) and triphenyltin (TPhT) as biocides in antifouling paint formulations, certain textile products (e.g., carpets), hard surface disinfectants for fish hatcheries and poultry/swine farm facilities, construction materials including drywall, sterilants for nonfood processing equipment, agricultural pesticides and preservatives for wood and other materials (e.g., stone, leather, and paper protection) (Gajda and Jancsó, 2015; Rosenberg, 2013; RPA, 2005; US EPA, 2008). These compounds can enter aquatic systems directly through leaching from the hulls of vessels covered by OTCs-based antifouling paints, runoff from agricultural fields, and wastewater effluent discharges, leading to concentrations in water and sediments that can be toxic to aquatic biota (Hoch, 2001; Sousa et al., 2017; Zhang et al., 2021). TBT and TPhT are lipophilic and highly toxic synthetic organometallic chemicals that induce a variety of adverse effects on non-target aquatic organisms even at very low concentrations (Meador, 2011). Moreover, studies have shown that OTCs can be accumulated in fish, crustaceans, and mollusks (Chung and Wu, 2017; Jadhav et al., 2011), which may impact human health due to the ingestion of seafood (Chung et al., 2020; Mattos et al., 2017). Thus, due to their toxicity, persistence, and accumulation in aquatic organisms, the use of OTCs-based antifouling paints on vessels was banned worldwide in September 2008 by the International Convention on the Control of Harmful Antifouling Systems on Ships (AFS Convention) of the International Maritime Organization (IMO). Furthermore, the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade (Rotterdam Convention) restricted the international trade of TBT compounds as pesticides in February 2009, and as industrial chemicals in September 2017 (UNEP, 2019). Despite these international restrictions, TBT-based antifouling products are still being manufactured in the United States (US) and marketed (in some cases illegally) in several countries in Latin America and the Caribbean (LAC), including Mexico (Uc-Peraza et al., 2022a).

Currently, Mexico is a member state of both the AFS and Rotterdam Conventions, and OTCs-based antifouling paints and TBT compounds as pesticides are restricted in this country by the General Health Law and regulated by the Federal Commission for the Protection Against Sanitary Risk (COFEPRIS) and the Ministry of Environment and Natural Resources (SEMARNAT) (DOF, 2014). Unfortunately, these restrictions are not fully applied and/or complied with in Mexico. Uc-Peraza et al. (2022a) reported that imported TBT-based antifouling products are still offered for sale by national companies based in the Yucatán Peninsula. Meanwhile, TPhT compounds (e.g., triphenyltin acetate - TPhTAc) are also used for agricultural purposes in Mexico (Bejarano, 2017).

Recent studies have reported the occurrence of OTCs in muscle tissues of fish and other seafood from several countries. For instance, Chung et al. (2020) reported OTCs, mostly TPhT, in fish, crustaceans, and mollusks collected in the Hong Kong market. Chen et al. (2019) found high TPhT and moderate TBT levels in commercial and wild oysters from China. Similarly, Mukhtar et al. (2020) in Malaysia and Ashraf et al. (2017) in Saudi Arabia have found phenyltins (PhTs) in seafood species at concentrations higher than butyltins (BTs). In contrast, Harino and Yamato (2021) determined that organotin levels in fish were lower than in gastropods and oysters from Japan, which were more contaminated by TBT. Lastly, Mattos et al. (2017) found high levels of TBT in tissues of an edible gastropod from sites in Chile under the influence of maritime traffic, indicating a potential risk related to TBT intake through seafood.

Sediments and gastropods from coastal areas of the Yucatán Peninsula have been reported to be contaminated by organotins, such as butyltins and phenyltins (Uc-Peraza et al., 2022b). In addition, high imposex incidence has also been detected in five marine gastropod species along the coastal zone. However, investigation of the occurrence of OTCs in marine organisms, in particular edible species, is scarce in Mexico (Uc-Peraza et al., 2021a). Based on this scenario, the present study assessed organotin contamination (TBT, TPhT, and their degradation products) in tissue samples of commercially important seafood species (e.g., fish and shellfish) of the Yucatán Peninsula and estimated their potential health risk for consumers.

2. Materials and methods

2.1. Sample collecting and processing

Sampling was performed in the Yucatán Peninsula (Mexico) during February and March 2018. According to the National Commission of Aquaculture and Fishing (CONAPESCA) of the Mexican Federal Government, all seafood samples considered in the present study are widely consumed by the Mexican population (Table S1). Fish and shellfish samples were obtained at five fishing sites (SS1 - Playa El Niño (21°11′33.70″ N/86°48′21.57″ W); SS2 - Yucalpetén (21°16′36.13″ N/89°41′10.92″ W); SS3 - Celestún (20°51′6.13″ N/90°24′1.73″ W); SS4 - Champotón (19°21′23.93″ N/90°43′32.78″ W); SS5 - Ciudad del Carmen (18°37′54.62″N/91°49′23.94″W)) directly from local fishermen immediately after arrival (Fig. 1). Samples included demersal and pelagic fish, gastropods, oysters, and shrimps (Table S1). They were identified, wrapped in pre-processed aluminum foil (450 °C for 8 h), placed in individual plastic bags (tin-free polyethylene), labeled, and kept cold until reaching the laboratory. In the lab, samples were taxonomically confirmed to the species level, measured (e.g., biometric parameters such as weight and length in fish and shrimps, or shell length and height in mollusks, see Table S1), and dissected to separate the edible tissue (i.e., the dorsal muscle tissue in fish, tail and carapace-free muscle tissue in shrimps and all soft tissue of oysters and gastropods).

For organotins analysis, tissue samples were freeze-dried, homogenized, and stored at −20 °C until analysis.

2.2. Total lipids and moisture content in biota tissues

Lipids (expressed as a percentage of total lipids in dry weight, %L) were analyzed in homogenized freeze-dried tissue using chloroform/methanol (2:1, v/v) and were gravimetrically determined as described by Svensson and Förlin (2004). For the determination of moisture content, fresh tissue samples were weighed before and after being oven-dried (105 °C) until constant weight, and results were expressed as a percentage of wet weight (w.w., %M).

2.3. Organotin analysis

Butyltins (TBT, DBT, and MBT) and phenyltins (TPhT, DPhT, and MPhT) were analyzed in seafood samples according to Uc-Peraza et al. (2022b). Briefly, 0.5 g of homogenized freeze-dried tissue was weighed in a 40 mL glass vial and spiked with a surrogate standard (100 ng of tripropyltin - TPrT). After 30 min, 9 mL of tropolone solution in methanol (0.03%, w/v) and 1 mL of glacial acetic acid were added. Samples were vortex mixed for 1 min, ultrasonicated for 30 min (40 kHz, 132 W, 30 °C) and, finally, left in the dark for equilibration (30 min). The supernatants (7 mL) were collected in a 15 mL glass tube with 100 mg of PSA (Primary Secondary Amine) silica, mixed on a vortex for 1 min and, subsequently, centrifuged (3000 rpm) for 10 min. The supernatants (5 mL) were derivatized by adding 0.1 mL sodium tetraethyl borate ($NaEt_4B$) in water and 1 mL of sodium acetate/acetate acid buffer (pH = 4.5), and ultrasonicated for 10 min (40 kHz, 132 W, 30 °C). After derivatization, the ethylated BTs and PhTs were liquid-liquid extracted with hexane (5 mL). Extracts were cleaned up in an activated silica (2.5 g) column. BTs were eluted with 10 mL of hexane, while the PhTs were eluted with another 10 mL of hexane/toluene (2:1, v/v). Extracts were finally concentrated down to 200 μL (N_2 flow) and tetrabutyltin was added as an internal standard (100 μL of 1000 ng Sn mL^{-1}). Extracts (2 μL) were analyzed in a PerkinElmer Clarus 500MS gas chromatograph equipped with a mass spectrometer detector (GC/MS) and an Elite-5MS (5% diphenyl dimethylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 μm).

Quality assurance and quality control (QA&QC) were based on regular analyses of blanks, spiked matrices, and certified reference material ERM-CE477 (mussel tissue). Results obtained from the ERM-CE477 were 82 ± 4.0% for TBT, 75 ± 5.5% for DBT and 95 ± 9.6% for MBT (n = 6). Recoveries for the surrogate standards varied between 89% and 108%, while RSD was below 20%. Limits of detection (LOD) for TBT, DBT, MBT, TPhT, DPhT, and MPhT in seafood samples were ≤0.7 ng Sn g^{-1}. Limits of quantification (LOQ) were ≤1.3 ng Sn g^{-1} (DBT, MBT, and DPhT) and ≤3.3 ng Sn g^{-1} (TBT, TPhT, and MPhT). All concentrations were reported as ng Sn g^{-1} in dry weight (d.w.).

2.4. Butyltin and phenyltin degradation index

To determine the predominance of TBT or TPhT over their degradation products, and thus the occurrence of recent TBT or TPhT inputs into the environment, the degradation index of butyltins (BDI) and phenyltins (PhDI) were calculated for fish and shellfish samples based on the following equations: BDI = (MBT + DBT)/TBT and PhDI = (MPhT + DPhT)/TPhT, as originally proposed by Díez et al. (2002) for sediments and later used in marine organisms (Ho et al., 2016; Kim et al., 2008; Sousa et al., 2009). BDI or PhDI values > 1 may suggest legacy inputs of TBT or TPhT, respectively, while those <1 could indicate recent inputs (Díez et al., 2002).

2.5. Human health risk assessment

The estimated daily intake (EDI), maximum safe seafood

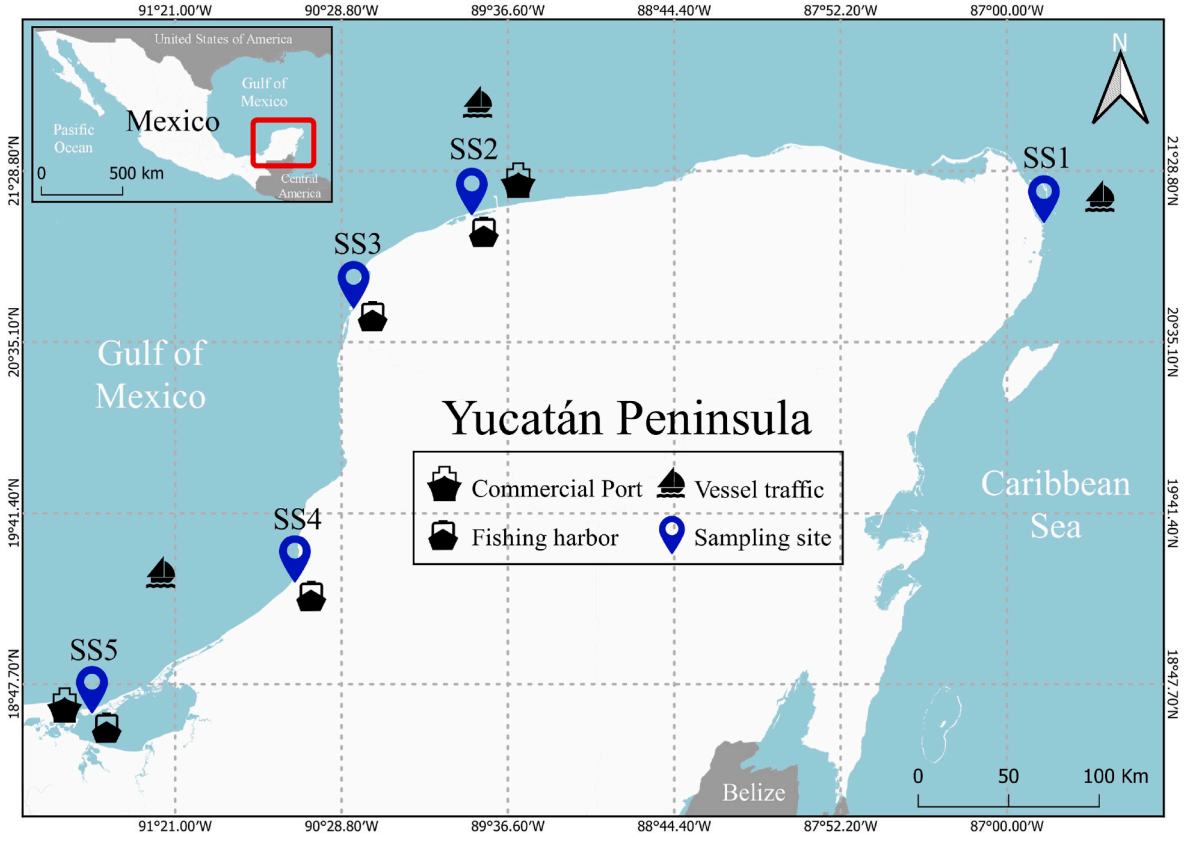

Fig. 1. Sampling sites of fish and shellfish in the Yucatán Peninsula, Mexico.

consumption (MSSC), hazard quotient (HQ), and hazard index (HI) were calculated to determine the potential human health risk associated with organotin contamination of seafood from the Yucatán Peninsula.

2.5.1. Estimated daily intake of organotins

The estimated daily intake (EDI) of organotins through seafood consumption (e.g., fish, mollusks and crustaceans) was determined for adults using the following equation (Uc-Peraza et al., 2021b; US EPA, 2000):

$$\text{EDI (ng Sn kg}^{-1}\text{ body weight day}^{-1}\text{ wet weight)} = \text{(C x IR)/BW.}$$

where C is the sum of organotin concentrations (e.g., ΣOTCs or ΣTBT, DBT, TPhT) in each seafood species (ng Sn g^{-1} in w.w.). IR is the daily uptake rate (g day^{-1}) and BW refers to the average body weight (kg). The present calculations were done assuming an average body weight of 70 kg for Mexican adults (both genders) (Uc-Peraza et al., 2021b). The daily uptake rate for fish, mollusks and crustaceans was obtained from the estimate for the general population (CONAPESCA, 2018) and fishing communities in Mexico (Ramírez-Ayala et al., 2021; Romo-Piñera et al., 2018; Zamora-Arellano et al., 2017) (Table S2). Thus, considering IR values, three consumption scenarios were established: low (e.g., national average), medium and high consumption (e.g., fishing communities). The tolerable daily intake (TDI) established by EFSA (2004) for the sum of TBT, DBT, and TPhT (100 ng Sn kg^{-1} body weight day^{-1}) was used to evaluate whether the EDI values obtained here exceeded the health guidance threshold.

2.5.2. Maximum safe seafood consumption

Although seafood is an excellent source of nutrients for humans, the organotin content (on an Sn basis) in the edible parts can cause health problems. In this case, the quantity of seafood that may be consumed safely must be such that the organotin levels consumed do not exceed the Provisional Tolerable Weekly Intake (PTWI) value established by EFSA (2004) for the sum of TBT, DBT, and TPhT. The maximum safe seafood consumption (MSSC) of each seafood category was calculated for adult men, adult women, and children, using the following equation (Uc-Peraza et al., 2021b; US EPA, 2000):

$$\text{MSSC (g week}^{-1}\text{ wet weight)} = \text{(PTWI x BW)/C}$$

where PTWI is 720 ng Sn kg^{-1} body weight $week^{-1}$, C is the highest concentration of organotins (sum of TBT, DBT, and TPhT, ng Sn g^{-1} w.w.) of each seafood category (e.g., fish, gastropods, oysters, and shrimps), and BW is the average body weight (kg) of the consumer. In this case, body weights of 70, 60, and 16 kg were assumed for adult men, adult women and children, respectively (Romo-Piñera et al., 2018; Uc-Peraza et al., 2021b).

2.5.3. Hazard quotient and hazard index

A non-carcinogenic hazard quotient (HQ) was used to determine the health risk of TBT and TPhT in adults (both genders) and was calculated using the following equation (Ho and Leung, 2014):

$$\text{HQ} = \text{[(CF x IR x EF x ED)/(BW x AT)]/RfD}$$

where CF is the concentration of organotins in the samples (ng g^{-1} w.w.), IR is the seafood ingestion rate (g d^{-1}) (Table S2), EF is the exposure frequency (365 days $year^{-1}$), ED is the exposure duration (70 years), BW is the average body weight (70 kg, both genders), AT is the average exposure time for non-carcinogens (365 days $year^{-1}$ x 70 years = 25,550 days), and RfD is the oral reference dose (ng kg^{-1} body weight d^{-1}). RfD for TBT, TBT + DBT and TPhT is 250 (US EPA, 1997), 250 (Penninks, 1993) and 500 ng kg^{-1} body weight d^{-1} (WHO, 1992), respectively.

The Hazard index (HI) is the sum of all individual HQs (e.g., $HQ_{TBT + DBT}$ and HQ_{TPhT}), which has been used to assess the overall potential for non-carcinogenic effects posed by more than one chemical (Lee et al., 2005; Mukhtar et al., 2020). If an HQ value or an HI value is lower than one, means that no adverse health risks are expected. HQ and HI values were estimated considering the three seafood consumption scenarios (low, medium and high) (Table S2).

2.6. Statistical analysis

Data were analyzed (biometric parameters, % moisture, % lipids and organotin concentrations) using one-way analysis of variance (ANOVA) coupled with a post hoc assay (Tukey) to infer the differences among seafood species. Data normality and homogeneity were verified by Shapiro-Wilk and Levene tests, respectively, and when data did not meet the assumptions (normality and homogeneity) of ANOVA, non-parametric Kruskal Wallis followed by Dunn's tests were performed. Spearman's correlation coefficients were estimated to assess the relationship between biometric parameters, % moisture, % lipids and organotin concentrations. All tests were performed using R (R Core Team, 2021) with $\alpha = 0.05$.

3. Results and discussion

3.1. Total lipids and moisture content

The percentage of moisture content and total lipids in the analyzed seafood samples are shown in Table 1. The moisture content ranged from 66.6 ± 1.7% (*Euthynnus alletteratus*) to 83.5 ± 3.6% (*Haemulon plumierii*) in fish, from 71.5 ± 0.6% (*Melongena bispinosa*) to 74.5 ± 0.5% (*Strombus pugilis*) in gastropods, and was 83.7 ± 0.2% (*Crassostrea virginica*) in oysters and 80.0 ± 0.5% (*Penaeus duorarum*) in shrimps. Regarding lipids, levels ranged from 2.0 ± 0.7% (*Haemulon plumierii*) to 9.4 ± 2.1% (*Euthynnus alletteratus*) in fish, from 3.2 ± 0.4% (*Strombus pugilis*) to 4.1 ± 0.3% (*Melongena bispinosa*) in gastropods, and was 4.5 ± 0.0% (*Crassostrea virginica*) in oysters and 2.6 ± 0.0% (*Penaeus duorarum*) in shrimps. Although no significant differences were seen between fish and shellfish ($p > 0.05$), lipid contents were significantly higher in pelagic than demersal fish species ($p < 0.001$). Similar results were observed by Ball et al. (2007), where pelagic fish (e.g., Clupeidae, Osmeridae) showed higher lipid contents than demersal fish (e.g., Gobiidae, Cottidae). Overall, total lipid and moisture levels are in agreement with those found in the literature for fish (Kristensen and Andersen, 1987; Mathew et al., 1999; Salgado-Ramírez et al., 2017; Sidwell et al., 1974) and shellfish (Aldana-Aranda and Colas-Marrufo, 1999; Belisle and Stickle, 1978; Dabrowski et al., 1969; Mathew et al., 1999; Zhu et al., 2018) species, which may vary according to the season, water temperature, geographical area, diet, size, sex, age, and reproductive cycle stage (Leu et al., 1981; Nurnadia et al., 2011).

3.2. Organotin concentration in seafood

Butyltin and phenyltin compounds were detected in all seafood samples analyzed, suggesting a widespread occurrence of organotins in the region. Mean concentrations of BTs and PhTs are shown in Table 1. Total butyltins (ΣBTs) and total phenyltins (ΣPhTs) ranged from 16.2 ± 3.1 to 71.1 ± 20.2 ng Sn g^{-1} and from 5.1 ± 1.7 to 164.7 ± 23.1 ng Sn g^{-1}, respectively, in seafood. PhTs were the most predominant compounds in 5 out of 7 fish species, while BTs were predominant in all shellfish (Fig. 2). Similar patterns were found in fish from Taiwan and Hong Kong, where PhTs levels were higher than those of BTs (Ho and Leung, 2014; Lee et al., 2005).

3.2.1. Fish samples

Among fish species, concentrations of TBT, DBT, and MBT varied from 9.0 ± 0.0 to 28.8 ± 9.4 ng Sn g^{-1}, 7.6 ± 0.2 to 21.7 ± 6.3 ng Sn g^{-1} and 6.0 ± 1.2 to 20.6 ± 5.5 ng Sn g^{-1}, respectively. For phenyltins, TPhT, DPhT, and MPhT varied from ≤0.7 to ≤3.3 ng Sn g^{-1}, 3.1 ± 1.8 to 33.1 ± 0.6 ng Sn g^{-1}, ≤0.7 to 129.9 ± 23.7 ng Sn g^{-1}, respectively. These levels revealed the bioaccumulation of BTs and PhTs in fish

Table 1
Percentage of moisture (%M) and total lipids (%L), butyltin (TBT, DBT and MBT) and phenyltin (TPhT, DPhT and MBT) levels (mean ± standard deviation, ng Sn g^{-1} dry weight), the sum of butyltins (ΣBTs) and phenyltins (ΣPhTs) (ng Sn g^{-1} dry weight), total organotins (ΣOTCs) (ng Sn g^{-1} dry weight), butyltin degradation index (BDI) and phenyltin degradation index (PhDI) in seafood samples from the Yucatán Peninsula, Mexico.

Species	N	%M	%L	Butyltins (mean ± standard deviation, ng Sn g^{-1})				Phenyltins (mean ± standard deviation, ng Sn g^{-1})				ΣOTCs[a] (ng Sn g^{-1})	BDI[a]	PhDI[a]
				TBT	DBT	MBT	ΣBTs[a]	TPhT	DPhT	MPhT	ΣPhTs[a]			
Fish														
Lutjanus synagris	12	78.3 ± 3.7	3.3 ± 0.6	12.9 ± 0.4	8.7 ± 2.8	9.9 ± 1.0	31.5 ± 3.9	≤3.3	15.7 ± 4.7	23.5 ± 6.0	40.8 ± 10.4	72.3 ± 6.5	1.4	23.7
Lutjanus campechanus	4	77.6 ± 0.2	2.8 ± 1.6	21.8 ± 10.9	19.5 ± 2.8	15.7 ± 1.7	57.0 ± 14.6	≤0.7	16.0 ± 0.0	119.6 ± 27.2	136.0 ± 27.0	192.9 ± 31.7	1.6	387.5
Calamus pennatula	5	73.0 ± 2.3	3.6 ± 2.3	28.8 ± 9.4	21.7 ± 6.3	20.6 ± 5.5	71.1 ± 20.2	≤3.3	33.1 ± 0.6	129.9 ± 23.7	164.7 ± 23.1	235.9 ± 40.5	1.5	98.8
Haemulon plumierii	4	83.5 ± 3.6	2.0 ± 0.7	17.0 ± 12.8	16.4 ± 5.3	13.1 ± 2.1	46.6 ± 18.5	≤3.3	17.2 ± 1.0	104.7 ± 39.2	123.6 ± 39.9	170.1 ± 48.9	1.7	121.9
Rhomboplites aurorubens	4	73.2 ± 1.5	3.9 ± 0.7	22.7 ± 17.2	21.1 ± 0.7	10.32 ± 0.4	54.1 ± 18.2	≤3.3	5.5 ± 0.3	≤0.7	7.5 ± 0.6	62.6 ± 18.7	1.4	3.5
Euthynnus alletteratus	4	66.6 ± 1.7	9.4 ± 2.1	24.6 ± 2.0	19.4 ± 3.1	11.3 ± 1.6	55.3 ± 4.7	≤3.3	5.2 ± 5.8	96.4 ± 12.3	103.3 ± 14.2	158.6 ± 12.1	1.2	61.6
Opisthonema oglinum	4	71.9 ± 3.7	4.7 ± 1.0	9.0 ± 0.0	7.6 ± 0.2	6.0 ± 1.2	22.6 ± 1.4	≤0.7	3.1 ± 1.8	≤3.3	5.1 ± 1.7	27.6 ± 2.2	1.5	13.5
Gastropod														
Melongena bispinosa	2 pools of 6	71.5 ± 0.6	4.1 ± 0.3	26.9 ± 0.1	17.7 ± 0.8	13.0 ± 1.4	57.6 ± 0.5	≤3.3	7.2 ± 1.2	32.0 ± 3.0	40.8 ± 4.2	98.4 ± 3.6	1.1	23.0
Strombus pugilis	2 pools of 7	74.5 ± 0.5	3.2 ± 0.4	9.1 ± 0.0	5.6 ± 0.7	2.7 ± 0.0	17.3 ± 0.7	≤3.3	4.2 ± 0.3	≤0.7	6.2 ± 0.3	23.5 ± 1.0	0.9	2.7
Oyster														
Crassostrea virginica	5 pools of 6	83.7 ± 0.2	4.5 ± 0.0	39.0 ± 0.1	8.9 ± 0.5	17.2 ± 1.1	65.1 ± 1.7	≤3.3	8.3 ± 0.0	≤3.3	11.6 ± 1.1	76.7 ± 2.6	0.7	6.0
Shrimp														
Penaeus duorarum	4 pools of 10	80.0 ± 0.5	2.6 ± 0.0	8.3 ± 0.7	5.7 ± 1.7	2.3 ± 0.6	16.2 ± 3.1	≤3.3	3.0 ± 1.2	7.9 ± 1.0	12.6 ± 1.2	28.8 ± 2.7	1.0	6.6

[a] To estimate ΣBTs, ΣPhTs, ΣOTCs, BDI and PhDI, half of LOD and LOQ values were used whenever results were < LOD or < LOQ; < LOD = ≤ 0.7 ng Sn g^{-1} (TBT, DBT, MBT, TPhT, DPhT, MPhT), < LOQ = ≤ 1.3 ng Sn g^{-1} (DBT, MBT, DPhT) and < LOQ = ≤ 3.3 (TBT, TPhT, MPhT).

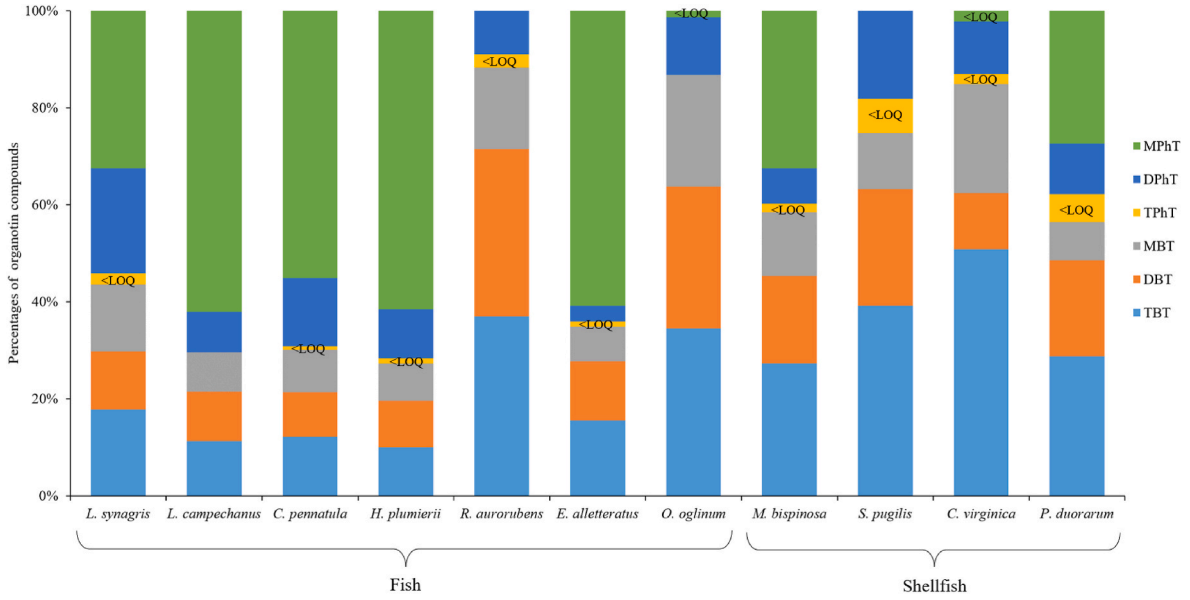

Fig. 2. Relative percentages of individual butyltin and phenyltin compounds in the edible tissue of fish, gastropod, oyster, and shrimp samples from the Yucatán Peninsula, Mexico. Levels below limit of quantification (<LOQ) were indicated.

muscle collected from the region. The highest values of ΣBTs and ΣPhTs were found in the demersal fish *C. pennatula* (71.1 ± 20.2 ng Sn g^{-1} and 164.7 ± 23.1 ng Sn g^{-1}, respectively) and *L. campechamus* (57.0 ± 14.6 ng Sn g^{-1} and 136.0 ± 27.0 ng Sn g^{-1}, respectively), while the lowest values were found in the pelagic fish *O. oglinum* (22.6 ± 1.4 ng Sn g^{-1} and 5.1 ± 1.7 ng Sn g^{-1}, respectively). The species *O. oglinum* is planktivorous and feeds on a variety of zooplankton (e.g., copepods), but also ingests small fish, crabs, and shrimps (Robertson and Van Tassell, 2019). *C. pennatula* is a carnivorous fish that lives over rocky areas or reefs, but also on soft bottoms, and feeds on crabs, shrimps, mollusks (bivalves and gastropods), mobile benthic worms, starfish, cucumbers, urchins, and hermit crabs (Robertson and Van Tassell, 2019). *L. campechanus* is a planktivorous and carnivorous fish that feeds on zooplankton, mollusks, crabs, shrimps, and other benthonic organisms, and adult species are associated with rocky bottoms (Robertson and Van Tassell, 2019). It is known that benthic organisms (e.g.,

bivalves and gastropods) accumulate contaminants (e.g., metals, organometals, and organic compounds) stored in sea-bottom sediments, resulting in high concentrations at top predators (Gray, 2002; Wang, 2002). Thus, the accumulation of organotins in fish species can be influenced by their diet. For instance, Lee et al. (2005) found that concentrations of PhTs in demersal fish were higher than in pelagic fish from Taiwan, while Ho and Leung (2014) reported the highest concentration of organotins in a demersal fish from Hong Kong.

Among butyltin residues, TBT was the predominant compound in all fish samples with a mean percentage of 40% of total butyltins, while DBT and MBT represented 34 and 26%, respectively (Fig. 2). Spearman correlation analysis showed significant positive correlations between TBT and DBT ($rho = 0.75$, $p < 0.001$), and TBT and MBT ($rho = 0.72$, $p < 0.001$), suggesting that butyltin metabolites were originated from tributyltin dealkylation. In addition, BDI levels in fish samples ranged from 1.2 to 1.7 (Table 1), suggesting that fish are capable of metabolizing TBT to less toxic metabolites (e.g., DBT and MBT) (Lee, 1985), and/or that legacy inputs probably exist in this region. However, the predominance of TBT in fish samples suggests that recent inputs of TBT have also occurred into the coastal environment, which is likely associated with the current use of TBT-based antifouling products. Uc-Peraza et al. (2022a) have shown that four TBT-based antifouling products (Island 44 Plus™ Harder, Tin Booster™, BioTin™, and ClearGear™) are offered for sale in México, and Uc-Peraza et al. (2022b) have found high butyltin levels in sediments and gastropod tissues at the Yucatán Peninsula.

For phenyltins, TPhT levels were below the LOD or LOQ, while its degradation products (DPhT or MPhT) predominated in most fish samples. MPhT was the dominant analyte in demersal fish samples (e.g., *L. campechanus*, *C. pennatula,* and *H. plumierii*), suggesting a relatively higher rate of dephenylation and/or direct uptake of this compound from the environment, as reported by Sham et al. (2021) in marine fish samples from Hong Kong waters. The PhDIs were > 3 in fish samples (Table 1), indicating that old TPhT inputs and/or fast degradation processes are taking place in the region. However, the lack of correlations ($p > 0.05$) between TPhT and its degradation products, while DPhT and MPhT concentrations were significantly correlated ($rho = 0.56$, $p < 0.001$), may indicate that DPhT and MPhT were derived not only from TPhT degradation but also from other sources, such as domestic wastewater, industrial effluents (e.g., refineries and textile companies), and urban and agricultural run-off (Dong et al., 2015; Lu et al., 2012; Yi et al., 2012).

Although the uptake and bioaccumulation of lipophilic compounds can be related to the lipid contents of biota tissues (Hoch, 2001), no significant Spearman correlations ($p > 0.05$) were found between organotins residues (e.g., TBT, TPhT, BTs, PhTs or OTCs) and %L in fish samples. The lack of relationship between OTCs and lipids has already been reported for marine fish (Harino et al., 2000; Sham et al., 2021), which may be explained by their higher affinity to proteins than to lipids (Antizar-Ladislao, 2008). The low lipid content in muscle tissue of some fish might have influenced the relationship as well.

When compared to other studies performed after the global OTCs ban (Table S3), BTs and PhTs levels were higher than those found in three marine fish species from the Gulf of Gdańsk (Baltic Sea), which presented average levels ranging from <1.2 to 19.8 ng Sn g^{-1} d.w. for BTs, and <1.0 ng Sn g^{-1} d.w. for PhTs (Filipkowska et al., 2016). BTs levels were also lower (6.8–21.5 ng Sn g^{-1} d.w.) in seven fish species of Tunisia (Abidli et al., 2016). However, higher organotin levels were detected in fish species of the Arabian Gulf (BTs - 137.2 to 228.4 ng Sn g^{-1} d.w.; PhTs - 203.6 to 281.9 ng Sn g^{-1} d.w.) (Ashraf et al., 2017), Taiwan (Lee et al., 2016) and Hong Kong (Ho and Leung, 2014). In general, these studies reveal that coastal fish species have been widely contaminated with organotins, which depends on the species, diet, physiology, season, geographical area, and degree of current use of tin-based products in different regions, as shown by Uc-Peraza et al. (2022a).

3.2.2. Shellfish samples

Concentrations of BTs and PhTs in gastropod, oyster, and shrimp samples ranged from 16.2 ± 3.1 to 65.1 ± 1.7 ng Sn g^{-1} and from 6.2 ± 0.3 to 40.8 ± 4.2 ng Sn g^{-1}, respectively (Table 1). PhTs levels were lower than BTs in all species. In gastropods, the highest BTs and PhTs levels were found in *M. bispinosa* (57.6 ± 0.5 ng Sn g^{-1} and 40.8 ± 4.2 ng Sn g^{-1}, respectively), while the lowest values were found in *S. pugilis* (17.3 ± 0.7 ng Sn g^{-1} and 6.2 ± 0.3 ng Sn g^{-1}, respectively). *S. pugilis* is a herbivorous gastropod that lives over sandy and muddy bottoms, and feeds on complex plants and macroalgae (Stoner and Waite, 1991), while *M. bispinosa* is a carnivorous species that lives in muddy habitats and preys on small bivalves (clams, mussels, and oysters) (Rosenberg et al., 2009). Chen et al. (2017) showed that different dietary patterns or habitats can result in different organotins bioaccumulation factors in seafood. Hence, the accumulation of organotins in gastropod species in the present study may have been influenced by their diet. In oyster (*Crassostrea virginica*), BTs and PhTs levels were 65.1 ± 1.7 ng Sn g^{-1} and 11.6 ± 1.1 ng Sn g^{-1}, respectively. The concentration of BTs in oysters was higher than in gastropod species, which could be explained by the fact that bivalves filter large amounts of seawater, potentially accumulating higher levels of organotins present in the water column and/or suspended materials (Ruiz et al., 2005). Abidli et al. (2016) also found higher BTs levels in bivalves than in gastropods from the Bizerte lagoon, Tunisia. Regarding lipids in mollusks, no significant differences were found among the four species. Despite the greater affinity of BTs for proteins than lipids (Antizar-Ladislao, 2008), lipids were significantly correlated with TBT ($rho = 0.92$, $p = < 0.05$) and BTs ($rho = 0.93$, $p < 0.05$). Similar results have also been observed for other mollusk species from Argentina (Del Brio et al., 2016) and Chile (Mattos et al., 2017).

To compare OTC levels, studies performed in shellfish (preferably the same genus or class) after their global ban by IMO (September 2008) were considered here. Concerning the gastropods, concentration of BTs in *M. bispinosa* and *S. pugilis* were lower than those registered in Argentina (*Odontocymbiola magellanica*) (Del Brio et al., 2016), the British Virgin Islands (*Strombus gigas*) (Titley-O'Neal et al., 2011) and Chile (*Thaisella chocolata*) (Mattos et al., 2017) (Table S3). However, BTs levels in the gastropod *M. bispinosa* and the oyster *C. virginica* were higher than those found in gastropods and bivalves from Tunisia (Abidli et al., 2016) and China (Chen et al., 2019). For PhTs, the present results were much lower than those registered in three gastropod species from Hong Kong (Ho and Leung, 2014). Nevertheless, higher concentrations of BTs and PhTs were found in cultivated oyster species from China (Chen et al., 2019). Concerning shrimps, concentration of BTs and PhTs in *P. duorarum* was 16.2 ± 3.1 ng Sn g^{-1} and 12.6 ± 1.2 ng Sn g^{-1}, respectively. These levels were lower than those registered for the gastropods and oysters, which is likely because shrimp present a high enzymatic capacity for metabolizing and excreting organotins (Abidli et al., 2016; Lee, 1985). These levels were also lower to those registered in shrimp species from Taiwan (Lee et al., 2016), but higher than those found in Tunisia (Abidli et al., 2016) and the North Sea (Verhaegen et al., 2012) (Table S3).

BDI and PhDI in shellfish samples ranged from 0.7 to 1.1 and from 2.7 to 23, respectively (Table 1). As seen for fish samples, TBT levels in gastropods, oysters, and shrimps were higher than their degradation products (Fig. 2). BDI values were equal or close to one in gastropods (*M. bispinosa* (1.1) and *S. pugilis* (0.9)) and shrimp (*P. duorarum* (1.0)), suggesting that a combination of recent and old inputs or a moderate degradation rate was probably taking place in the coastal environment. Nevertheless, a BDI of 0.7 in the oyster *C. virginica* (0.7) pointed to recent inputs and/or low degradation rates of TBT. Lee (1985) reported that *C. virginica* has a lower capacity to metabolize TBT than other organisms tested (e.g., fish and crabs), influencing its accumulation. Similar results were found by Mattos et al. (2017) and Abidli et al. (2016), who reported BDI values lower than 1 for gastropods and bivalves, respectively. Regarding PhDI, values were higher than one in all shellfish samples which, in combination with the relatively low TPhT concentration and

the predominance of its degradation products, suggests old inputs and/or that the degradation process has already occurred in the studied region. Differently, Ho and Leung (2014) reported PhDI values < 1 in shellfish species (gastropods and bivalves) from Hong Kong, indicating recent inputs of PhTs and/or low degradation rates of TPhT.

3.3. Human health risk assessment from consuming fish, mollusks, and crustaceans

The estimated daily intake (EDI) for the sum of all organotins (ΣOTCs) and for the organotins (ΣTBT, DBT, and TPhT) considered by the European Food Safety Authority (EFSA, 2004) in three exposure scenarios are shown in Table 2. Considering the results for ΣTBT, DBT, and TPhT, the EDI values varied between 0.2 and 4.7, 0.7 to 22, and 2.0–65 ng Sn kg^{-1} body weight day^{-1} for the low, medium, and high seafood consumption estimates, respectively. These values are lower than the EDI values registered for food samples in Portugal (Sousa et al., 2017); however, the medium and high fish consumption estimates are higher than those reported for fish samples from the Arabian Gulf (Ashraf et al., 2017) and Taiwan (Lee et al., 2016).

Considering the three exposure scenarios, the highest EDI values of ΣOTCs and ΣTBT, DBT, and TPhT were registered for the fish *C. pennatula* and *E. alletteratus*, while the lowest values were found for the gastropod *S. pugilis* and shrimp *P. duorarum* (Table 2). Overall, fish samples were the major contributors to organotin intake, since the average EDI values of ΣOTCs and ΣTBT, DBT, TPhT for fish in low, medium, and high consumption estimates were higher (10.1 and 2.9, 47 and 13.7, and 139.5 and 40.7 ng Sn kg^{-1} body weight day^{-1}, respectively) than those for shellfish (0.7 and 0.4, 2.5 and 1.4, and 6.8 and 3.7 ng Sn kg^{-1} body weight day^{-1}, respectively).

Regarding ΣTBT, DBT, and TPhT in the three consumption scenarios (low, medium and high), average EDI values through the consumption of fish (2.9, 13.7 and 40.7 ng Sn kg^{-1} body weight day^{-1}, respectively), mollusks (0.4, 1.3 and 4.0 ng Sn kg^{-1} body weight day^{-1}, respectively), and crustaceans (0.3, 1.5 and 2.9 ng Sn kg^{-1} body weight day^{-1}, respectively) were lower than the Tolerable Daily Intake (TDI) value (100 ng Sn kg^{-1} body weight day^{-1}) established by the European Food Safety Authority (EFSA, 2004). Additionally, low EDI values, below the TDI threshold limit set by the EFSA, were also seen for Finnish consumers (3.2 ng Sn kg^{-1} body weight day^{-1} of fish) (Airaksinen et al., 2010), Arab consumers (8.1 ng Sn kg^{-1} body weight day^{-1} of fish) (Ashraf et al., 2017) and high seafood French consumers of fish, mollusks, and crustacean (Guérin et al., 2007).

In addition, to establish a safe human consumption limit for the Yucatán Peninsula considering OTCs levels in the analyzed species, the maximum safe seafood consumption (MSSC) was calculated for threes age groups (men, women, and children) using the highest average concentration of OTCs (sum of TBT, DBT, and TPhT) detected in the seafood samples for the worst-case scenario. For adult men (~70 kg), the MSSC that could be consumed per week was 3.6 kg $week^{-1}$ of fish or 3.8 kg $week^{-1}$ of gastropods or 6.2 kg $week^{-1}$ for oysters or 16.2 kg $week^{-1}$ for shrimps, or a combination considering the relative contribution of each type of seafood. For adult women (~60 kg), the MSSC was 3.1 kg $week^{-1}$ of fish or 3.3 kg $week^{-1}$ of gastropods or 5.4 kg $week^{-1}$ of oysters or 13.9 kg $week^{-1}$ of shrimps, or their combination, while the MSSC for children (~16 kg) was 0.8 kg $week^{-1}$ of fish or 0.9 kg $week^{-1}$ of gastropods or 1.4 kg $week^{-1}$ of oysters or 3.7 kg $week^{-1}$ of shrimps, or their combination. These MSSC values were above the per capita national consumption rate of 7.9 kg yr^{-1} (0.15 kg $week^{-1}$) of fish, 1.2 kg yr^{-1} (0.02 kg $week^{-1}$) of mollusks, and 2.3 kg yr^{-1} (0.04 kg $week^{-1}$) of crustaceans for the Mexican population (CONAPESCA, 2018) (Table S2). Moreover, MSSC values of mollusks and crustaceans for adults were higher than the medium (0.08 and 0.23 kg $week^{-1}$, respectively) and high (0.23 and 0.46 kg $week^{-1}$, respectively) consumption rates of the adult population in Mexico (Table S2). However, the high average fish consumption rates reported for adults (300 g day^{-1} or 2.1 kg $week^{-1}$) (Ramírez-Ayala et al., 2021) and children (105 g day^{-1} or 0.7 kg $week^{-1}$) for coastal fishing communities in Mexico (Zamora-Arellano et al., 2018) were close to the MSSC values of adults and children.

Estimated hazard quotients (HQs) of TBT, TBT + DBT, and TPhT and hazard indices (HIs) of TBT + DBT + TPhT in seafood for the three exposure scenarios are shown in Table S4. Values of both hazard parameters were lower than one for the consumption of fish, mollusks, and crustaceans from the Yucatán Peninsula in all scenarios. Thus, the daily exposure to these concentrations of TBT, DBT, and TPhT is unlikely to cause any harmful effects during the lifetime of the seafood consumers from the Yucatán Peninsula. Other studies found HQ and HI values lower than one, suggesting no risk for human health associated with the intake of fish and shellfish (Abidli et al., 2016; Ho and Leung, 2014; Lee et al., 2005). Nevertheless, coastal populations with predominantly seafood-based diets can have consumption rates above the national average. Consumption rates as high as 471 g day^{-1} were reported for

Table 2
Estimated daily intake (EDI, ng Sn kg^{-1} body weight day^{-1}) for the sum of all organotins (ΣOTCs) and the sum of TBT, DBT, and TPhT (ΣTBT, DBT, TPhT) from three consumption scenarios (low (e.g., national average), medium and high consumption (e.g., fishing communities)).

Species	ΣOTCs (ng Sn g^{-1} w. w.)	ΣTBT, DBT, TPhT (ng Sn g^{-1} w. w.)	EDI (ng Sn kg^{-1} bw day^{-1})[a]					
			Low consumption		Medium consumption		High consumption	
			ΣOTCs	ΣTBT, DBT, TPhT	ΣOTCs	ΣTBT, DBT, TPhT	ΣOTCs	ΣTBT, DBT, TPhT
Fish								
Lutjanus synagris	15.7	5.0	4.9	1.6	22.6	7.3	67.2	21.6
Lutjanus campechanus	43.2	9.3	13.4	2.9	62.3	13.5	185.1	40.0
Calamus pennatula	63.6	14.1	19.8	4.4	91.8	20.3	272.6	60.3
Haemulon plumierii	28.1	5.8	8.7	1.8	40.6	8.4	120.5	24.9
Rhomboplites aurorubens	16.5	12.2	5.1	3.8	23.8	17.6	70.7	52.2
Euthynnus alletteratus	53.0	15.3	16.5	4.7	76.4	22.0	226.9	65.4
Opisthonema oglinum	7.7	4.7	2.4	1.5	11.2	6.8	33.2	20.3
Average for fish	**32.5**	**9.5**	**10.1**	**2.9**	**47.0**	**13.7**	**139.5**	**40.7**
Mollusks								
Melongena bispinosa	28.1	13.2	1.3	0.6	4.4	2.1	13.2	6.2
Strombus pugilis	6.0	4.2	0.3	0.2	0.9	0.7	2.8	2.0
Crassostrea virginica	12.5	8.1	0.6	0.4	2.0	1.3	5.9	3.8
Crustaceans								
Penaeus duorarum	5.8	3.1	0.5	0.3	2.7	1.5	5.4	2.9
Average for shellfish	**13.1**	**7.1**	**0.7**	**0.4**	**2.5**	**1.4**	**6.8**	**3.7**

[a] Calculated for an adult of 70 kg; w.w. - wet weight; bw – body weight.

women of a fishing community of Sonora (García-Hernández et al., 2018). Such a high rate of seafood ingestion could pose a potential risk to human health, especially if a realistic scenario of multi-contaminants in seafood, and their additive or synergistic effects, is taken into account.

4. Conclusions

The present study showed organotin contamination in different seafood species from the Yucatán Peninsula. PhTs, especially MPhT, were the predominant compounds among fish species, while BTs, especially TBT, were among shellfish. Regarding the toxic OTCs, TBT followed by DBT were the predominant compounds in all seafood species, while TPhT was below the limit of quantification in most samples. BDI $\leq$1 for shellfish suggested possible recent inputs of TBT in the coastal environment. Among seafood species, fish were the main contributors to the estimated daily intake (EDI) of OTCs. Even so, the EDI of OTCs through seafood consumption was below the tolerable daily intake (TDI) established by the European Food Safety Authority (EFSA). In addition, HQ and HI values were lower than one, suggesting that the consumption of seafood does not pose any risk to the health of seafood consumers in the investigated region. However, given the increasing coastal urbanization, maritime activities, and the likely illegal use of OTCs along the coastal zones of Mexico, monitoring of organotin residues should not only continue, but also be extended to other areas along the coast. To ensure the safety of the Mexican population, different species of seafood and populations with different profiles and rates of seafood consumption must also be appraised.

Credit author statement

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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Research Paper

Mexican paradise under threat: The impact of antifouling biocides along the Yucatán Peninsula

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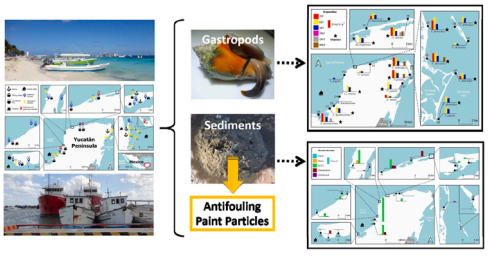
HIGHLIGHTS

- First comprehensive study of antifouling residues and biological effects in the Yucantán Peninsula.
- Imposex and organotins were reported for the first time in gastropods from the Yucatán Peninsula.
- Marinas, shipyards and fishing harbors are relevant sources of antifouling biocides.
- TBT, Irgarol, diuron and DCOIT levels exceeded threshold limits set by international guidelines.
- Antifouling residues are a relevant environment issue in the coastal areas of the Yucantán Peninsula.

GRAPHICAL ABSTRACT



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ABSTRACT

Levels of booster biocides (Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT), organotins (TBT, DBT, MBT, TPhT, DPhT and MPhT) and antifouling paint particles (APPs) were assessed in sediments of sites under the influence of maritime activities along the coastal zone of the Yucatán Peninsula, Mexico. Imposex incidence and organotin levels were also evaluated in seven caenogastropod species. The incidence of imposex was detected in five species from sites nearby fishing harbors and marinas, including the first reports to *Gemophos tinctus* and *Melongena bispinosa*. Butyltins levels were higher than phenyltins in gastropod tissues, sediments, and APPs. Regarding booster biocides, chlorothalonil was the most frequently detected compound and DCOIT was the most abundant biocide in sediments. DCOIT levels were registered in APPs from fishing harbors and marina areas. In addition, the highest levels of TBT, Irgarol, diuron and DCOIT exceeded the threshold limits set by international sediment quality guidelines, indicating that toxic effects could be expected in some of the studied areas, thus

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being a potential threat to marine life. Based on such outputs, Mexico urgently needs to adopt restrictive actions aiming at conserving the rich biological heritage of the Yucatán Peninsula.

1. Introduction

Antifouling paints based on toxic compounds or elements have been historically used to protect ship and boat hulls from the biofouling attack. Organotin compounds (OTCs), such as tributyltin (TBT) and triphenyltin (TPhT), were extensively used as active ingredients in antifouling paints since the 1960 s (Castro et al., 2012b; Lam et al., 2017). In 2004, estimates pointed out that TBT-based antifouling paints were applied in about 70% to 80% of the world's fleet (Kotrikla, 2009). Thus, OTCs have been largely released to the aquatic environment, resulting in high levels of parental and metabolite compounds in water, sediment and biota from coastal zones under the influence of ship traffic (Okoro et al., 2011). OTCs are highly toxic to non-target aquatic life, and are also known as endocrine disruptor chemicals (EDC) that induce imposex, which is characterized by the development of male morphological features, penis and/or vans deferens, in marine gastropod females (Meador, 2011). This syndrome has been reported for more than 260 species of gastropods worldwide (Titley-O'Neal et al., 2011), and it is used as a biomarker in OTCs biomonitoring studies (Cacciatore et al., 2018). In addition, several studies have reported deleterious effects of OTCs on aquatic organisms, such as shell malformation in bivalves and gastropods (Alzieu, 2000; Márquez et al., 2011), genotoxic damage in mussels (Hagger et al., 2005), obesogenic metabolic syndrome and immunotoxicity in fish (Meador, 2011; O'Halloran et al., 1998) and hormonal imbalance in aquatic mammals (Choi et al., 2011). Such evidence triggered subsequent restrictions to OTCs-based antifouling paints since the early 1980 s in many countries. Later, these compounds were globally banned from these paints in 2008 by the International Marine Organization (IMO), through the Convention on the Control of Harmful Antifouling Systems on Ships (AFS Convention) (IMO, 2021). Despite this global ban, TBT-based antifouling paints are still being manufactured in the United States and offered for sale in several countries, including Mexico (Turner and Glegg, 2014; Uc-Peraza et al., 2022).

As an alternative, manufacturers have developed tin-free paint formulations based on copper (e.g., cuprous oxide and copper thiocyanate) and/or zinc (e.g., zinc oxide and zinc acrylate) compounds in combination with one or more co-biocides to enhance their effectiveness (Paz-Villarraga et al., 2021). These biocides are known as "booster biocides", and include non-metallic organic compounds and organometallic compounds (Martins et al., 2017; Thomas and Brooks, 2010). However, the use of these biocides in antifouling systems have also led to contamination of the aquatic environment (Kim et al., 2015). Indeed, Irgarol, diuron, DCOIT, chlorothalonil, and dichlofluanid have been frequently detected at high concentrations in water and sediment from harbors, shipyards, marinas, and navigation channels, where boat activities are intense (Abreu et al., 2020; Konstantinou and Albanis, 2004; Lam et al., 2017; Lee et al., 2015). Although some booster biocides are rapidly degraded in the water column, they can accumulate in surface sediments and, in addition, be present and preserved in antifouling paint particles (APPs) (Konstantinou and Albanis, 2004). APPs are released into the aquatic environment and, consequently, accumulated in sediments during repair, cleaning and painting procedures on vessel hulls in marinas and ship/boatyards (Abreu et al., 2020; Turner, 2010). In this regard, studies have shown that APPs are toxic residues and may act as significant long-term secondary sources of metals and biocides to aquatic environments (Abreu et al., 2020; Soroldoni et al., 2020).

Mexico is the second largest economy in Latin America and the fifteenth largest economy in the world (World Bank, 2021). Maritime traffic has been increasing along the coastlines, particularly in fisheries and tourist regions (i.e., Yucatán Peninsula) (Rivera-Arriaga and Villalobos, 2001). For example, the Mexican Caribbean is the main tourist destination in the country and this activity has increased rapidly in recent years, receiving over 400,000 visitor per year since 2014 (González-De Zayas et al., 2020). Currently, the national port system consists of 117 active ports and terminals: 58 in the Pacific, and 59 in the Gulf of Mexico-Caribbean Sea region (Zepeda-Ortega et al., 2017). Notably, a significant number of marinas, fishing harbors and international ports (receiving passenger vessels and cruise ships) lead to intense maritime traffic along the Yucatán Peninsula coastline. Moreover, Mexico is a state member of IMO since 1954 and it is one of the few signatories countries in Latin American and the Caribbean (LAC) that already ratified the AFS Convention (IMO, 2021).

Several studies have assessed organotin compounds and booster biocides in coastal areas with intense marine traffic worldwide (Abreu et al., 2020; Batista-Andrade et al., 2018; Lam et al., 2017; Mukhtar et al., 2019; Romanelli et al., 2019). Despite high ship traffic along the Yucatán Peninsula, the levels and impacts of antifouling residues have not been assessed in the region (Uc-Peraza et al., 2021). Thus, the present study assessed sedimentary levels of organotins (TBT, TPhT and their degradation products) and booster biocides (Irgarol, diuron, clorotalonil, DCOIT and dichlofluanid) seeking to elucidate the present status of contamination by antifouling biocides in the Yucatán Peninsula. In addition, imposex incidence and organotin levels were also assessed in gastropods as proxies of biological damages related to TBT exposure.

2. Materials and methods

2.1. Study area

The Yucatán Peninsula, which includes the states of Campeche, Yucatán and Quintana Roo, is in the southeast of Mexico between the Gulf of Mexico and the Caribbean Sea (Fig. 1). It has a total surface area of approximately 145,000 km^2 with about 1500 km of coastline (Cinco-Castro and Herrera-Silveira, 2020). Its coastal and marine zones are the most important environments to the economic development in the country. Hence, the main economic activities in this area are focused on tourism, fisheries, offshore oil and gas extraction, maritime transportation and port operations (Herrera-Silveira et al., 2004). Therefore, this region has intense ship and boat traffic along its shoreline. In Quintana Roo state, the main economic activity is coastal tourism, with many recreational boats in operation, while in Yucatán state fisheries and port are the most relevant naval operations. Finally, the most important coastal activities in Campeche state are related to fisheries and offshore oil and gas extraction. In addition, other relevant economic activities distributed in the Yucatán Peninsula include those related to agricultural, hospitality services and commercial activities.

2.2. Sampling

Sampling of gastropod mollusks and surface sediments was carried out during February and March of 2018 (Fig. 1 and Table S1). Surface sediments (upper 2 cm) were collected in triplicate using a stainless steel "Van-veen" dredge at 19 sites (Q8, Q9, Y1, Y2, Y3, Y4, Y5, Y8, Y9, Y11, Y12, C1, C2, C3, C4, C5, C8, C10 and C11), while gastropod specimens were collected manually at 17 sites (Q1, Q2, Q3, Q4, Q5, Q6, Q7, Q8, Y1, Y6, Y7, Y8, Y10, C4, C6, C7 and C9) from the intertidal zone during low tide (Fig. 1). Gastropod samples were transferred alive to the laboratory, identified and narcotized using a 3.5% solution of $MgCl_2$ in 1:1 distilled water and local seawater. After approximately 2 h, shell lengths were measured using a calliper (to the nearest 0.1 mm) and soft tissues were removed using a bench vice. Then, each specimen was sexed under a

stereomicroscope, and the imposex parameters in females were evaluated. Finally, all samples were freeze-dried, homogenized, and stored at − 20 °C for posterior chemical analysis.

2.3. Imposex characterization

Imposex was evaluated in seven gastropods species belonging to the families Muricidae (*Stramonita haemastoma*, *Plicopurpura patula*, *Vasula deltoidea*), Fasciolariidae (*Leucozonia nassa*), Pisaniidae (*Gemophos tinctus*), Strombidae (*Strombus pugilis*) and Melongenidae (*Melongena bispinosa*), according to Mattos et al. (2017). Levels of imposex development in females were estimated using the following indices: the percentage occurrence of imposex (I%), the Relative Penis Length Index (RPLI) and the Vas Deferens Sequence Index (VDSI) (Bryan et al., 1987). The VDSI was classified according to the general scheme for the determination of vas deferens stage (VDS) proposed by Gibbs et al. (1987) and Stroben et al. (1992).

2.4. Sediment characterization

Granulometry was determined as described by Gray and Elliott (2014) and results were expressed as percentage of fine fraction (< 63 μm; %F). Sediment samples were pre-treated with 1 M HCl for inorganic carbon elimination, and total organic carbon (%TOC) was determined using a total carbon analyzer (Shimadzu TOC-L SSMM-5000A). The Limits of Detection (LOD) and Quantification (LOQ) were ≤ 0.02% and ≤ 0.06%, respectively. In order to determine the accuracy of the TOC method, standard reference material (marine sediment, NIST SRM-1941b) was analyzed and the mean percentage recovery was 96.50 ± 0.1% (n = 3).

2.5. Antifouling paint particles (APPs) characterization

APPs identification in sediments samples was carried out according to Takahashi et al. (2012). A fraction of each sample (100 g wet) was sieved through 500 μm open mesh using distilled water, dried at 45 °C overnight, and then examined under a stereomicroscope (5 x magnification; Opticam OPZTS). The visible APPs of each fraction, by color and shape, were retrieved and stored until chemical analysis.

2.6. Chemical analyses

2.6.1. Butyltins and phenyltins

Organotin compounds (TBT and TPhT) and their degradation products (DBT, MBT, DPhT and MPhT) were analyzed as described by Chen et al. (2019a), with modifications. Briefly, 2 g of freeze-dried sediment samples (or 0.5 g of freeze-dried gastropod tissue or 0.01 g of APPs) were accurately weighed in 40 mL glass vials and spiked with tripropyltin (TPrT, 100 ng) as a surrogate standard. After 30 min, 9 mL of tropolone in methanol (0.03%, w/v) and 1 mL of glacial acetic acid was added to each sample. Samples were vortex mixed for 1 min, ultrasonicated for 30 min (40 kHz, 132 W, 30 °C) and, finally, left in the dark for equilibration (30 min). For gastropod tissue samples, a pre-cleaning phase before derivatization was performed. Seven mL of supernatant was collected in a 15 mL glass tube with 100 mg of PSA Silica (Primary Secundary Amine, PSA) and mixed on a vortex for 1 min and, subsequently, centrifuged (3000 rpm for 10 min) to obtain a liquid/solid phase separation. Then, 5 mL of supernatant was derivatized by adding 0.1 mL sodium tetraethylborate ($NaEt_4B$) in water and 1 mL of sodium acetate/acetate acid buffer (pH = 4.5), and ultrasonicated for 10 min (40 kHz, 132 W, 30 °C). After derivatization, the ethylated butyltins and phenyltins were recovered by a liquid-liquid extraction with hexane (5 mL). The supernatants were cleaned up in an activated silica (2.5 g) column, and analytes were eluted with 10 mL of hexane (butyltins - BTs) and 10 mL of hexane/toluene (2:1) (phenyltins - PhTs). Finally, the solution was concentrated down to 200 μL (N_2 flow) and 100 μL of tetrabutyltin solution (1000 ng Sn mL^{-1}) was added as an internal standard. Extracts (2 μL) were analyzed in a Perkin Elmer Clarus 500MS gas chromatograph equipped with a mass spectrometer detector (GC/MS), a split/splitless injector, an auto sampler and an Elite-5MS (5% diphenyldimethylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 μm).

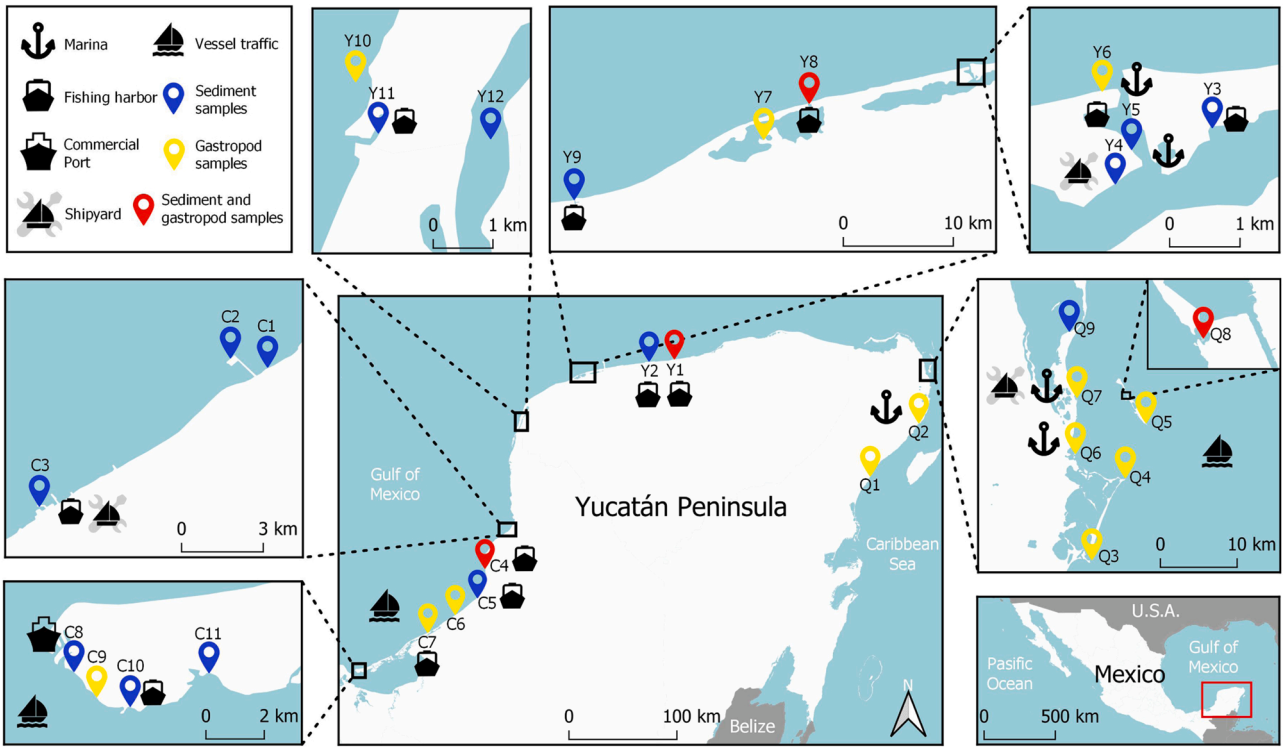

Fig. 1. Locations of sampling sites of surface sediments and gastropods in the Yucatán Peninsula, Mexico, along with indication of potential sources of contaminants.

The quality assurance and quality control were based on regular analyses of blanks, spiked matrices and certified reference material for sediments (PACS-3/National Research Council of Canada, Ottawa, Canada) and mussel tissue (ERM-CE477/European Commission). Recoveries for the surrogate standards varied between 76% and 111%, while RSD was below 20%. For sediment and tissue samples, certified values were 88% and 96.4% for TBT, 97% and 75.7% for DBT and, 75% and 89.4% for MBT, respectively. LODs for OTCs (TBT, DBT, MBT, TPhT, DPhT and MPhT) in sediment and tissue samples were ≤ 0.3 ng Sn g^{-1} and ≤ 0.7 ng Sn g^{-1}, respectively, and ≤ 100 ng Sn g^{-1} for APPs. LOQ were ≤ 0.7 ng Sn g^{-1} (DBT, MBT, TPhT and DPhT) and ≤ 1.7 ng Sn g^{-1} (TBT and MPhT) for sediment, and ≤ 1.3 ng Sn g^{-1} (DBT, MBT and DPhT) and ≤ 3.3 ng Sn g^{-1} (TBT, TPhT and MPhT) for tissue samples. For APPs, LOQ was ≤ 500 ng Sn g^{-1} for TBT, DBT, MBT, TPhT, DPhT and MPhT. All concentrations were reported as ng Sn g^{-1} (dry weight).

2.6.2. Booster biocides

Extraction of the booster biocides from sediment samples and APPs was performed according to Abreu et al. (2020). An exact mass of freeze-dried sediment samples (~1 g) or APPs (~0.01 g) were accurately weighed in 40 mL glass vials. Samples were spiked with 10 ng L^{-1} of atrazine-d5 and 20 ng L^{-1} of PCB112 as surrogate standards for LC/MS/MS and GC-ECD analysis, respectively. Samples were mixed twice with 15 mL of acetonitrile for 1 min, sonicated (50 °C for 30 min) and centrifuged (4000 rpm for 10 min). Supernatants were combined and concentrated in a vacuum vortex evaporator down to 1 mL. Then, they were diluted with 50 mL of ultrapure water (Milli-Q®) and cleaned-up by solid phase extraction (SPE) using C18 cartridges previously active with 4 mL of ethyl acetate and ultrapure water. Afterwards, SPE cartridges were dried for 1 h and then eluted twice using 2 mL of ethyl acetate. Eluates were concentrated and divided in two fractions. The first fraction was solvent exchanged to methanol (LC-ESI-MS/MS) and the second fraction to hexane (GC-ECD), using PCB 30 (10 ng mL^{-1}) as the internal standard. Irgarol, diuron, dichlofluanid and DCOIT were analyzed by LC-ESI-MS/MS (Alliance Separations, model 2695, Waters – Milford, MA, USA) and chlorothalonil by GC-ECD (Perking Elmer Clarus 500; Waltham, MA, USA). Recoveries for the surrogate standards varied between 78% and 139% with RSD below 20%. For sediments, LOD and LOQ were ≤ 0.2 and ≤ 0.6 ng g^{-1} for diuron and Irgarol, ≤ 0.2 and ≤ 0.7 ng g^{-1} for chlorothalonil, ≤ 0.9 and ≤ 2.7 ng g^{-1} for dichlofluanid, and ≤ 0.8 and ≤ 2.5 ng g^{-1} for DCOIT, respectively. For APPs, LOD and LOQ were 1000 times higher than reported for sediments. All concentrations were reported as ng g^{-1} (dry weight).

2.7. Butyltins and phenyltins degradation index

Degradation indexes of butyltins (BDI) and phenyltins (PhDI) were calculated for sediment and gastropod samples, based on the following equations: BDI = (MBT + DBT) / TBT and PhDI = (MPhT + DPhT) / TPhT. BDI or PhDI values > 1 are associated to old inputs of TBT or TPhT, respectively (Díez et al., 2002).

2.8. Data analysis

Data normality and homogeneity (shell length, imposex indices, biocide concentrations, %TOC and %F) were verified by Shaphiro-Wilks and Levene tests, respectively. Pearson's correlation coefficient was used to assess associations between %TOC and %F. Furthermore, Spearman's non-parametric correlation analysis (*rho*) was performed to investigate the relationship between biocides levels and sediment parameters, between organotin levels (TBT and TPhT) and their degradation products (DBT, MBT, DPhT and MPhT), and between organotin levels in female tissues and imposex parameters (RPLI and VDSI). Principal component analysis (PCA) was used to evaluate the relationships between antifouling residues (organotin compounds and booster biocides) and other measured parameters. Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity were applied to evaluate the suitability of the data set for PCA. All statistical analyses were conducted using R (R Core Team, 2021), and the significance level was set at $\alpha = 0.05$.

3. Results and discussion

3.1. Sediment samples characterization

Sediment samples presented %F (clay and silt: < 63 μm) ranging between 4.1 ± 1.5% and 37 ± 1.7%, and %TOC between ≤ 0.06% and 4.7 ± 0.1% (Table 1). No significant correlation was found between %F and %TOC ($p > 0.05$), as reported by Batista-Andrade et al. (2018) and Mattos et al. (2017), which can be explained by the multiple and intense processes of mechanical mixing (local hydrodynamics, dredging, etc.) and differences in organic matter input from each site (Radke et al., 2012).

3.2. Organotin levels in sediments

Organotin compounds (TBT, TPhT and their degradation products) were detected in all sediment samples except at site Y12 (Table 1 and Fig. S1). The sum of butyltins (ΣBTs) and phenyltins (ΣPhTs) varied from ≤ 0.5–324.5 ng Sn g^{-1} and ≤ 0.5–35.6 ng Sn g^{-1}, respectively. Particularly, the contribution of butyltins (84%) was higher than phenyltins (16%) to the OTCs total, while the individual compounds were as follow: MBT (36%) > DBT (25%) > TBT (23%) > TPhT (7%) > MPhT (6%) > DPhT (3%). Hence, the order of abundance found for organotin species was as follows: MBT > DBT > TBT > TPhT > MPhT > DPhT.

TBT, DBT, and MBT concentrations ranged from ≤ 0.3–90.6 ± 7.4 ng Sn g^{-1}, ≤ 0.3–79 ± 0.8 ng Sn g^{-1}, and ≤ 0.3–195.8 ± 9.6 ng Sn g^{-1}, respectively (Table 1). Significant Spearman non-parametric correlations were observed between TBT and DBT ($rho = 0.65$, $p < 0.05$), and TBT and MBT ($rho = 0.71$, $p < 0.05$), suggesting that BT metabolites were originated from tributyltin dealkylation. Similar results have been found by other studies that indicate TBT as the main source of DBT and MBT to aquatic environments (Batista-Andrade et al., 2018; Batista et al., 2016; Mattos et al., 2017). In addition, no significant Spearman correlations were found between BTs levels with %COT and %F in sediment ($p > 0.05$). Similarity, Del Brio et al. (2016) did not find correlations between BTs and sediment parameters in Golfo Nuevo (Argentina). This behavior can be explained by inherent physicochemical sediment complexity, variation in pH and salinity of the surrounding waters, as well as by the magnitude of local TBT sources (Mattos et al., 2017).

The highest BTs value was found at site Y4 (ΣBTs – 324.5 ng Sn g^{-1}), in an area under the influence of shipyards (buildings and repair). The second highest value was detected at site C3 (ΣBTs – 280.8 ng Sn g^{-1}), at the inner part of a fishing harbor with boat maintenance activities (repair). The main BTs sources are probably related to maintenance procedures and/or the presence of antifouling paint particles, mainly in areas under direct influence of maritime traffic. Indeed, APPs are generated during the maintenance of vessel hulls, and then leach out into local aquatic systems via run-off where they become significant long-term secondary sources of biocides to aquatic environments (See Section 3.5). Such pattern was reported by Abreu et al. (2021a), reporting high BTs levels in sediments from the Vitoria estuarine system (Brazil) in sites receiving run-offs from fishing port/boatyards used for the maintenance of fishing boats. In general, areas under the influence of shipyards, marinas and fishing boats have been highlighted as hotspots of recent butyltins inputs (Abreu et al., 2020; Batista et al., 2016; Maciel et al., 2018; Paz-Villarraga et al., 2015). Additionally, organotins such as TBT can accumulate in sediments over many years due to their hydrophobicity and slow degradation (Lam et al., 2017). The biodegradation of TBT to DBT and MBT takes days to weeks in water, years in oxic sediments and one or more decades in anoxic sediments (Dowson et al., 1993). Thus, contaminated sediments represent a long-term source of

Table 1
Percentage of fine fraction (< 63 μm, %F), total organic carbon (%TOC), butyltin (TBT, DBT, MBT) and phenyltin (TPhT, DPhT, MPhT) average levels (sd = standard deviation, N = 2, ng Sn g^{-1}), sum of butyltins (ΣBTs) and phenyltins (ΣPhTs) (ng Sn g^{-1}), butyltin degradation index (BDI), phenyltin degradation index (PhDI), triorganotins normalized to 1% organic carbon (TBT_n and $TPhT_n$; ng Sn g^{-1}), and booster biocides levels (Diuron, Irgarol, Chlorothalonil (Chlor), Dichlofluanid (Dichlo) and DCOIT) (sd = standard deviation, N = 2, ng g^{-1}) for sediment samples from the Yucatán Peninsula, Mexico.

Site	%F	%TOC	Butyltin levels (± sd, ng Sn g^{-1})				BDI*	TBTn	Phenyltin levels (± sd, ng Sn g^{-1})				PhDI*	TPhTn	Booster biocides (± sd, ng g^{-1})				
			TBT	DBT	MBT	ΣBTs*			TPhT	DPhT	MPhT	ΣPhTs*			Diuron	Irgarol	Chlor	Dichlo	DCOIT
Q8	28.2 ± 0.0	1.0 ± 0.0	≤ 0.3	1.4 ± 0.0	11.7 ± 0.0	13.3	79.9	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.5	2.0	≤ 0.3	≤ 0.2	≤ 0.6	≤ 0.2	≤ 0.9	≤ 2.5
Q9	4.1 ± 1.5	≤ 0.06	≤ 0.3	≤ 0.3	6.2 ± 0.2	6.5	38.5	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.5	2.0	≤ 0.3	≤ 0.6	≤ 0.2	3.3 ± 4.5	≤ 2.7	≤ 0.8
Y1	10.9 ± 3.7	1.6 ± 0.5	24.8 ± 5.4	29.6 ± 6.9	17.1 ± 5.1	71.6	1.9	15.5	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.5	2.0	≤ 0.3	≤ 0.2	≤ 0.2	≤ 0.7	≤ 2.7	≤ 0.8
Y2	19.3 ± 12.3	1.2 ± 0.3	6.6 ± 1.7	7.3 ± 0.2	6.7 ± 0.1	20.8	2.2	5.5	6.2 ± 3.2	4.7 ± 0.1	6.1 ± 0.1	17.1	1.7	5.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.9	8.1 ± 0.9
Y3	14.6 ± 0.7	3.9 ± 0.0	17.9 ± 2.1	24.5 ± 3.7	13.2 ± 1.2	55.7	2.1	4.6	9.1 ± 0.5	≤ 0.3	6.9 ± 0.1	16.1	0.8	2.3	2.1 ± 0.4	3.9 ± 0.4	≤ 0.2	≤ 2.7	≤ 0.8
Y4	19.8 ± 8.5	1.8 ± 0.1	82.9 ± 3.9	63.7 ± 2.6	195.8 ± 9.6	324.5	2.9	46.1	6.2 ± 0.1	4.8 ± 0.1	5.8 ± 0.0	16.8	1.7	3.4	1.7 ± 0.3	0.7 ± 0.0	1.0 ± 0.8	≤ 0.9	≤ 0.8
Y5	21.1 ± 3.6	2.1 ± 0.6	6.8 ± 1.1	7.3 ± 2.9	6.7 ± 0.4	20.8	2.1	3.2	15.8 ± 0.7	≤ 0.3	5.6 ± 0.0	21.6	0.4	7.5	1.7 ± 0.2	≤ 0.6	≤ 0.2	≤ 0.9	4.0 ± 0.1
Y8	18.7 ± 10.8	2.5 ± 0.0	3.5 ± 0.7	16.1 ± 6.7	6.4 ± 0.0	26.0	6.5	1.4	3.8 ± 1.0	1.8 ± 0.3	4.0 ± 2.6	9.6	1.5	1.5	1.7 ± 0.9	≤ 0.6	17.1 ± 24.0	≤ 2.7	≤ 2.5
Y9	8.9 ± 0.0	0.6 ± 0.4	7.4 ± 1.3	1.7 ± 1.9	7.4 ± 0.3	16.6	1.2	12.3	2.9 ± 0.7	1.5 ± 0.0	5.1 ± 0.1	9.5	2.3	4.8	≤ 0.6	0.8 ± 0.1	9.3 ± 12.1	7.9 ± 0.3	28.1 ± 2.3
Y11	11.0 ± 0.1	1.8 ± 1.4	2.4 ± 1.0	13.9 ± 0.5	9.8 ± 3.2	26.2	9.7	1.3	4.8 ± 0.7	0.9 ± 0.8	2.8 ± 0.7	8.5	0.8	2.7	10.2 ± 4.1	≤ 0.2	≤ 0.7	≤ 2.7	43.1 ± 3.1
Y12	23.3 ± 12.6	1.9 ± 0.1	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.5	2.0	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.5	2.0	≤ 0.3	≤ 0.2	≤ 0.2	2.4 ± 3.2	≤ 0.9	≤ 2.5
C1	20.6 ± 6.5	4.4 ± 0.1	7.9 ± 0.9	2.3 ± 2.8	7.0 ± 0.1	17.3	1.2	1.8	1.4 ± 0.5	4.6 ± 0.0	5.5 ± 0.4	11.7	7.2	0.3	1.9 ± 0.8	≤ 0.6	0.8 ± 0.9	≤ 2.7	8.6 ± 10.5
C2	26.2 ± 0.5	3.8 ± 0.0	9.0 ± 2.4	1.5 ± 2.8	6.7 ± 0.1	17.2	0.7	2.4	4.3 ± 4.3	3.1 ± 2.2	≤ 1.7	8.3	0.9	1.1	≤ 0.2	≤ 0.2	≤ 0.7	≤ 0.9	≤ 0.8
C3	16.6 ± 7.9	4.7 ± 0.1	90.6 ± 7.4	79.0 ± 0.8	111.2 ± 2.7	280.8	2.1	19.3	17.7 ± 0.4	5.1 ± 0.0	12.7 ± 1.6	35.6	1.0	3.8	1.1 ± 0.1	0.7 ± 0.3	1.7 ± 0.2	≤ 0.9	10.7 ± 5.3
C4	23.5 ± 1.0	2.1 ± 0.2	≤ 0.3	≤ 0.3	6.6 ± 0.0	6.9	40.9	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.5	2.0	≤ 0.3	≤ 0.6	≤ 0.2	≤ 0.2	≤ 2.7	76.2 ± 15.5
C5	25.0 ± 9.1	3.8 ± 0.3	7.9 ± 0.0	21.2 ± 3.8	6.2 ± 0.2	35.3	3.5	2.1	3.4 ± 0.6	5.1 ± 0.0	5.6 ± 0.6	14.3	3.1	2.1	2.5 ± 0.3	≤ 0.6	8.6 ± 9.3	≤ 0.9	33.3 ± 5.3
C8	15.0 ± 6.3	0.5 ± 0.0	2.8 ± 0.0	4.1 ± 1.4	6.9 ± 0.7	13.9	3.8	5.6	1.0 ± 0.1	1.5 ± 0.0	≤ 0.3	2.8	1.6	5.6	≤ 0.2	≤ 0.2	≤ 0.7	≤ 0.9	≤ 2.5
C10	33.2 ± 8.4	2.2 ± 0.2	≤ 0.3	17.5 ± 1.5	5.8 ± 0.1	51.6	139.7	≤ 0.3	1.9 ± 0.1	≤ 0.3	≤ 1.7	2.4	0.3	0.9	≤ 0.2	≤ 0.2	10.1 ± 12.0	≤ 0.9	≤ 0.8
C11	37.1 ± 1.7	1.2 ± 0.1	≤ 0.3	10.3 ± 0.0	5.9 ± 0.0	22.5	98.0	≤ 0.3	1.1 ± 0.0	1.5 ± 0.0	≤ 1.7	3.6	2.0	0.9	≤ 0.2	≤ 0.6	≤ 0.7	≤ 0.9	≤ 0.8

*To estimate ΣBTs, ΣPhTs, BDI and PhDI, half of LOD and LOQ values were used whenever results were < LOD or < LOQ; < LOD = ≤ 0.3 ng Sn g^{-1} (TBT, DBT, MBT, TPhT, DPhT, MPhT), < LOQ = ≤ 0.7 ng Sn g^{-1} (DBT, MBT, TPhT, DPhT) and ≤ 1.7 ng Sn g^{-1} (TBT, MPhT); For COT, < LOQ = ≤ 0.06%

contamination to the water column, releasing contaminants whenever they are resuspended. This may occur due to local hydrodynamics or shipping densities that generate turbulence and vertical mixing in the water column or during dredging activities that can resuspend contaminants buried deeper in the sediments (Buggy and Tobin, 2006; Lam et al., 2017). Intermediate BTs values ranging from 16.6 to 71.6 ng Sn g^{-1} were found in fishing harbors (sites Y1, Y2, Y3, Y5, Y8, Y9, Y11, C2, C5 and C10), fishing moorings (site C1) and urban areas (sites C1 and C11). Furthermore, lower BTs values were detected in sites (sites Q8, Q9 and Y12) with lower maritime traffic when compared to other studied areas, combined with the lower sorption capability of their coarse sediments (Del Brio et al., 2016).

Levels of TPhT, DPhT, and MPhT ranged from ≤ 0.3–17.7 ± 0.4 ng Sn g^{-1}, ≤ 0.3–5.1 ± 0.0 ng Sn g^{-1}, and ≤ 0.3–12.7 ± 1.6 ng Sn g^{-1}, respectively. As seen for BTs, significant Spearman non-parametric correlations were observed between TPhT and DPhT (*rho* = 0.60, $p < 0.05$), and TPhT and MPhT (*rho* = 0.90, $p < 0.05$), indicating that phenyltin metabolites originated from triphenyltin dephenylation. MPhT was the dominant degradation product, indicating a relevant TPhT degradation in surface sediments (Sheikh et al., 2007). TPhT is mainly used as a broad-spectrum agricultural fungicide (e.g., triphenyltin acetate and triphenyltin hydroxide) in agriculture (Schulte-Oehlmann et al., 2000) and aquaculture, and to a lesser extent (< 10% of total formulation) as a co-biocide in antifouling systems (Anastasiou et al., 2016; Chen et al., 2019a). In México, triphenyltin acetate is sold as Brestan 60 HP® (COFEPRIS registration number: RSCO-MEZC-1308–301–002–078) and is used to eliminate a range of fungal diseases in various crops, particularly potatoes, peanuts and carrots. Furthermore, levels of TPhT were significantly correlated (*rho* = 0.61, $p < 0.05$) with TBT concentrations. A similar positive correlation between TPhT and TBT ($r = 0.64$, $p < 0.05$) was found by Sheikh et al. (2007) in sediments of the Manko estuary in Japan, suggesting that organotins came from the same source. Thus, TPhT levels in sediment of the present study may also be the result of maritime activities, as well as the use of TPhT-based pesticides in agriculture (Bejarano, 2017). As seen for BTs, no significant Spearman correlations were found between phenyltin levels with %COT and %F in sediment ($p > 0.05$).

The highest ΣPhTs concentrations were found in sites under the influence of fishing harbors and shipyards, as well as marinas (sites Y5 - 21.6 ng Sn g^{-1}, C3 - 35.6 ng Sn g^{-1}, Y2 - 17.1 ng Sn g^{-1}, Y4 - 16.8 ng Sn g^{-1} and Y3 - 16.1 ng Sn g^{-1}). The highest TPhT concentrations were also found at sites C3 (17.7 ± 0.4 ng Sn g^{-1}), Y5 (15.8 ± 0.7 ng Sn g^{-1}), Y3 (9.1 ± 0.5 ng Sn g^{-1}), Y2 (6.2 ± 3.2 ng Sn g^{-1}) and Y4 (6.2 ± 0.1 ng Sn g^{-1}). These results indicate that fishing harbors (sites Y2, Y3, Y5 and C3), shipyards (sites Y4 and C3) and marinas areas (site Y5) act as sources of PhTs to the study area. The lowest concentrations were found at sites Q8, Q9, Y1 and Y12, probably associated with the dominance of coarse sediments and relatively low %TOC, but also by higher hydrodynamics, which can rapidly disperse local OTCs inputs (Abreu et al., 2020).

BDI and PhDI were estimated for each sampling site (Table 1). For butyltins, 18 out of 19 sites shown BDI > 1, indicating either old TBT inputs or high degradation rates of butyltins in the sediments. However, levels of TBT > 60 ng Sn g^{-1} suggest that a combination of recent and old (chronic) inputs is probable taking place (Abreu et al., 2020), especially for the samples collected from two sites under the influence of boat repairing activities and intense boat traffic (sites Y4 and C3). Additionally, BDI < 1 was only observed at site C2 (fishing and recreational boats), indicating possible recent input of TBT. In this case, it may be stated that TBT is still being introduced into the aquatic environment due probably to the use of TBT-based antifouling paint in fishing and recreational boats and by APPs. For phenyltins, PhDI values < 1 were registered in five sites (sites Y3, Y5, Y11, C2 and C10), indicating some recent inputs or poor TPhT degradation. However, some ratios could be biased by the very low levels detected. On the other hand, fourteen sites showed PhDI > 1, which along with the relatively low TPhT concentrations indicate legacy inputs.

In México, there are no sediment quality guidelines (SQG) for TBT and TPhT contamination. Therefore, to evaluate the ecotoxicological risk associated with OTCs in sediments, SQG from other countries were used for comparison. According to the SQG of Australia, sedimentary TBT levels of 9 and 70 ng Sn g^{-1} indicate low and high trigger values, respectively (Simpson et al., 2013). Based on this guideline, TBT levels (normalized to 1% total organic carbon, 1%TOC) in the sites Y1, Y4, Y9 and C3 were between the low and high trigger values (Table 1), which indicates possible adverse effects on benthic biota. Such sites are characterized by the traffic of fishing and recreational boats, and ship maintenance activities (shipyards), which were probably the main sources of OTCs in the study area; the other sites presented TBT concentrations below the low trigger values. The Norwegian Environment Agency (NEA) established a threshold value of 14.3 ng Sn g^{-1} (1%TOC) for ecological risk in sediments contaminated by TBT and TPhT (M-, 1132, 2018). Considering this SQG, TBT level at sites Y1 (Fishing harbor), Y3 (Fishing harbor), Y4 (shipyard) and C3 (Fishing harbor and Shipyard) were above the threshold value.

According to the U.S. Environmental Protection Agency (USEPA, 1996), the lower screening value (LSV) and higher screening value (HSV) for TBT were 5.15 and 72.04 ng Sn g^{-1} (1% TOC), respectively (Chen et al., 2017). Six out of nineteen sites (Y1, Y2, Y4, Y9, C3, C8) had TBT levels between LSV and HSV, indicating low ecological toxicity, while levels in sediment in the other sites were below LSV, presenting none or negligible ecological toxicity. According to the classification proposed by the OSPAR for TBT levels in sediments, 6 out of 19 sampled sites (sites Q8, Q9, Y12, C4, C10 and C11) were categorized in class B (TBT levels < 2 ng g^{-1}), whereas sites Y2, Y3, Y5, Y8, Y9, Y11, C1, C2, C5 and C8 were within class C (TBT levels ≥ 2 - < 50 ng g^{-1}), site Y1 (Fishing harbor) was classified as class D (TBT levels ≥ 50 - $<$ 200 ng g^{-1}), and sites Y4 (Shipyard) and C3 (Fishing harbor and shipyard) were classified as class E (TBT levels $\geq$ 200–500 ng g^{-1}) (OSPAR, 2011). Sites classified as class C can induce some negative biological effect because of TBT levels above the environmental assessment criteria (EAC), while higher assessment classes (D and E) indicate levels able to cause reproductive effects on the sensitive gastropod species. Finally, using the TBT contaminated sediments classification proposed by Dowson et al. (1993), sites can be ranked as follows: Y4 and C3 as highly contaminated (41–205 ng Sn g^{-1}); Y1, Y3 and C2 as moderately contaminated (8–41 ng Sn g^{-1}); Y2, Y5, Y8, Y9, Y11, C1, C5 and C8 as lightly contaminated (1–8 ng Sn g^{-1}); and Q8, Q9, Y12, C4, C10 and C11 as uncontaminated (< 1 ng Sn g^{-1}). As seen above, these results indicate that sediments from sites under high influence of maritime traffic can pose a threat to marine life, particularly to benthic organisms. In general, the wide occurrence of OTCs in sediment samples in the study area, especially BTs, suggests that the international legislation against OTCs usage (AFS Convention) has not been fully effective in Mexico.

A compilation of levels of OTCs reported for surface sediments in coastal areas from several regions of the world are shown in Table 2. Compared with other studies, TBT levels were lower than the concentrations reported in US Virgin Island (Hartwell et al., 2016), Venezuela (Paz-Villarraga et al., 2015), Argentina (Quintas et al., 2021), Brazil (Abreu et al., 2020), Malta (Romeo et al., 2015) and France (Briant et al., 2013), but higher than Poland (Filipkowska et al., 2018), India (Garg et al., 2011), Tunisian (Anastasiou et al., 2016), Portugal (Anastasiou et al., 2016), China (Chen et al., 2019b) and Malaysia (Mukhtar et al., 2019). Regarding TPhT, there are few scientific publications available, but concentrations measured in sediments from Korea (Lam et al., 2017), China (Chen et al., 2019b) and Malaysia (Mukhtar et al., 2019) showed to be higher than those detected in the present study. However, levels found in Tunisian, Italy, Portugal (Anastasiou et al., 2016) and Saudi Arabian (Hassan et al., 2019) were relatively lower.

3.3. Imposex and organotin levels in marine gastropods

The preset study is the first to evidence imposex and organotin levels in soft tissues of marine gastropods from the Yucatán Peninsula (Table 3 and Fig. S2). Levels of imposex were observed in five caenogastropod species (i.e., *P. patula*, *V. deltoidea*, *S. haemastoma*, *G. tinctus* and *M. bispinosa*) of three families (Muricidae, Pisaniidae and Melongenidae) from sites nearby potential organotins sources, such as fishing and commercial harbors, and marinas. In Mexico, some imposex studies have been conducted in coastal areas (Uc-Peraza et al., 2021), particularly with muricid species (e.g. *Plicopurpura pansa*) from the Pacific coast (Ahumada-Martínez et al., 2018; Domínguez-Ojeda et al., 2015; Liñán-Cabello et al., 2020), but this phenomenon had never been reported for *G. tinctus* and *M. bispinosa* of the Pisaniidae and Melongenidae families, respectively. However, imposex has already been recorded in Colombia (Caribbean Sea) for these gastropod families. Hernández and Stotz (2004), for instance, observed imposex in *Melongena melongena*

Table 2
Concentration of organotins (ng Sn g^{-1} dry weight) in surface sediments from several regions of the world.

Location	TBT	DBT	MBT	TPhT	DPhT	MPhT	References
Benner Bay, United State Virgin Island	5.57–993	3.93–700	6.25–940	–	–	–	Hartwell et al. (2016)
Peruvian coast, Peru	52.2–171	4.4–26.6	< 2.5–53.2	–	–	–	Castro et al. (2018)
Gulf of Guayaquil, Ecuador	13–99	1.8–58	44–340	–	–	–	(Castro et al., 2012a)
Venezuelan coast, Venezuela	57–517	14.8–990	10.4–456	–	–	–	Paz-Villarraga et al. (2015)
Caribbean coast, Panama	< 1–24	3–32	2–37	–	–	–	Batista-Andrade et al. (2018)
Pacific coast, Panama	21–149	11–46	7–32	–	–	–	Batista-Andrade et al. (2018)
Coquimbo, Chile	0.8–2.0	32–57	0.6–9.5	–	–	–	Batista et al. (2016)
Concepción, Chile	1.2–122	4.7–103	3.1–118	–	–	–	Batista et al. (2016)
Bahía Blanca Estuary, Argentina	< 0.78–259	< 1.08–782	< 3.5–831	–	–	–	Quintas et al. (2021)
Santos-São Vicente Estuarine System, Brazil	< 0.5–688	< 0.5–304	< 0.5–809	–	–	–	Abreu et al. (2020)
Valletta, Malta	8–5654	5–929	4–489	–	–	–	Romeo et al. (2015)
Port Camargue, France	0.24–10738	0.55–5066	5.02–710	–	–	–	Briant et al. (2013)
Gulf of Gdansk, Poland	0.9–28.5	0.5–18.9	1.2–15.2	–	–	–	Filipkowska et al. (2018)
German coast, Germany	nd - 380	nd - 955	nd - 490	–	–	–	Abraham et al. (2017)
Gujarat, Karwar and Marmugao Ports, India	2.5–32	1.4–110	3.6–72	–	–	–	Garg et al. (2011)
Egyptian Red Sea coast, Egypt	7–220	3.5–48.8	4.2–78	–	–	–	Younis (2020)
El Kantaoui, Tunisian	6.1	8.4	7.2	0.3	–	–	Anastasiou et al. (2016)
Cagliari, Italy	74.5	79.9	43.8	0.9	–	–	Anastasiou et al. (2016)
Ria Formosa and Olhão, Portugal	1.8–2.6	2.0–2.4	3.4–3.9	0.4	–	–	Anastasiou et al. (2016)
South Hangzhou Bay, China	< 1–28	< 1–16.2	< 1–51.8	< 1–22.6	< 1–21.1	–	Chen et al. (2019b)
Arabian Gulf, Saudi Arabian	nd	nd - 60	nd – 160	12	nd - 260	nd - 330	Hassan et al. (2019)
Gwangyang, Busan and Ulsan Bays, Korea	nd - 2304	nd - 160	nd - 56.9	nd - 68.5	nd - 2.3	nd - 46.7	Lam et al. (2017)
Sungai Pulai Estuary, Malaysia	8.1–10.6	< 0.1–6.1	6.5–12.2	17.1–19.4	9.3–89.1	< 0.1–16	Mukhtar et al. (2019)
Yucatán Peninsula, Mexico	≤ 0.3–90.6	≤ 0.3–79	≤ 0.3–195.8	≤ 0.3–17.7	≤ 0.3–5.1	≤ 0.3–12.7	Present study

nd not detected

Table 3
Biometric parameters (N = sample size, M/F = male/female, SL = shell length, sd = stand deviation), imposex levels (%I = percentage of imposex in females, RPLI = Relative Penis Length Index, VDSI = Vas Deferens Sequence Index), butyltin (TBT, DBT, MBT) and phenyltin (TPhT, DPhT, MPhT) levels (ng Sn g^{-1}) in gastropod samples from the Yucatán Peninsula, Mexico.

Site	Species	N	M/F	SL (mm ± sd)	Imposex levels			Butyltin levels (ng Sn g^{-1})				BDI*	Phenyltin levels (ng Sn g^{-1})				PhDI*
					%I	RPLI	VDSI ± sd	TBT	DBT	MBT	ΣBTs*		TPhT	DPhT	MPhT	ΣPhTs*	
Q1	*P. patula*	28	13/15	31.3 ± 6.5	40.0	2.0	0.4 ± 0.5	≤ 0.7	7.8	5.7	13.9	38.7	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
	L. nassa	30	5/25	33.4 ± 2.5	0.0	0.0	0.0 ± 0.0	3.5	2.6	1.9	8.1	1.2	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Q2	*P. patula*	29	14/15	35.9 ± 13.7	100.0	5.8	1.3 ± 0.4	10.6	8.2	6.6	25.5	1.4	≤ 0.7	≤ 0.7	5.3	6.3	16.9
	V. deltoidea	30	12/18	26.5 ± 5.0	100.0	3.5	1.0 ± 0.0	11.6	8.8	5.9	26.4	1.3	≤ 0.7	≤ 0.7	5.6	6.3	16.9
Q3	*P. patula*	7	1/6	33.5 ± 4.1	0.0	0.0	0.0 ± 0.0	≤ 0.7	7.6	5.7	13.7	38.2	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
	V. deltoidea	27	15/12	26.9 ± 5.3	8.0	2.7	0.1 ± 0.3	≤ 0.7	7.5	5.5	13.4	37.1	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Q4	*V. deltoidea*	27	13/14	25.3 ± 4.0	71.0	3.6	0.7 ± 0.4	3.5	7.7	5.8	17.1	3.8	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Q5	*V. deltoidea*	33	16/17	30.9 ± 6.9	0.0	0.0	0.0 ± 0.0	≤ 0.7	2.5	1.9	4.8	12.8	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Q6	*V. deltoidea*	14	9/15	26.7 ± 6.5	100.0	8.8	1.3 ± 0.5	10.9	10.4	7.0	28.3	1.6	≤ 0.7	≤ 0.7	5.6	6.3	16.9
Q7	*V. deltoidea*	30	10/20	27.7 ± 5.9	100.0	20.0	1.7 ± 0.6	11.9	14.7	8.6	35.3	1.9	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Q8	*V. deltoidea*	30	11/19	34.8 ± 4.6	100.0	3.2	1.1 ± 0.2	≤ 0.7	7.7	5.8	13.9	38.6	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Y1	*G. tinctus*	30	15/15	20.9 ± 1.3	0.0	0.0	0.0 ± 0.0	≤ 0.7	2.6	1.9	4.9	13.0	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Y6	*G. tinctus*	30	8/22	20.2 ± 1.7	95.0	1.5	1.0 ± 0.2	10.6	8.9	5.9	25.5	1.4	≤ 0.7	4.4	5.6	10.3	28.5
Y7	*M. bispinosa*	30	9/21	47.4 ± 4.7	76.0	1.7	0.8 ± 0.6	≤ 0.7	7.9	5.7	14.0	38.9	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Y8	*S. haemastoma*	30	14/16	51.4 ± 3.6	100.0	1.9	1.2 ± 0.4	3.5	7.7	5.0	16.3	3.6	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
Y10	*S. pugilis*	28	12/16	70.7 ± 10.9	0.0	0.0	0.0 ± 0.0	≤ 0.7	≤ 0.7	1.9	2.6	7.4	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
C4	*G. tinctus*	30	9/21	22.7 ± 1.5	52.0	1.3	0.5 ± 0.5	≤ 0.7	7.8	5.7	13.9	38.6	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
C6	*S. haemastoma*	9	4/5	37.6 ± 3.0	0.0	0.0	0.0 ± 0.0	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
C7	*S. haemastoma*	19	10/9	46.9 ± 3.6	100.0	1.4	1.0 ± 0.0	≤ 0.7	7.7	5.8	13.8	38.5	≤ 0.7	≤ 0.7	≤ 0.7	≤ 1.0	2.0
C9	*S. haemastoma*	25	11/14	35.2 ± 3.1	93.0	2.0	0.9 ± 0.3	10.6	3.9	5.8	20.3	0.9	≤ 0.7	4.4	≤ 0.7	5.1	13.6

* To estimate ΣBTs, ΣPhTs, BDI and PhDI, half of LOD and LOQ values were used whenever results were < LOD or < LOQ; < LOD = ≤ 0.7 ng Sn g^{-1} (TBT, DBT, MBT, TPhT, DPhT, MPhT), < LOQ = ≤ 1.3 ng Sn g^{-1} (DBT, MBT, DPhT) and ≤ 3.3 ng Sn g^{-1} (TBT, TPhT, MPhT).

(Melongenidae family), and Rodríguez-Grimón et al. (2020) reported this endocrine disruption in Muricid (*P. patula*, *V. deltoidea* and *S. haemastoma*) and Pisaniid (*Gemophos auritulus*) gastropds.

The highest imposex incidences (%I = 100) were seen in muricid species (*P. patula*, *V. deltoidea* and *S. haemastoma*) collected inside or close to marinas (sites Q2, Q6, Q7) and fishing harbors (sites Y8 and C7), and under a high influence of traffic of leisure boats (site Q8). Considering these three muricid species, the RPLI and VDSI ranged from 1.4 to 20.0 and 0.1 ± 0.3–1.7 ± 0.6, respectively (Table 3). Similar results were reported by Rodríguez-Grimón et al. (2020) and Sierra-Marquez et al. (2018) when studying *P. patula* and *S. haemastoma* from Colombia, respectively, and Paz-Villarraga et al. (2015) in *P. patula* from Venezuela; these studies pointed out that imposex incidence was highly influenced by maritime activities. Furthermore, the relationship between the intensity of maritime traffic and imposex levels has also been previously reported for several muricid species, such as *Stramonita brasiliensis* from Brazil (França et al., 2021), *Thaisella chocolata* and *Xanthochorus buxeus* from Peru (Enrique et al., 2019), *Thais biserialis* from Ecuador (Rodríguez-Grimón et al., 2016), *Thais deltoidea* and *Thaisella kiosquiformis* from Panama (Batista-Andrade et al., 2018), and *Acanthina monodon* and *Xanthochorus cassidiformis* from Chile (Batista et al., 2016). In addition, the high levels of imposex in the study area are due probably to the sensitivity of muricid species to organotins exposure (e.g., TBT), as reported by Castro et al. (2012b).

High levels of imposex in *G. tinctus* (%I = 95, RPLI = 1.5, VDSI = 1.0 ± 0.2) and *S. haemastoma* (%I = 93, RPLI = 2.0, VDSI = 0.9 ± 0.3) were found at sites Y6 and C9, respectively. Y6 is located nearby marinas and is under high influence of recreational/fishing boats, while C9 is under the influence of a commercial port and fishing harbor. Furthermore, low or moderate imposex incidence (%I = 8 – 76%) were detected in *P. patula* (site Q1), *V. deltoidea* (sites Q3 and Q4), *M. bispinosa* (site Y7) and *G. tinctus* (site C4), while no imposex signs were observed in *L. nassa* (site Q1), *P. patula* (site Q3), *V. deltoidea* (site Q5), *S. pugilis* (site Y10) and *S. haemastoma* (site C6) from areas under little or no influence of maritime traffic. Imposex incidence in areas far from potential sources may suggest environmental mobility of TBT, as already reported by Mattos et al. (2017). In this regard, although site Q1 was initially chosen as a reference area – since it is inside a terrestrial and marine protected area "Santuario de la Tortuga Marina Xcacel - Xcacelito" without maritime traffic or human settlements – levels of butyltins (including TBT) in tissues of *P. patula* and *L. nassa*, and imposex incidence (*P. patula*), were observed in this site. This indicates that the southern coastal currents (Suárez-Morales and Rivera-Arriaga, 1998) are probably transporting TBT residues from more contaminated areas (e.g., site Q2) towards this region. Thus, the marine life of this protected area is probably under threat of organotin contamination. Similar situations were reported for the Galapagos Marine Reserve in Ecuador (Rodríguez-Grimón et al., 2016) and other 52 marine protected areas in Latin American coastal areas (Castro et al., 2021). On the other hand, despite detecting levels of TBT (24.8 ± 5.4 ng Sn g^{-1}) in sediments from site Y1, low levels of butyltin residues (without TBT) and no imposex were observed in *G. tinctus*. This may suggest that TBT was not bioavailable or the environment levels were not high enough to elicit imposex in this species (Mattos et al., 2017).

Organotin residues were detected in tissues of all analyzed gastropods (Table 3 and Fig. S2). The ΣBTs and ΣPhTs ranged from ≤ 1.0–35.3 ng Sn g^{-1} and ≤ 1.0–10.3 ng Sn g^{-1}, respectively. As seen for sediments, the contribution of butyltins (86%) to the total OTCs was also higher than phenyltins (14%) in biological samples. TBT levels ranged from ≤ 0.7–11.9 ng Sn g^{-1}, while DBT and MBT levels varied from ≤ 0.7–14.7 and < 0.7–8.6 ng Sn g^{-1}, respectively. Furthermore, DBT (42%) was the prevalent butyltin compound in tissues, followed by MBT (31%) and TBT (27%). The predominance of metabolites in marine gastropods may indicate a high environmental degradability of TBT or/and an ability to metabolize TBT by these organisms (Batista-Andrade et al., 2018).

The highest levels of butyltins were found in four gastropod species (*P. patula*, *V. deltoidea*, *G. tinctus* and *S. haemastoma*) collected inside or close to marinas and zones under high influence of recreational/fishing boats: sites Q2 (ΣBTs - 25.5 and 26.4 ng Sn g^{-1} in *P. patula* and *V. deltoidea*, respectively), Q6 (ΣBTs - 28.3 ng Sn g^{-1} in *V. deltoidea*), Q7 (ΣBTs - 35.3 ng Sn g^{-1} in *V. deltoidea*), Y6 (ΣBTs - 25.5 ng Sn g^{-1} in *G. tinctus*) and C9 (ΣBTs - 20.3 ng Sn g^{-1} in *S. haemastoma*). High levels of TBT have also been reported in *P. patula* (Paz-Villarraga et al., 2015) and *Thaisella chocolata* (Castro et al., 2018) collected in the vicinity of marinas in Venezuela and Peru, respectively. Although lower than butyltins, the highest levels of phenyltins were also found at sites Q2 (ΣPhTs - 6.3 and 6.3 ng Sn g^{-1} in *P. patula* and *V. deltoidea*, respectively), Q6 (ΣPhTs - 6.3 ng Sn g^{-1} in *V. deltoidea*), Y6 (ΣPhTs - 10.3 ng Sn g^{-1} in *G. tinctus*) and C9 (ΣPhTs - 5.1 ng Sn g^{-1} in *S. haemastoma*). Thus, the results showed that marinas (e.g., sites Q2, Q6, Q7 and Y6) and some fishing harbors (e.g., site Y8 and C9) were the most contaminated sites and are probably acting as sources of organotin compounds to the Peninsula. In this sense, BDI values ranging from 1.2 to 38.9 and PhDI values varying from 2.0 to 28.5 for all sites, except C9 (BDI = 0.9) (Table 3), indicate legacy inputs.

The RPLI and VDSI values were significantly ($p < 0.001$) and positively correlated with ΣBTs (RPLI vs ΣBTs: *rho* = 0.83; VDSI vs ΣBTs: *rho* = 0.87) and total OTCs (RPLI vs total OTCs: *rho* = 0.80, VDSI vs total OTCs: *rho* = 0.86) in female gastropods of caenogastropod species. Spearman's correlations were not as strong with ΣPhTs, since TPhT levels were < LOD in all analyzed tissues and TPhT is less efficient in inducing imposex (Yi et al., 2012). Thus, imposex indexes were strongly related to levels of butyltins and total OTCs in the investigated gastropod species. In general, the impact of organotin contamination in marine gastropods from the Yucatán Peninsula were related to marinas and fishing harbors, where almost all female gastropod species were affected by imposex associated to butyltins accumulation.

3.4. Booster biocides levels in sediments

Levels of booster biocides in sediment samples are shown in Table 1 and Fig. S3. The extent of contamination varied widely, but higher levels were found inside or close to fishing harbors. Overall, chlorothalonil was the most frequently detected compound in sediment samples (74%), followed by DCOIT (63%), diuron (58%), Irgarol (53%) and dichlofluanid (42%). Chlorothalonil is commonly used in agricultural activities at the Yucatán Peninsula (Bejarano, 2017) and may be released into aquatic ecosystems via leaching and agricultural run-off. However, although widely used in agriculture as a fungicide, its use in antifouling paints has increased after the global ban on organotins (Martins et al., 2017). For example, the chlorothalonil-based antifouling paint Islands 99 Plus™ (https://www.todovelamexico.com/product-page/islands-99-plus) has been found available for sale in Mexico. Regarding DCOIT, it was the second most frequent and the most abundant booster biocide measured in the study area. DCOIT is the active ingredient most frequently present in contemporary formulations registered for use as antifouling paints (9.3%), followed by Irgarol (4.5%), diuron (3.9%) and dichlofluanid (1.9%) (Paz-Villarraga et al., 2021). Also, Irgarol and diuron are heavily used in agriculture as herbicides, and are both highly toxic to primary producer communities (Ali et al., 2021; Bejarano, 2017). In general, leaching from vessels hulls and agriculture use could be the main sources of these five biocides to the study area.

The high organic carbon/water partition coefficients of booster biocides indicate their preferential association to organic matter (particulate phase), leading to a consequent deposition in sediments (De Campos et al., 2021; Yebra et al., 2004). Thus, sediment layers will serve as sinks for these compounds, acting as secondary contamination sources to the water column. However, booster biocides can be affected by natural transformation processes such as biodegradation, photolysis and hydrolysis, which influence their fate once released into the aquatic environment (Kaonga et al., 2016; Thomas et al., 2002). Non-significant Spearman correlations were found between concentrations of each target booster biocide with %COT and %F, suggesting that their partition into the aqueous phase is probably relevant as well (Abreu et al., 2020). Similarity, a lack of correlation between biocide levels and sediment parameters was observed in two estuarine systems from Southeastern Brazil (Abreu et al., 2021a, 2020).

Chlorothalonil levels ranged from ≤ 0.2–17.1 ± 24.0 ng g^{-1}, with a mean concentration of 3.1 ± 4.7 ng g^{-1}. The highest concentrations of chlorothalonil were measured at sites located under the influence of fishing harbors, such as sites Y8 (17.1 ± 24.0 ng g^{-1}), C10 (10.1 ± 12.0 ng g^{-1}), Y9 (9.3 ± 12.1 ng g^{-1}) and C5 (8.6 ± 9.3 ng g^{-1}). This can be explained by the poor water exchange regime in these shallow semi-closed environments, which favors sedimentation processes. On the contrary, biocide levels in open water sites tend to be quickly diluted and flushed away by local hydrodynamics (e.g., tides or currents). Although lower, levels of chlorothalonil were also observed at sites Q9 (3.3 ± 4.5 ng g^{-1}), Y4 (1.0 ± 0.8 ng g^{-1}), Y12 (2.4 ± 3.2 ng g^{-1}), C1 (0.8 ± 0.9 ng g^{-1}) and C2 (1.7 ± 0.2 ng g^{-1}), which present higher water renewal rates and/or less boating activities. Similar patterns were found in surface sediments of the Santos and São Vicente estuarine systems (< 0.1–9.2 ng g^{-1}; Abreu et al., 2020) and the Vitória estuarine system (< 0.1–8.6 ng g^{-1}; Abreu et al., 2021a) in Brazil. Other studies reported similar concentrations in an estuary of Malaysia (< 0.1–6.2 ± 0.3 ng g^{-1}, Mukhtar et al., 2019), and a saline lake in the USA (< 0.1–8.9 ng g^{-1}, Sapozhnikova et al., 2004), while higher values were found in a coastal area (1.2–99 ng g^{-1}) and bays (1.3–422 ng g^{-1} and 22–1065 ng g^{-1}) of South Korea (Lee et al., 2015), marinas and ports of Greece (< 8.0–165 ng g^{-1}, Albanis et al., 2002), and an estuary of the United Kingdom (< 4.0–47 ng g^{-1}, Voulvoulis et al., 2000).

Concentrations of chlorothalonil in sediment samples did not exceed the environment risk limit (ERL) of 50.6 ng g^{-1}, established by Van Wezel and Van Vlaardingen (2004). However, two sites (sites Y8 and C10) exceed the Predicted No Effect Concentration (PNEC) value of 9.5 ng g^{-1} for chlorothalonil (Abreu et al., 2021b). Studies have shown that chlorothalonil is highly toxic to aquatic organisms, such as crustaceans, fish, and invertebrates. Furthermore, chronic tests at low concentrations have shown that chlorothalonil can negatively affect the shell growth rate of marine oysters (*Crassostrea virginica*) with EC_{50} values of 5 μg L^{-1} (Van Wezel and Van Vlaardingen, 2004) and 7.3 μg L^{-1} (Voulvoulis et al., 2000). Thus, the higher levels of chlorothalonil in sites Y8 (17.1 ± 24.0 ng g^{-1}) and C10 (10.1 ± 12.0 ng g^{-1}) may cause long-term effects on some sensitive species, especially benthic organisms.

DCOIT was detected in 12 out of 19 sites with levels ranging from ≤ 0.8–76.2 ± 15.5 ng g^{-1} with a mean concentration of 11.9 ± 19.9 ng g^{-1}. The highest concentration was detected at site C4 (76.2 ± 15.5 ng g^{-1}), which is located inside of a fishing harbor with intense maritime traffic. High concentrations were also detected in other fishing harbors at sites Y9 (28.1 ± 2.3 ng g^{-1}), Y11 (43.1 ± 3.1 ng g^{-1}) and C5 (33.3 ± 5.3 ng g^{-1}), while lower levels were seen at Y2, Y5, C1 and C3 (4.0 ± 0.1–10.7 ± 5.3 ng g^{-1}). These findings confirm that fishing harbors are acting as important sources of DCOIT to the aquatic system. Comparing the present data with literature, higher concentrations were found in a coastal area (30–281 ng g^{-1}) and bay (61–269 ng g^{-1}) of South Korea (Lee et al., 2015) and in an estuary (9.1 ± 0.5–170 ± 8.0 ng g^{-1}) of Malaysia (Mukhtar et al., 2019).

DCOIT used to be considered an eco-friendly alternative by the marine industry due to its fast degradation in natural seawater and lower bioaccumulation factor (Batista-Andrade et al., 2018). Currently, it is one of the main antifouling agents in the formulations of many marine paints in use (Paz-Villarraga et al., 2021). However, ecotoxicological studies have reported its high toxicity to non-target organisms, especially crustacean and fish (Amara et al., 2018; Chen and Lam, 2017). For example, the Lowest Observed Effect Concentration (LOEC) value of DCOIT for teleost fish (*Oryzias melastigma*) was of 0.76 μg L^{-1} (Chen et al., 2017). For sediments, toxicity tests with the marine polychaete

Perinereis nuntia showed LC_{50} and No Observed Effect Concentration (NOEC) values of 110 ng g^{-1} and 9.7 ng g^{-1}, respectively (Onduka et al., 2013), while Abreu et al. (2021b) estimated a PNEC value of 0.97 ng g^{-1} for DCOIT. Considering this last threshold limit, DCOIT may cause biological effects to the sediment-dwelling biota of 12 sites of Yucatan Peninsula, especially in sediments from sites Y9, Y11, C3, C4 and C5, which presented DCOIT levels > 10 ng g^{-1}.

The concentration of diuron, detected in 11 out of 19 sites, ranged from ≤ 0.2–10.2 ± 4.1 ng g^{-1} with a mean concentration of 1.4 ± 2.3 ng g^{-1}. The highest diuron concentration was measured at site Y11 (10.2 ± 4.1 ng g^{-1}), which is located in a fishing harbor with high flow of small boats. Diuron was also found in sites under the influence of fishing harbors (sites Y3, Y8, C3, C5) and shipyard and marinas (sites Y4 and Y5). Similar levels of diuron were reported by Batista-Andrade et al. (2018) in areas of Panama with intense vessel traffic (up to 14.1 ± 1.3 ng g^{-1}), by Abreu et al. (2020) in an estuarine system of Brazil (< 0.5–9.9 ng g^{-1}) and by Mukhtar et al. (2019) in an estuary of Malaysia (< 0.1–22.9 ± 1.1 ng g^{-1}). Regarding toxic effects of diuron, the mean level measured in Y11 (10.2 ± 4.1 ng g^{-1}) was above the threshold limit (Maximum Permissible Concentration - MPC) proposed by Dutch authorities (9 ng g^{-1}) (Crommentuijn et al., 2000). Consequently, organisms from this site could exhibit toxic effects following short-term exposure to this biocide. In addition, sites Q9, Y4, Y5, Y9 and Y11 can be classified as class III "moderate" (0.7–6.4 ng g^{-1}, normalized to 1% TOC) by the Norwegian guidelines for marine sediments (Bakke et al., 2010), suggesting that benthic organisms from these sites may exhibit toxic effects following chronic exposure to diuron. In fact, diuron levels in all sediment samples were above the PNEC value of 0.15 ng g^{-1} estimated by Abreu et al. (2021b). Thus, diuron levels at 10 out of 19 sites had levels above the limit detection (0.2 ng g^{-1}), which could cause toxic effects to marine organisms.

Irgarol and dichlofluanid levels ranged from ≤ 0.2–3.9 ± 0.4 ng g^{-1} (mean 0.6 ± 0.8 ng g^{-1}) and ≤ 0.9–7.9 ± 0.3 ng g^{-1} (mean 1.9 ± 1.7 ng g^{-1}), respectively. The highest levels were measured at sites Y3, Y4, Y9 and C3 for Irgarol (0.7 ± 0–3.9 ± 0.4 ng g^{-1}) and Y9 for dichlofluanid (7.9 ± 0.3 ng g^{-1}). Comparing with others studies, similar levels of Irgarol were reported by Lam et al. (2017) in South Korea (< 0.02–8.0 ng g^{-1}) and by Batista-Andrade et al. (2018) in Panama (< 0.08–2.8 ng g^{-1}). Regarding dichlofluanid, higher levels were found in surface sediments of Santos and São Vicente estuarine system (< 0.7 to up 16.0 ng g^{-1}; Abreu et al., 2020) in Brazil. In addition, Irgarol levels did not exceed the PNEC value of 16 ng g^{-1}, established by Abreu et al. (2021b). However, Irgarol level at site Y3 (3.9 ± 0.4 ng g^{-1}) exceeded the ERL of 1.4 ng g^{-1} proposed by the Dutch authorities (Van Wezel and Van Vlaardingen, 2004). This result indicates that Irgarol may induce deleterious effects on aquatic organisms (e.g., photosynthetic organisms) in this site. For dichlofluanid, levels of this biocide in sediment samples did not exceed the PNEC value of 16.6 ng g^{-1} estimated by Abreu et al. (2021b).

3.5. Paint particles in sediments

APPs are residues that contain toxic biocides generated during vessel maintenance and removal of old antifouling coatings of boat hulls and other submerged structures (Soroldoni et al., 2018; Turner, 2010). Once incorporated into sediments, APPs can act as a significant log-term secondary source of contemporary and legacy biocides, and likely be toxic to benthonic organisms (Muller-Karanassos et al., 2021; Soroldoni et al., 2020; Ytreberg et al., 2010). In the present study, APPs (> 500 μm) levels ranging between 9.9 and 2935 μg g^{-1} were observed in 15 out of 19 sediment samples from the Yucatán Peninsula (Table 4). Overall, the highest APPs levels were found in marinas, fishing harbors and shipyards. Levels > 1600 μg g^{-1} were found at Y1 (fishing harbor) and C3 (fishing harbor and shipyard), which are associated to boat maintenance activities. Relevant amounts of these residues (> 100 μg g^{-1}) were also found in a marina area (site Y5), fishing harbors (sites Y2, Y3, Y8 and Y11) and shipyard (site Y4). A recent study showed that paint fragments in coastal sediments from Campeche State are probably attributed to fisheries and boat maintenance activities (Ramirez et al., 2019). Thus, the present results suggest that these sites are acting as potential sources of APPs to coastal environments and, consequently, as sources of biocides and other compounds present in antifouling paint formulations (Gaylarde et al., 2021). Indeed, the occurrence of APPs in coastal sediments influenced by fishing harbors, marinas and boatyards has been reported in other regions (Abreu et al., 2021a, 2020; Soroldoni et al., 2018).

Butyltins were detected in 5 out of 9 samples of APPs, with levels varying from ≤ 100–3829 ng Sn g^{-1} for TBT, ≤ 100–3171 ng Sn g^{-1} for DBT and ≤ 100–15,366 ng Sn g^{-1} for MBT, while phenyltins were all ≤ 100 ng Sn g^{-1} (Table 4). TBT and DBT were detected in APPs from Y4 (shipyard), the site with the highest butyltin levels in sediments; DBT and MBT were detected in APPs from Y3 and Y8; and MBT only was

Table 4
Occurrence (μg g^{-1}) of antifouling paint particles (APPs) in sediment samples from the Yucatán Peninsula, and butyltin (TBT, DBT, MBT) and phenyltin (TPhT, DPhT, MPhT) levels (ng Sn g^{-1}), and booster biocides (Diuron, Irgarol, Chlorothalonil (Chlor), Dichlofluanid (Dichlo) and DCOIT) levels (ng g^{-1}) in the corresponding APPs.

Site	APPs (μg g^{-1})	Butyltin levels (ng Sn g^{-1})			Phenyltin levels (ng Sn g^{-1})			Booster biocides (ng g^{-1})				
		TBT	DBT	MBT	TPhT	DPhT	MPhT	Diuron	Irgarol	Chlor	Dichlo	DCOIT
Q8	≤ 0.01	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Q9	≤ 0.01	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Y1	1609.2	≤ 100	≤ 100	≤ 100	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
Y2	175.0	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
Y3	164.8	≤ 100.0	967.3	906.3	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
Y4	300.7	3828.7	2581.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
Y5	138.8	≤ 100.0	≤ 100.0	1641.6	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	7074.7
Y8	190.1	≤ 100.0	3170.7	2653.8	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
Y9	≤ 0.01	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Y11	231.5	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
Y12	14.6	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
C1	41.4	≤ 100.0	≤ 100.0	15365.9	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
C2	9.9	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
C3	2935.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
C4	32.1	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	4001.4
C5	37.9	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 100.0	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
C8	28	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
C10	30.2	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	≤ 200	≤ 200	≤ 200	≤ 900	≤ 800
C11	≤ 0.01	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. = not analyzed (lack of APPs); < LOD = ≤ 100 ng Sn g-1 (TBT, DBT, MBT, TPhT, DPhT, MPhT), < LOQ = ≤ 500 ng Sn g-1 (TBT, DBT, MBT, TPhT, DPhT, MPhT); < LOD and < LOQ for booster biocides were 1000 times higher than reported for sediments.

detected in APPs from Y5 and C1. These findings together with sediment results reinforce the hypothesis that TBT-based antifouling paints are still being trade and used in this region, as shown by Uc-Peraza et al. (2022). These authors found that TBT-based antifouling products have been offered for sale in Quintana Roo State (Mexican Caribbean), pointing that current international legislation against TBT usage has not been effective in Mexico. However, it cannot be ruled out that APPs presenting high TBT levels may have been generated from historical applications. Regarding booster biocides in APPs, concentrations were ≤ LOD in most sites, but DCOIT levels were 4001 and 7075 ng g^{-1}, respectively, in C4 and Y5 (Table 4). Sites Y5 and C4 were under the influence of traffic of fishing/recreational boats and activities associated with maintenance and repair of vessels. As already pointed out by Batista-Andrade et al. (2018) in Panama, by Soroldoni et al. (2017) in Southern Brazil, and by Abreu et al. (2021a), (2020) in Southeastern Brazil, APPs are highly toxic residues releasing associate compounds to the sediment layers, particularly butyltins and DCOIT in the case of the study area.

3.6. Data integration

Chemical contamination along coastal zones may be affected by the intensity of anthropogenic sources in combination with local hydrodynamics and biogeochemical factors (Abreu et al., 2020; Ruiz et al., 1996). In the case of antifouling biocides, different types of maritime activities such as commercial and recreational navigation, oil extraction, fisheries, tourism and the presence of vessel maintenance facilities (dockyards) can also affect contamination levels in the investigated region. In relatively confined zones, such as fishing harbors and marinas areas (e.g., sites Q2, Q6, Q7, Y1, Y2, Y3, Y4, Y5, Y8, Y9, Y11, C3, C4, C5 and C10), water exchange with open waters is limited, leading to increase of biocide concentrations in the water column and consequent accumulation in sediments, which may end up causing adverse effects on local marine organisms (e.g., imposex in marine gastropods – sites Q2, Q6, Q7, Y7, Y8, C4 and C7). Under these conditions, APPs generated at sites Y1, Y4, Y11 and C3 can reach the sediments due to their high settling velocity associated to the low turbulence in these sites (Soroldoni et al., 2018). Thus, sites under the influence of fishing harbors and marinas presented higher levels of biocides, APPs and %COT in sediments. Moreover, in the Caribbean region, the Yucatán current (YC) is able to transport contaminants released from ship and boat traffic areas (e.g., Playa del Carmen and Cozumel) to the northeastern coast of the Yucatan Peninsula (Puerto Morelos – Q2) (Jordán-Dahlgren et al., 2005). However, counter-currents that flow southward along the coast of Quintana Roo may also play a role in this transportation (Suárez-Morales and Rivera-Arriaga, 1998). Hence, the antifouling residues could be transported to other sites, as observed at Q1, a Marine Reserve that probably received inputs released nearby (e.g., Q2). Organotins and booster biocides can also be re-introduced into the water column through resuspension of contaminated sediments. Local hydrodynamics, such as tides and high speed winds, or even the maritime traffic and eventual dredging activities, can resuspend local sediments and thus favor the desorption of antifouling residues (Buggy and Tobin, 2006; Lam et al., 2017).

Using a PCA to further analyze the data variance, the first two principal components (PC1 – 44.9% and PC2 – 12.2%) explained 57.1% of the total variance (Fig. 2). The most import variables at PC1 were TBT (loading value: 0.35), DBT (0.34), ΣOTCs (0.34), ΣBTs (0.33), ΣPhTs (0.31), MPhT (0.30), MBT (0.29), TPhT (0.27), APPs (0.26) and DPhT (0.23), all of them associated with tin-based antifouling system or pesticide use (e.g., TPhT). PC2 was mainly related to dichlofluanid (0.49), diuron (0.39), DCOIT (0.33), Irgarol (0.28) and chlorothalonil (0.15), which are used in contemporary antifouling paint formulations, while diuron has also been used as a pesticide in the region. The PCA showed that levels of organotins, APPs and %COT in sediments were associated with sites (e.g., Y4 and C3) under specific conditions such as coastal morphology, hydrodynamics, and anthropogenic pressure (e.g., fisheries and boat maintenances), among others. Furthermore, booster biocides were associated to sites under the influence of harbors, marinas and multiple activities (e.g., Y3, Y5, Y8, Y9, Y11, C1, C4 and C5), which were contaminated by Irgarol, diuron, DCOIT chlorothalonil and dichlofluanid.

4. Conclusions

Fishing harbors, marinas, and shipyards areas were identified as sources of antifouling residues, particularly of butyltins, Irgarol, diuron, chlorothalonil, DCOIT and APPs, in the Yucatán Peninsula. Furthermore, imposex incidence in five caenogastropod species was observed in 13 out of 17 studied sites, indicating that TBT levels are still producing deleterious effects on marine organisms. Levels of butyltins were higher than phenyltins in sediment, and the highest organotin concentrations were registered at sites under the influence of fishing and boat maintenance activities. Thus, the widespread occurrence of OTCs in sediment, especially butyltins, suggests that the international restrictions against the use of tin-based antifouling paints may not be completely effective in this region. Chlorothalonil was the most frequently detected booster biocide in sediment samples, while DCOIT was the most abundant. Both biocides were mainly found in sediments from sites under the direct influence of fishing harbors and marinas, regardless of being vessel maintenance areas. APPs were registered in 15 out of 19 studied sites, and the highest levels (> 1600 μg g^{-1}) were found in sites under the

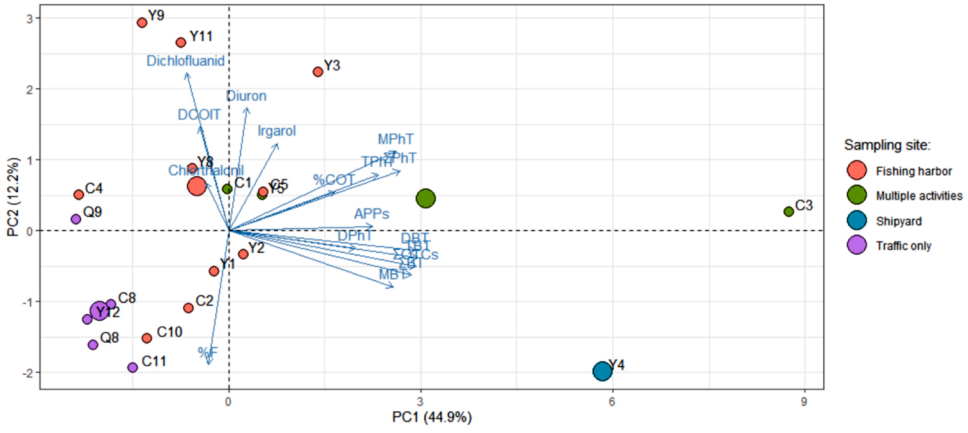

Fig. 2. Principal component analysis (PCA) of contaminants (organotins, booster biocides and APPs) and other parameters (Percentage of fine fraction - %F and total organic carbon - %COT) in sediments related to anthropogenic activities in the Yucatán Peninsula, Mexico.

influence of fishing harbors. In addition, butyltins and DCOIT were measured in APPs, suggesting that these residues may be a source of contemporary and legacy biocides in the investigated sites. In general, TBT, Irgarol, diuron and DCOIT levels exceed the threshold values established by international sediment quality guidelines, indicating that adverse effects could be expected in some studied areas, thus posing a serious environment threat. Finally, Mexico urgently needs to adopt restrictive actions in order to protect marine life in the Yucatán Peninsula.

CRediT authorship contribution statement

Russell Giovanni Uc-Peraza: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft preparation, Writing – original draft, Writing – review & editing preparation. **Victor Hugo Delgado-Blas:** Investigation, Writing – review & editing preparation. **Jaime Rendón-von Osten:** Investigation, Writing – review & editing preparation. **Maíra Carneiro Proietti:** Writing – review & editing, Supervision. **Ítalo Braga Castro:** Conceptualization, Investigation, Formal, Writing – original draft preparation, Writing – review & editing. **Gilberto Fillmann:** Conceptualization, Investigation, Writing – original draft preparation, Writing – review & editing, Supervision, Funding acquisition.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.128162.

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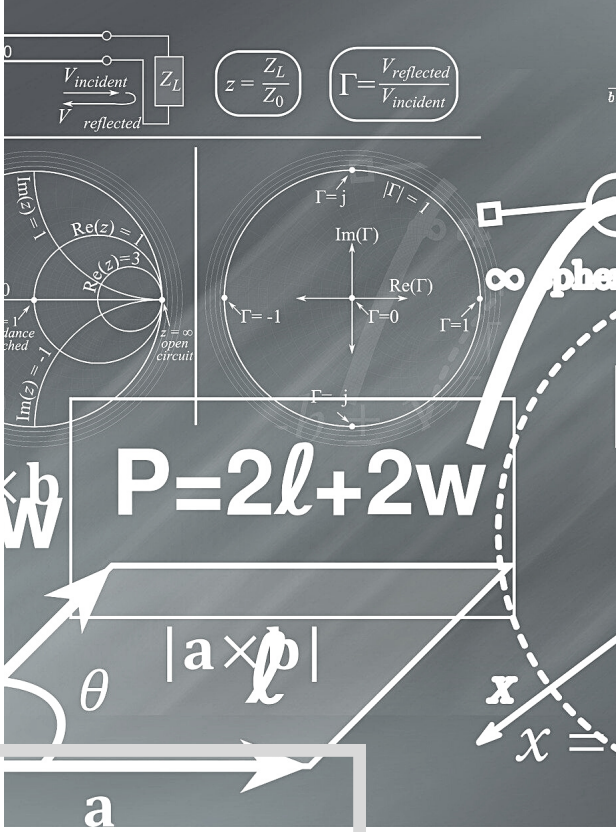

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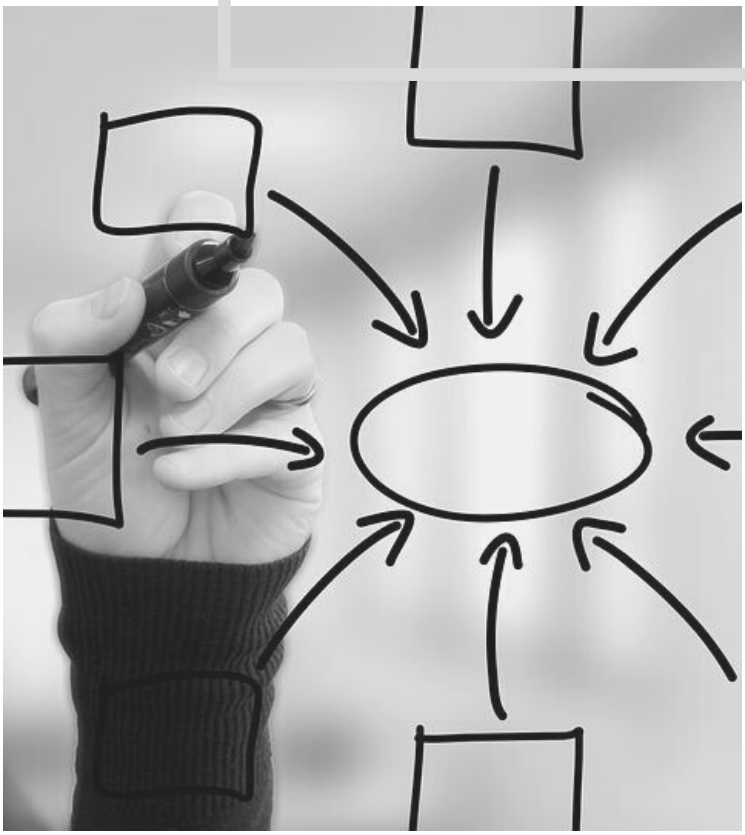

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Especies Marinas Comerciales de las Pescaderías de la Ciudad de Chetumal, Quintana Roo, México

Dra. Jennifer Denisse Ruiz Ramírez[1], Dr. Víctor Hugo Delgado Blas[2] y
Dr. Adrián Cervantes Martínez[1]

***Resumen*—** La explotación pesquera en Quintana Roo, está dirigida principalmente hacia recursos de alto valor económico como la langosta, el caracol rosado, la escama y el tiburón. Debido a las actividades de sobrepesca y otros factores, ha ocasionado que el abastecimiento de los recursos marinos sea insuficiente para la población. El objetivo fue investigar y conocer las especies de peces, moluscos y crustáceos que se comercializan en Chetumal, y su lugar de procedencia. Los resultados muestran que las pescaderías ofrecen en total 29 especies marinas, de las cuales 16 corresponden a escama, 7 a moluscos y 6 a crustáceos. En Quintana Roo se concentran tres sitios de extracción: Banco Chinchorro, Punta Herrero, e Xcalak; y el resto lo compensan los Estados de Yucatán, Campeche y Veracruz. Se puede concluir, que las pescaderías ofrecen productos que son del gusto del público, pero se necesita el abastecimiento de otros Estados.

***Palabras clave*—** Caribe Mexicano, Crustáceos, Moluscos, Peces, Pescaderías

Introducción

Desde ya hace algunas décadas, la explotación pesquera en Quintana Roo, es dirigida principalmente hacia recursos de alto valor económico como la langosta (*Panulirus argus*), el caracol rosado (*Strombus gigas*), de escama (peces pertenecientes a diversas familias) y tiburón (Camarena-Luhrs y Salazar-Vallejo, 1991). Debido a las actividades de sobrepesca, pesca furtiva, vedas permanentes, aumento de la población y crecimiento de hoteles y restaurantes, el abastecimiento de los recursos marinos provenientes de las costas del Estado es insuficiente para el abastecimiento de la población en general. Las especies tienen mayor demanda y son altamente consumidos, principalmente los fines de semana, y el suministro y consumo se incrementan en las vacaciones de Semana Santa y de Verano.

El objetivo principal fue investigar y conocer cuáles especies de peces, moluscos y crustáceos se venden en las pescaderías de Chetumal, de los principales mercados de la ciudad y el lugar de procedencia de los mismos.

Descripción del Método

Las pescaderías de la ciudad de Chetumal se concentran en los mercados Lázaro Cárdenas (Nuevo) e Ignacio Manuel Altamirano (Viejo), por lo que se procedió a entrevistar a los dueños de los locales expendedores, se elaboró una lista de las especies que expenden y el sitio de extracción de donde los obtienen. Para cada etapa, se tienen:

Expendios de pescados y "mariscos"

Se visitaron las pescaderías de ambos mercados para conocer cuáles especies de peces, moluscos y crustáceos se comercializan, para ello se tomaron los nombres comunes de las especies, en algunos casos se cuestionó la venta de especies que estaban en época de veda, así como el lugar de procedencia de las mismas (Figura 1). Para ello, se aplicaron encuestas dirigidas a los expendedores de los dos principales mercados de la ciudad de Chetumal.

Consulta bibliográfica y base de datos

Se revisó la literatura para encontrar los nombres científicos de las especies en general. Se consultaron el Catálogo de especies marinas de interés económico actual o potencial para América Latina (1982), las listas de peces e invertebrados en Biodiversidad Marina y Costera de México (1993), las listas de fauna marina contenida en el Programa de Manejo Parque Marino Nacional Arrecifes de Cozumel (1998), la lista de crustáceos y peces arrecifales de Cozumel (2008) y la base de datos para peces en línea (www.fishbase.org)

Principales sitios de extracción

Se anotaron los sitios de extracción de las especies marinas comerciales, obtenidos de los datos ofrecidos por los locatarios, tanto del Estado de Quintana Roo, como de otros estados del país.

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Figura 1. Pescadería Huachinango (Foto de: Punto de quiebre, 2022).

Resultados

Las pescaderías de los mercados de la ciudad de Chetumal ofrecen a los usuarios, pescados y "mariscos" que incluyen: moluscos (caracol, pulpo, almeja, mejillones, ostiones y chivitas) y crustáceos (langosta, jaiba y camarón). En total son 29 especies marinas que se comercializan en Chetumal, de las cuales 16 corresponden a escama, 7 a moluscos y 6 a crustáceos (Tabla 1).

Clasificación taxonómica	**Nombre común**	**MN**	**MV**	**Lugar de procedencia**
FILO MOLLUSCA				
Clase Bivalvia				
Mercenaria sp	Almeja		X	Coatzacoalcos, Veracruz
Brachidontes recurvus (Rafinesque, 1820)	Mejillón		X	Coatzacoalcos, Veracruz
Crassostrea virginica (Gmelin, 1791)	Ostión	X	X	Cd. del Carmen, Campeche, Veracruz
Clase Gastropoda				
Strombus gigas Linneo, 1758	Caracol rosado	X	X	Banco Chinchorro
Melongena corona bispinosa Philippi, 1844	Chivita		X	Yucatán
Clase Cephalopoda				
Octopus vulgaris Cuvier, 1797	Pulpo	X	X	Champotón Campeche
Loligo vulgaris Lamarck, 1798	Calamar	X		ND*

SUBFILO CRUSTACEA				
Clase Malacostraca				
Penaeus californiensis Holmes, 1900	Camarón	X	X	Campeche, Veracruz Coatzacoalcos, Veracruz
Penaeus setiferus (Linneaus, 1767)	Camarón blanco	X		Campeche, Veracruz
Antepenaeus californiensis Holmes, 1900	Camarón café	X	X	Champotón, Campeche Tampico
Penaeus duorarum Burkenroad, 1939	Camarón rosado	X	X	Campeche, Veracruz Champotón, Campeche
Callinectes sapidus Rathbun, 1896	Jaiba	X	X	Isla Aguada, Campeche Champotón, Campeche
Panulirus argus Latreille, 1804	Langosta		X	Banco Chinchorro
SUBFILO VERTEBRATA				
Peces				
Clase Chondrichthyes				
Rhisoprionodon terraenovae (Richardson, 1836)	Cazón	X	X	Punta Herrero, Yucatán Xcalak
Pristiophorus schroederi Springer & Bullis, 1960.	Sierra	X	X	Candelaria, Campeche
Clase Actinopterygii				
Mycteroperca microlepis (Goode & Bean, 1879)	Abadejo	X	X	Punta Herrero, Yucatán Xcalak
Lachnolaimus maximus (Walbaum, 1792)	Boquinete		X	Campeche; Celestún Yucatán
Epinephelus itajara (Lichtenstein, 1822)	Cherna	X	X	Punta Herrero, Yucatán Xcalak
Haemulon plumierii (Lacepede, 1801)	Chacchí	X	X	Huay Pix
Cyprinodon julimes (De la Maza-Benignos y Vela-Valladares, 2009)	Chihua	X	X	Punta Herrero, Yucatán Banco Chinchorro
Lutjanus campechanus (Poey, 1860)	Huachinango	X	X	Campeche
Mugil curema Valenciennes, 1836	Lisa o liseta	X		Celestún, Campeche
Epinephelus striatus (Bloch, 1792)	Mero	X		Xcalak
Eugerres plumieri (Cuvier, 1830)	Mojarra rayada	X		Campeche
Cichlasoma urophthalma (Günther, 1862)	Mojarra de río	X	X	Mahahual, Punta Herrero
Gerres cinereus (Walbaum, 1792)	Mojarra blanca		X	Celestún, Progreso, Yucatán; Punta Herrero
Lutjanus synagris Linnaeus, 1758	Pargo	X	X	Celestún, Campeche
Atractosteus tropicus Gill, 1863	Peje lagarto		X	Tabasco
Sphyraena barracuda (Walbaum, 1792)	Picuda	X		Mahahual
Centropomus undecimalis (Bloch, 1792)	Robalo		X	Punta Herrero, Yucatán
Oreochromis aureus (Steindachner, 1864	Tilapia	X	X	Sergio Butrón, Felipe Carrillo Puerto, Noh bec

Cuadro 1. Especies de peces, moluscos y crustáceos que se expenden en las pescaderías de los mercados Lázaro Cárdenas (Nuevo) e Ignacio Manuel Altamirano (Viejo) de la ciudad de Chetumal. * No Determinado
Nota: Mercado Nuevo (MN) y Mercado Viejo (MV)

Por otro lado, ambas pescaderías reciben parte de su venta de tres sitios de extracción del Estado de Quintana Roo que se concentran en:

- Banco Chinchorro: Se encuentra a unos 30 km frente a Mahahual, es considerado un atolón arrecifal de aproximadamente 700 km^2, tiene una laguna somera con parches de coral de diversos tamaños y zonas con pastos marinos. En el centro se localiza el Cayo Centro, al norte el Cayo Norte y al sur el Cayo Lobos. En la región del centro existe el ecosistema de mangle principalmente.

- Punta Herrero: Se localiza al norte de Mahahual con una laguna somera, cuyo ancho varía entre decenas y centenas de metros y una barrera arrecifal discontinua, con pastos marinos.

- Xcalak: Se localiza al sur de Mahahual, cuenta con una laguna somera, donde continúa la barrera arrecifal, en su frente se encuentra el Hoyo Azul y pastos marinos.

En menor proporción están Huay Pix, Mahahual y Candelaria; de Sergio Butrón, Felipe Carrillo Puerto y Noh bec, se obtiene a la tilapia como práctica de acuacultura. El resto de las especies lo compensan los Estados de Yucatán (Celestún y Progreso), Campeche (Champotón, Ciudad del Carmen e Isla Aguada) y Veracruz (Puerto y Coatzacoalcos), Tabasco y Tampico con una sola especie.

Comentarios Finales

Resumen de resultados

En este trabajo se resaltó el número de especies comerciales que se consumen en la ciudad de Chetumal, poniendo en evidencia que la demanda del consumo es tan alta, que el Estado provee insuficiente cantidad de especies, y éstas deben ser transportadas de otros estados del país. Además, las especies preferidas se concentran en los peces, moluscos (caracol, pulpo, almeja, mejillones, ostiones y chivitas) y crustáceos (langosta, jaiba y camarón).

Conclusiones

Ambas pescaderías ofrecen pescados, moluscos y crustáceos que son del gusto del público, pero debido a que las pesquerías en Quintana Roo son insuficientes para el abasto de productos marinos, es necesario conseguirlos de otros Estados, principalmente de Yucatán, Campeche y Veracruz, con aportación de Tabasco y Tampico. Es importante mencionar que, a lo largo del año, hay suficiente abastecimiento de los mismos, pero se complica en las vacaciones de Semana Santa, donde el público se queja mayormente sobre el aumento de los precios, que también coinciden con las vedas impuestas a las especies con categorías en peligro de extinción o amenazadas. En el caso específico de la tilapia, existen en el Estado de Quintana Roo, tres centros dedicados a la acuacultura rural o de subsistencia y que ha tenido aceptación entre el público.

Recomendaciones

Se sugiere realizar un estudio de las preferencias de las especies a consumir, así como el volumen de demanda por cada especie, a fin de conocer si han habido cambios significativos en las poblaciones marinas debido al aumento de la temperatura en el medio marino. También se podría proponer establecer programas de acuacultura para solventar la demanda de las especies marinas en la ciudad de Chetumal y con ello, ejercer menor presión de captura en las especies silvestres.

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Notas Biográficas

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Redescription of *Microspio moorei* (Gravier, 1911) (Annelida: Spionidae) with inclusion of a taxonomic key for all the species of the genus

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Abstract

Microspio moorei (Gravier, 1911), described from Admiralty Bay, King George Island (Antarctic Peninsula), has not been recorded since the original description, based on a single specimen, the holotype. Thus, subsequent comments and observations about the species have been made based only on the original description and with no information on morphological variations of the species. A re-description of this species is presented based on new material collected from a bay near the type locality, with detailed descriptions and illustrations of morphological characters. A key to all species of *Microspio* Mesnil, 1896 is provided. This research was part of the Colombian Antarctic Program, in collaboration with the Chilean Antarctic Institute (INACH).

Key words: Taxonomy, Antarctic Peninsula, morphological variation, Programa Antártico Colombiano

Introduction

Polychaetes from Antarctica have been poorly studied in both space and time, due to the relatively recent knowledge about the biodiversity of this continent. The study of Antarctic polychaetes began with the Challenger Expedition by McIntosh (1885). However, no species belonging to the family Spionidae were registered or newly described from this region during the Challenger Expeditions.

The study of the Antarctic spionids began with Gravier (1911b) with the French Expedition "Pourquoi-Pas", erecting the genus *Mesospio*, and describing the first new Spionidae species, *Mesospio moorei*, from Admiralty Bay, King George Island, South Shetland Islands (Antarctic Peninsula) (Gravier 1911a). Later, Ehlers (1912) described *Nerinopsis hystricosa* from Kaiser Wilhelm II Land, Davis Sea (Australian Antarctic Region, Eastern Antarctica), now considered *taxon inquirendum*, because it was based on a spionid planktonic larva (Read & Fauchald 2021). Augener (1932) reported *Mesospio moorei* from Deception Island, South Shetland Islands (Antarctic Peninsula), which became the most southern distribution for the species, and Blake (1983) mentioned that the Spionidae of Antarctica were represented by only 10 genera and 16 species, including *Microspio moorei*.

The genus *Microspio* has 21 valid species, described from worldwide locations, mainly from tropical waters (Maciolek & Blake 2021). The first species described was *M. mecznikowianus* (Claparède, 1869) from the Mediterranean Sea. Additional species have been described from the southern hemisphere subtropical region as *M. elegantula* Blake, 1984 from New Zealand.

In terms of its ecology, Sicinski (2004) commented, referring to an assemblage named Group D "*Microspio moorei*", that *M. moorei* was found as one of the most abundant polychaetes, in the sublittoral zone in King George Island. Petti *et al.* (2006), in a bathymetric distribution study in King George Island, found *M.* cf. *moorei* with low abundance at 6 m (3 individuals/10 cm^2) and with very low abundance at 18 m (1 individual/10 cm^2), suggesting that the species is likely to be found in shallow water. Barbosa *et al.* (2010) reported three species of spionids (*Mi-*

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crospio sp., *Scolelepis eltaninae*, and *Pygospiopis dubia*) during the summer 2003–2004. They mentioned that *M. moorei* (also considered *M*. cf. *moorei*) has been reported by several studies about the polychaete composition and distribution from Admiralty Bay, in shallow waters (Sicinski 1986; Wägele & Brito 1990; Sicinski 1993; Sicinski & Janowska 1993; Bromberg *et al.* 2000; Sicinski 2000; Echeverría *et al.* 2005).

Pabis & Sicinski (2010a) found *M. moorei* with very low abundance, and density, among the polychaetes associated with holdfasts of *Himantothallus grandifolius* (Phaeophyceae) in Admiralty Bay, between 10 and 75 m depth. They considered this spionid as a surface deposit feeder. In shallow waters, Pabis & Sicinski (2010b) found this species as the most abundant infaunal polychaete, together with *Apistobranchus glacierae* Hartman, 1978 (Apistobranchidae). Sicinski *et al.* (2011), although incorrectly calling this species *Mesospio moorei*, reported it as very common and abundant at 5–40 m depth.

No other ecological or taxonomic studies have been made recording this species. Material belonging to *M. moorei* presented above as *Microspio* cf. *moorei*, *Microspio* sp. or *Mesospio moorei*, might be in museum collections, but these were not available as loan for this study. The lack of morphological boundaries for the species is evident, resulting in it being reported under different designations. The focus of this paper is to improve the taxonomy of *M. moorei*, by redescribing known characters and documenting morphological variations. At the same time, the geographical distribution is confirmed by the material collected from Fildes Bay, which is contiguous with Admiralty Bay, the type locality.

Materials and methods

Abbreviations

ANNE	Annelida
CCO	Comisión Colombiana del Océano (In English: Ocean Colombian Comission).
CEMUA	Colección Estuarina y Marina, Universidad de Antioquia, Medellin, Colombia.
INACH	Instituto Antártico Chileno (Chilean Antarctic Institute).
MNHN	Muséum National d'Histoire Naturelle, Paris, France.
PAC	Programa Antártico Colombiano (Colombian Antarctic Program).
UDEA	Universidad de Antioquia, Medellin, Colombia

Study site. Live specimens of *M. moorei* were sampled at the Fildes Bay (62º12'31,32"S 58º57'45,86"W), King George Island, Western Antarctic Peninsula, at 30 km from the type locality, Admiralty Bay (Fig. 1A–C), in March 2017 during the Third Colombian Scientific Expedition to Antarctica "Almirante Padilla" Austral Summer 2016–2017. This expedition was coordinated by the PAC, led by the CCO, in collaboration with the INACH, with funds from the University of Antioquia (Medellín, Colombia).

Sampling method. Benthic samples were taken in the subtidal zone during low tide at 0.5–0.8 m in sandy to muddy substrate. Samples were taken with a shovel and the substrate placed into a 0.015 m^3 plastic bucket; then, with the container full of water, the sediment was resuspended by hand with circular movements, in order to get the soft sediment and animals floating in the water, which was then poured through a 240-µm-pore sieve. The worms were taken with tweezers directly from the sieve and transported in small plastic containers with seawater to the cold laboratory in the Chilean Antarctic Scientific Base "Profesor Julio Escudero" (INACH). Physicochemical parameters (oxygen, temperature and conductivity), as well as geographical data (coordinates) were registered *in situ*. Specimens for morphological studies were relaxed in 10% magnesium chloride ($MgCl_2$) in seawater; they were photographed using a Canon G9 camera on both dissecting and compound microscopes. Afterwards, these specimens were fixed in formalin, and preserved in 70% ethanol. All the specimens were deposited at the Collection CEMUA, in Medellin, Colombia.

Taxonomic treatment. A preliminary taxonomic identification was carried out at the Chilean Antarctic Scientific Base to establish the species identity of the samples. Specimens were preserved in 70% ethanol and transported to the laboratory in Colombia. Drawings and measurements of chaetae were made using a camera lucida attached via a Martin Microscope Universal adapter to a Olympus SZ stereomicroscope. The best and complete specimens were selected for the redescription; the remaining material was used for morphological variation and slide preparations. Since type material consists of only the holotype (MNHN-IA-TYPE0264) and that was not available for this

study, the organisms herein studied were compared with the literature (Foster 1971; Blake & Kudenov 1978; Blake 1983).

Total body length and thoracic width at the 14th to 17th chaetigers (excluding chaetae) were measured on 34 complete specimens. All measurements are given in millimeters using a scale reticule in the eyepiece of the microscope.

Simple and multivariate regression analyses were carried out to determine if the first appearance of hooded hooks is size dependent or not. The means and standard deviations of the body length and width, classified by the segment with the first appearance of the hooded hook, were calculated. A significance level of 0.05 was used in all test procedures. The P values were calculated using a null model with 1000 permutations. All statistics were performed using the software R version 4.0.4 (R Core Team 2021) and Rstudio version 1.4.1103 (Rstudio Team 2021).

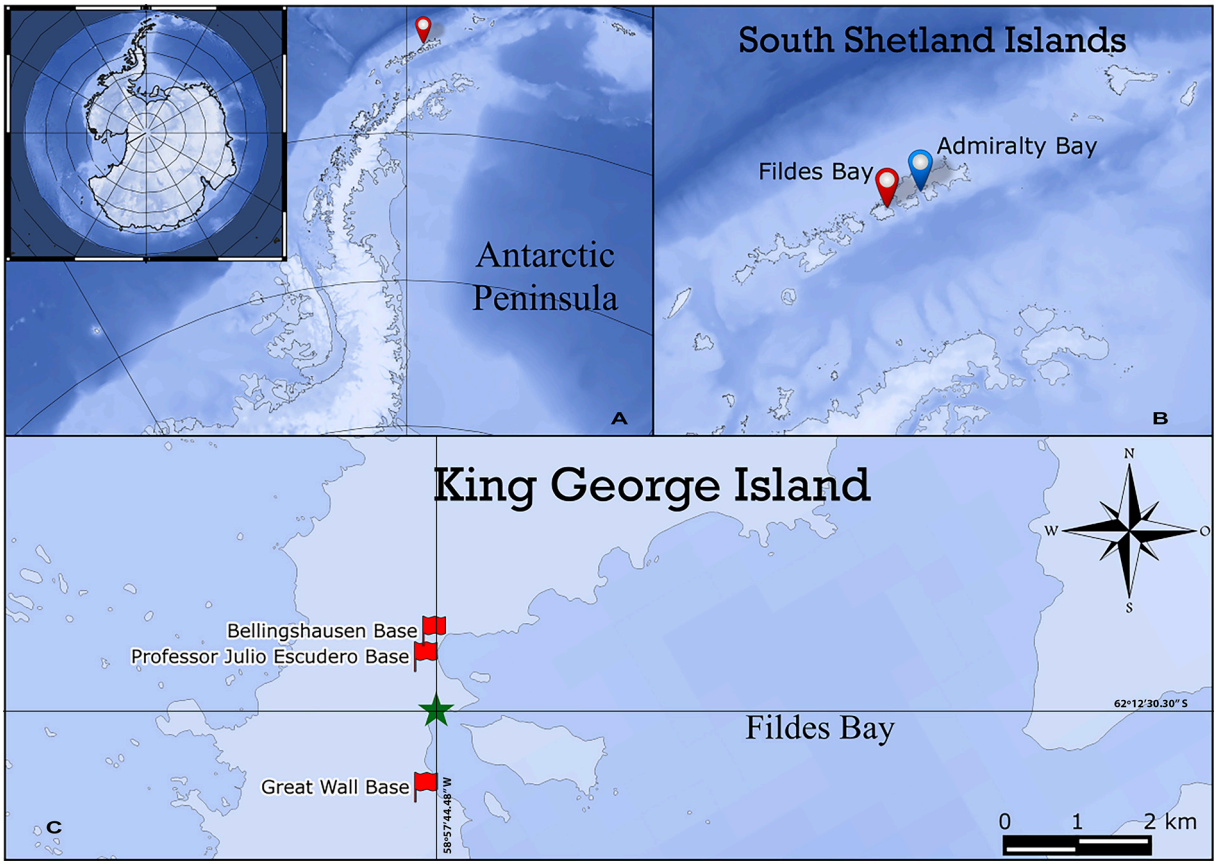

FIGURE 1. **A.** Antarctic Peninsula showing King George Island (Red Mark). **B.** South Shetland Islands: Fildes Bay, sampling bay (Red Mark); Admiralty Bay, Type locality of *Microspio moorei* (Blue Mark). **C.** Fildes Bay: Red flags indicate Antarctic Bases (Russian: Bellingshausen Base; Chinese: Great Wall Base; Chilean: Professor Julio Escudero Base). Green star indicates collecting site for *M. moorei* samples.

Results

Family Spionidae Grube, 1850

Genus *Microspio* Mesnil, 1896

Microspio Mesnil, 1896: 116, 119; type species: *Spio mecznikowianus* Claparède, 1869, (type by subsequent designation (Söderström, 1920)).
Mesospio Gravier, 1911: 313; type-species: *Mesospio moorei* Gravier, 1911, by monotypy. Fide Foster, 1971.

Diagnosis. Prostomium anteriorly rounded; or broadly rounded, only weakly incised; or conical or bilobed or bifid

(deeply incised along anterior margin, producing two widely diverging lobes), frontal or lateral horns absent; eyespots present or absent; occipital antenna present or absent. Peristomium reduced or well developed, not forming lateral wings. Nuchal organ with short median and long lateral ciliary bands, extending to chaetiger 2 or 3, sometimes an oval or U-shaped ciliated groove; metameric dorsal ciliated organs often present. Transverse ciliary bands present between bases of branchiae on some species. Branchiae from chaetiger 2, limited to anterior region of body or continuing to posterior end, partly fused or free to bases of anterior postchaetal notopodial lamellae and free on posterior lamellae. Ventral epidermal glands present or absent. Intensely pigmented with brown patches on prostomium, peristomium and branchiae, or/and spots scattered on dorsum continuing laterally and ventrally, or absent. Notopodium of chaetiger 1 present or absent; chaetiger 1 with or without notochaetae; with or without additional superior fascicle of long, capillaries from chaetiger 1 and continuing to middle chaetigers; notochaetae and anterior neurochaetae all capillaries. Neurochaetae include capillaries, hooded hooks; ventral sabre chaetae on middle and posterior chaetigers or absent. Hooded hooks bi-, tri-, or multidentate; hooks with a fang surmounted by unpaired or paired teeth; hooks with the hood closed, or completely or partially open. Pygidium with 2–4 anal cirri.

Remarks: *Microspio* was erected by Mesnil (1896) in order to include *Spio mecznikowianus* Claparède, 1869. *Mesospio* was erected by Gravier (1911b) to include *Mesospio moorei* Gravier, 1911; later, *Mesospio* was synonymyzed with *Microspio* by Foster (1971). *Microspio* is very closely related to *Spio*, they are distinguished mainly by having branchiae present from the chaetiger 2 in *Microspio* and from chaetiger 1 in *Spio*. Currently, the genus is recognized as having 21 species. The last revision of *Microspio* was made by Maciolek (1990), who recognized 15 species as valid. Blake (1996) and Zhou *et al.* (2009) added two species, *M. spinosa* Blake, 1996 and *M. multidentata* Zhou, Ji & Li, 2009. Recently, Maciolek & Blake (2021) described three new species, *M. fischeri*, *M. lydonia* and *M. ariena*, including comparative notes for all species in the genus.

Characters regarded as useful based on current information are the shape of the anterior margin of prostomium, presence or absence of caruncle, paired nuchal organs and occipital antenna, pigmentation in any part of the body, presence or absent of noto-lamellae and notochaetae on chaetiger 1, the starting and structure of hooded hooks, the shape of the nuchal organs, presence or absent of sabre chaetae, and the shape of pre- and postchaetal lamellae (Reish 1959; Blake & Kudenov 1978; Blake 1983, 1984, 1996; Maciolek 1990, Zhou *et al.* 2009; Bick & Meißner 2011; Maciolek & Blake 2021). Nevertheless, all characters mentioned above have not been described for some species. Therefore, it would be useful to redescribe them using type material or material from type locality or close to, using both morphological and molecular characters but this is beyond the scope of this study.

Microspio moorei (Gravier, 1911)

Figures 2A–G; 3A–K

Mesospio moorei Gravier, 1911a: 100–105, Plates VII, figs 80–83, VIII, 84–86.— Gravier, 1911b: 313.— Augener, 1932: 39–40.— Hartman, 1966: 17, Plate IV, figs 1–3.— Bellan, 1975: 789.— Blake, 1983: 241.— Sicinski *et al.* 2011: 35, Table 1: 37.
Microspio moorei Foster, 1971: 35.— Maciolek, 1990: 1113–1115, Table 1.
Microspio cf. *moorei* Petti *et al.* 2006: 166, Table 1.— Sicinski *et al.* 2011: 37.
Microspio sp. Barbosa *et al.* 2010: 1158, Table 1.

Material examined. All samples collected in Fildes Bay, King George Island, South Shetland Islands, Antarctic Peninsula; 62º12'31,32"S 58º57'45,86"W: UDEA:CEMUA:ANNE:001594 (8 specimens); 0.3 m depth, low tide. Coll. M. Londoño & I. Fonseca. Feb. 24, 2017. UDEA:CEMUA:ANNE:001595 (7); 0.3 m depth, low tide. Coll. M. Londoño & I. Fonseca. Feb. 24, 2017. UDEA:CEMUA:ANNE:001596 (2); 1.3 m depth low tide. Coll. M. Londoño & I. Fonseca. Feb. 26, 2017. UDEA:CEMUA:ANNE:001597 (9); 1.3 m depth low tide. Coll. M. Londoño & I. Fonseca. Feb. 24, 2017. UDEA:CEMUA:ANNE:001598 (3); 1.3 m depth low tide. Coll. M. Londoño & I. Fonseca. Feb. 24, 2017.

Description. Complete specimens with 4.1–12.3 mm long and 0.5–1.5 mm wide with 36–62 segments. In life and in alcohol, prostomium, peristomium, caruncle, and dorsum of first five chaetigers dark, subsequent segments with diminishing pigmentation, palps dark (Fig. 2A, B), ventral surface of first five segments with dark pigmentation, subsequent segments with pigmentation decreasing gradually, concentrated along midline up to chaetiger 12 (Fig. 2C); in life, specimens pink with visible blood vessel running inside the branchiae (Fig. 2D); ventral epidermal glands absent.

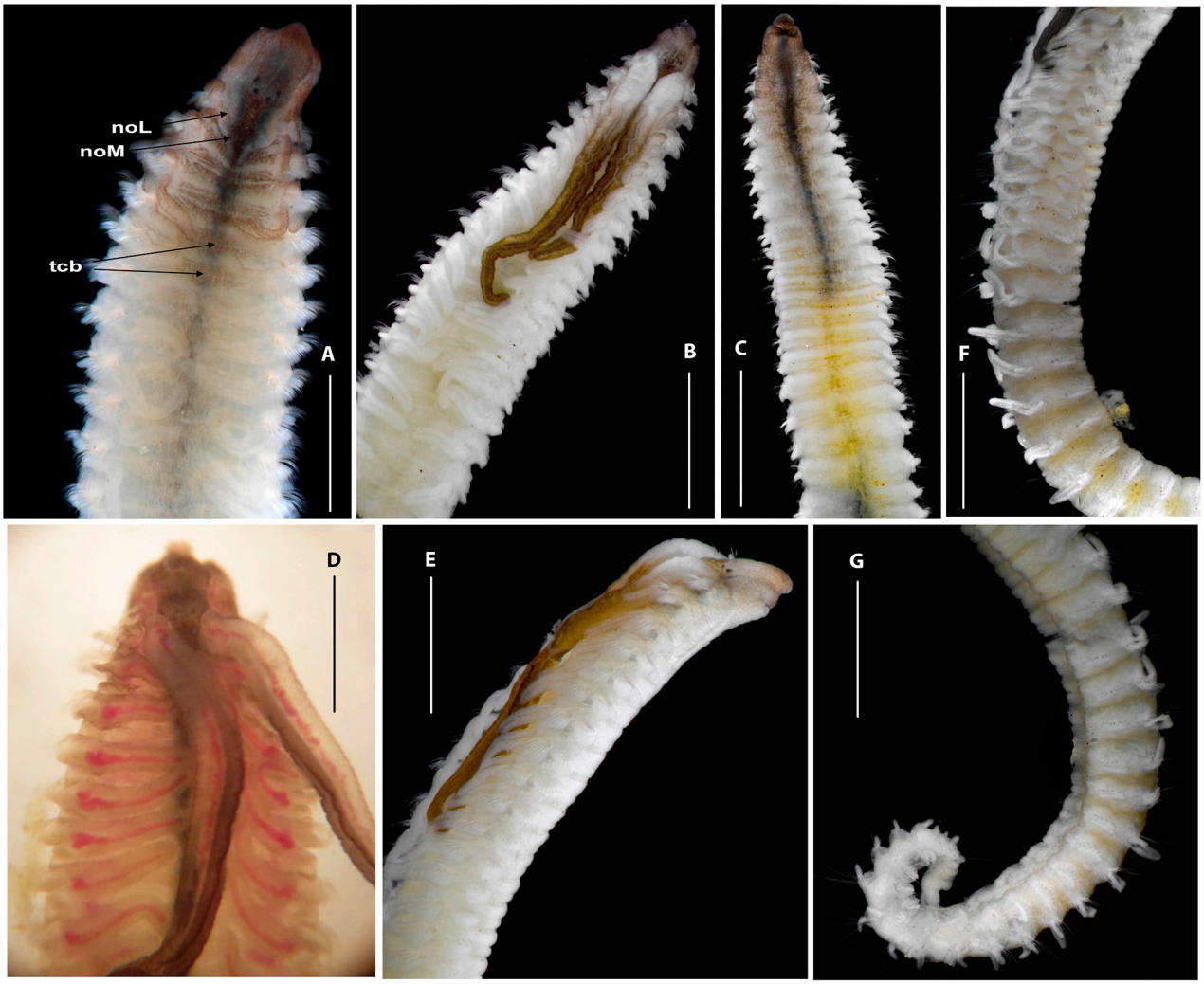

FIGURE 2. *Microspio moorei* (Gravier, 1911): **A.** Anterior region of best fixed specimen from UDEA:CEMUA:ANNE:001594, dorsal view; **B.** Anterior region of best fixed specimen from UDEA:CEMUA:ANNE:001595, dorsal view; **C.** Anterior end, ventral view, UDEA:CEMUA:ANNE:001595; **D.** Anterior end of best live specimen from UDEA:CEMUA:ANNE:001596, dorsal view; **E.** Anterior region, dorsolateral view, UDEA:CEMUA:ANNE:001595; **F.** Parapodium and branchiae from anterior and middle chaetigers, lateral view, UDEA:CEMUA:ANNE:001595; **G**. Posterior end, ventrolateral view, UDEA:CEMUA: ANNE:001595. Abbreviations: noL = nuchal organ, lateral band, noM = nuchal organ, medial band, tcb = transverse ciliary band. Scale bars: **A**–**G**: 1 mm.

Prostomium broadly rounded and tapered anteriorly (Figs 2A, 3A), posteriorly narrow, tapered in a narrow caruncle reaching the base of chaetiger 2 (Fig. 3A), with slightly elevated keel near base of palps (Fig. 3A). Occipital tentacle absent. Two pairs of black eyespots arranged in trapezoid, anterior pair larger, crescent-shaped, widely spaced; posterior pair smaller, rounded, closely spaced (Figs 2A, B, D, 3A). Peristomium long, collar-like, partially enveloping prostomium and extending around base of palps, not forming lateral wings (Figs 2A, 3A, B), separated from chaetiger 1. Palps long, thick, extending to chaetigers 8–11; palps longitudinally grooved, with dark brown pigment along both sides, except basally (Figs 2B, D, E, 3B); palpal sheath short, smooth, fused to anterior base of palps (Fig. 2B, D).

Nuchal organs with medial ciliary bands around caruncle, extending to chaetiger 2, then turning laterally, with small gap between this and the second lateral band. From chaetiger 3, dorsum with two transverse rows of ciliated patches; the first row extending between branchial bases; the second row widely separated from the first, near segmental groove (Figs 2A, 3A), transverse rows of ciliated patches visible (Fig. 2A) up to around chaetiger 22.

Branchiae from chaetiger 2 to almost posterior end; the first pair of branchiae slightly shorter and thinner or as long as those on following chaetigers (Figs 2A, D, 3A, C); longest through mid-body region, reaching dorsal

midline (Fig. 2A, E), then becoming very small; short posteriorly (Fig. 2F); branchiae partly fused at the base with notopodial postchaetal lamellae anteriorly (Figs 2A, B, 3A), increasingly separate from lamellae posteriorly, flattened, robust, elongate, distally rounded (Figs 2A, B, 3A), with long cilia on inner margin.

Notopodial postchaetal lamellae triangular, short on chaetiger 1; lamellae on chaetigers 2–8 small, subtriangular with rounded ventral edge (Figs 2A, E, 3A, C); thereafter becoming oval and slightly decreasing in size throughout the body (Fig. 2F). Notopodial prechaetal lamellae very short, rounded on chaetiger 1, robust, subtriangular on chaetigers 2–9 (Figs 2A, B, 3A, C); subsequent lamellae progressively decreasing in size, becoming round and smaller (Fig. 2F). Neuropodial postchaetal lamellae small, triangular on chaetiger 1 (Fig. 2E); subtriangular on chaetiger 2 (Figs 2E, 3B); subsequent neuropodial lamellae large, rounded, wider (Figs 2E, 3C), up to end of the body (Fig. 2F). Neuropodial prechaetal lamellae absent.

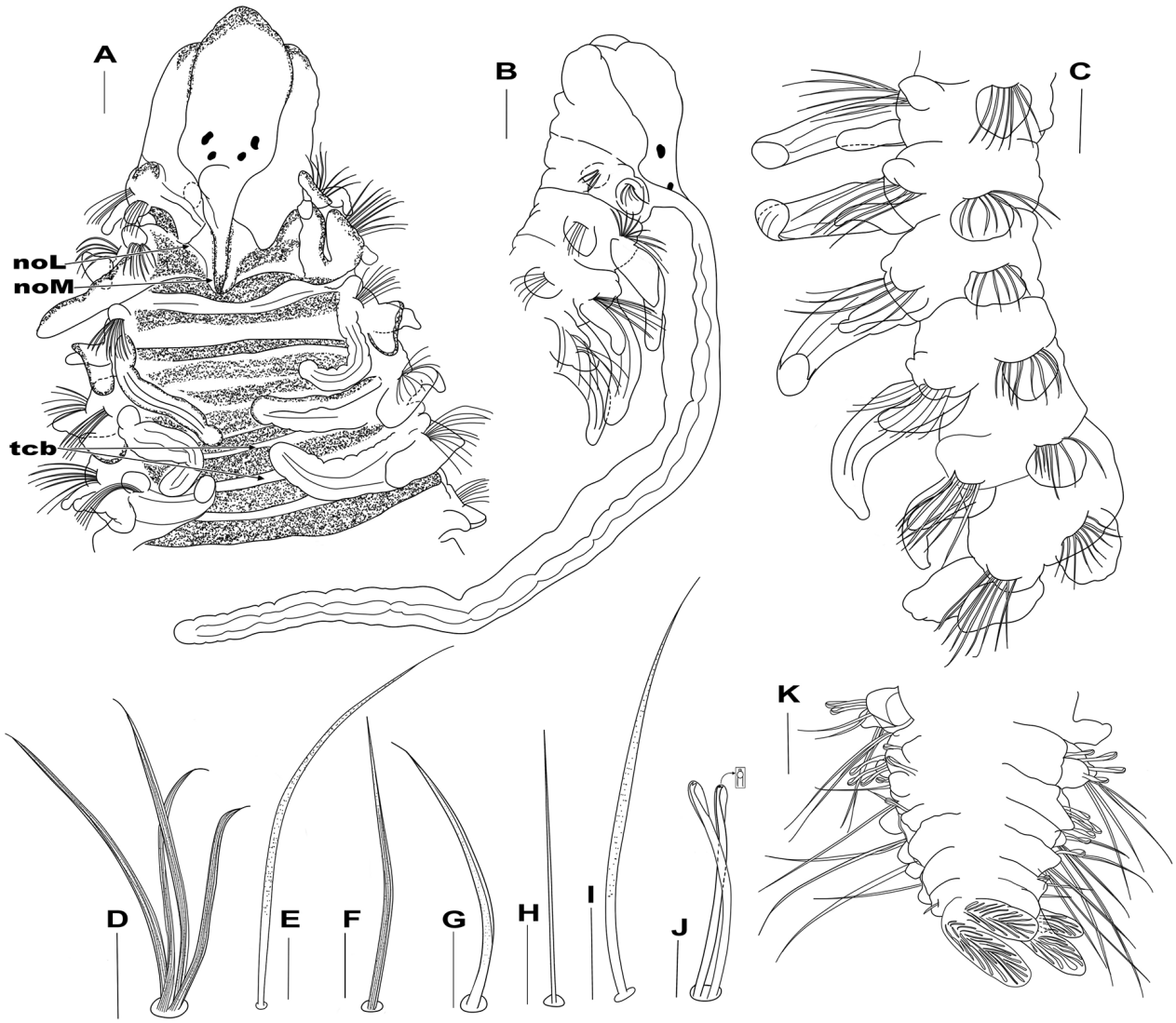

FIGURE 3. *Microspio moorei* (Gravier, 1911). (UDEA:CEMUA:ANNE:001594): **A.** Anterior region, dorsal view; **B.** Anterior region, lateral view; **C.** Parapodium and branchiae from chaetigers 2–7, lateral view; D. Notopodial capillaries from anterior and posterior rows of chaetiger 7; E. Notopodial capillary of superior fascicle from middle chaetiger. F. Neuropodial chaeta from chaetiger 7; G. Neuropodial capillary of ventral region from chaetiger 7; H. Neuropodial capillary from inferior fascicle of chaetiger 29; I. Sabre chaeta from middle chaetiger; **J.** Hooded hooks from chaetiger 29, K. Pygidium, ventral view. Abbreviations: noL = nuchal organ, lateral band, noM = nuchal organ, medial band, tcb = transverse ciliary band. Scale bars: **A, B, K:** 0.125 mm; **C:** 0,5 mm; **D**–**J**: 0.005 mm.

Notopodial capillary chaetae on chaetiger 1 longer, thinner and alimbate, arranged in one row; capillary chaetae from chaetiger 2 arranged in two rows; both rows with slightly granulated, striated, unilimbate chaetae (Fig. 3D); posterior row with very long and pointed chaetae. All chaetigers with an additional superior fascicle; anterior chaetigers with 4–7 long, granulated capillary chaetae (Fig. 3E); middle chaetigers with short, thin, smooth and alimbate chaetae; posterior chaetigers with slender, smooth, long and alimbate chaetae (Fig. 3K).

Neuropodial capillaries of chaetigers 1–3 arranged in one row; capillaries long, smooth, unilimbate; capillaries of subsequent chaetigers arranged in two rows, capillaries of both rows of same length, most dorsal capillaries stout, slightly granulated, striated and unilimbate (Fig. 3F); capillaries of ventral region slender, slightly granulated, unilimbate (Fig. 3G); inferior fascicle with 4–6 long, smooth, thin capillaries (Fig. 3H) in position of sabre chaetae usually present in most anterior chaetigers, around chaetiger 13 with granulate and long sabre chaetae (Fig. 3I), up to 3 per fascicle. Neuropodial hooded hooks (Fig. 3J) from chaetigers 14–17; up to 11 hooks per fascicle, accompanied by granulated, unilimbate capillaries in first chaetiger with hooks, thereafter only hooks. All hooks bidentate, with small tooth above main tooth (Fig. 3J). Pygidium long, with four short highly glandular digitate lobes surrounding the anal opening (Fig. 3K).

Methyl Green staining pattern. Body destains fairly rapidly; stain is retained briefly on anterior-most end of body. Anterior parapodial lamellae initially stain deeply but rapidly lose the stain.

Variation. The segments where the hooded hooks started varied from segment 14 in small specimens to segment 17 in the longest specimens.

A significant positive linear regression was found between the chaetiger where the hooded hooks first appeared and the body length ($R^2 = 0.22474$; Permutation $p = 0.0031$) (Fig. 4A). Specimens with an average length of 6.3 mm (SD±0.94) showed hooks starting on chaetiger 14, and as the body length increased, hooks first appeared on segments up to chaetiger 17 in individuals with a length close to 11 mm (SD±1.17).

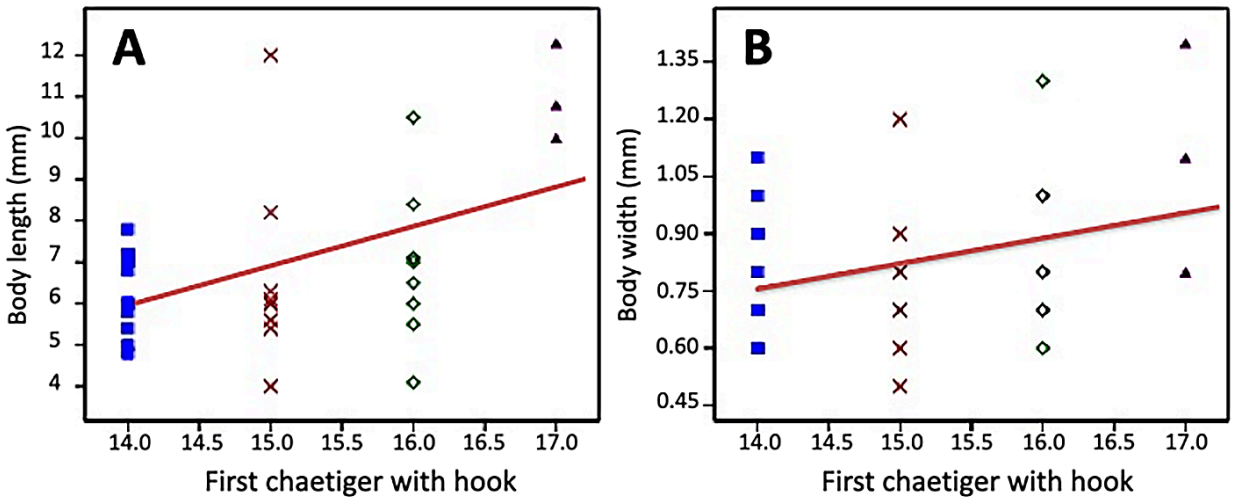

FIGURE 4. A. Relationships between body length, and **B.** Thoracic width of *Microspio moorei* and the first segment with hooded hoods.

Even though the statistical analysis was not significant ($R^2 = 0.10305$; Permutation $p = 0.0653$), it was also observed that in individuals with greater body width, hooded hooks first appeared on posterior segments, in such a way that specimens with a diameter greater than 1.1 mm (SD±0.30) had hooks starting in chaetiger 17 (Fig. 4B). A multivariate regression analysis supports these results and shows that as the polychaetes grow in length and width, the chaetiger where the hooded hooks first appear is progressively later ($R^2 = 0.2235$; p(regr) = 0.01907).

Discussion. Specimens herein described become the only additional material that has been used for taxonomic purpose, since none of the recent material used for ecological studies from the type locality, Admiralty Bay, and identified as *Microspio moorei* (Sicinski 2004; Pabis & Sicinski 2010a, b), *Microspio* cf. *moorei* (Petti *et al.* 2006), *Microspio* sp. (Barbosa *et al.* 2010), and *Mesospio moorei* (Sicinski *et al.* 2011), was available for checking their identity. Taxonomic information given by Hartman (1966) and Blake (1983) are based on the description by Gravier (1911a), and comparative notes by Maciolek & Blake (2021) are based on the description by Blake (1983). The quantity of individuals obtained from Fildes Bay, adjacent to the type locality, were sufficient to evaluate the

relationship between the segment where different types of chaetae first appear and the body length and number of segments presented by each complete individual. The original description, based on the holotype with 16 mm in length, considered chaetiger 15, where hooded hooks appear for the first time, as a character with taxonomic importance (Gravier, 1911a); nevertheless, regression analyses from additional material herein studied indicate that this character is size dependent, so increasing body length leads to hooded hooks occurring more posteriorly on the body (Fig. 4).

More analyses on ontogenetic development are needed to assess the physiological bases of this morphological variability in chaetation.

Type locality: Admiralty Bay, King George Island, South Shetland Islands, Antarctic Peninsula.

Distribution: This species has been identified only in the South Shetland Islands, Antarctic Peninsula, in Admiralty Bay by Gravier (1911a, b), Sicinski (2004), Pabis & Sicinski (2010a, 2010b), and in its different inlets (lagoons or fjord-like shaped bays), Mackellar, Martel, and Ezcurra (Barbosa *et al.* 2010), in Deception Island by Augener (1932), and in Fildes Bay, King George Island, in this study. From 0.3 m (this research) to 30 m depth (sensu Augener 1932).

Identification key to *Microspio* species

1. Prostomium rounded or straight on anterior margin; or broadly rounded, only very weakly incised in front; or conical 2
- Prostomium bilobed or bifid (deeply incised along anterior margin, producing two widely diverging lobes) on anterior margin 13
2. Prostomium conical on anterior margin . . . . . ***M. maori*** Blake, 1984
- Prostomium rounded or straight, slightly flared on anterior margin 3
3. Chaetiger 1 with notochaetae 4
- Chaetiger 1 without notochaetae 10
4. Eyes absent; four pairs branchiae present . . . . . ***M. tetrabranchia*** Maciolek, 1990
- Eyes present; branchiae continuing nearly to the end of the body 5
5. Hooded hooks with two teeth 6
- Hooded hooks with three or more teeth 7
6. Prostomium rounded, slightly expanded at anterolateral margin; hooded hooks beginning on chaetigers 18–23 . . . . . ***M. kussakini*** Chlebovitsch, 1959
- Prostomium anteriorly tapered; hooded hooks beginning on chaetigers 14–17. . . . . ***M. moorei*** (Gravier, 1911)
7. Hooded hooks with a fang surmounted by two unpaired teeth . . . . . ***M. hartmanae*** Blake, 1983
- Hooded hooks with a fang surmounted by paired teeth 8
8. Prostomium broad anteriorly, with curved, slightly incised margin; posterior notopodia include only long capillaries. . . . . ***M. elegantula*** Blake, 1984
- Prostomium rounded or truncate; posterior notopodia with numerous needle-like capillaries providing a distinct spinous appearance to the posterior end 9
9. Prostomium rounded; hooded hooks with two single teeth surmounted by three pairs of apical teeth; pygidium with four anal cirri . . . . . ***M. pigmentata*** (Reish, 1959)
- Prostomium truncate; hooded hooks with 2–3 single teeth surmounted by 1–2 pairs of apical teeth; pygidium with poorly developed cirri, if any. . . . . ***M. lydonia*** Maciolek & Blake, 2021
10. Eyes absent; hooded hooks multidentate beginning on chaetiger 26 . . . . . ***M. multidentata*** Zhou, Ji & Li, 2009
- Eyes present; hooded hooks uni-, bi-, or tridentate beginning on chaetiger 9–11 11
11. Occipital antenna present . . . . . ***M. occipitalis*** Hartmann-Schröder, 1983
- Occipital antenna absent. 12
12. Hooded hooks tridentate. . . . . ***M. rolasiana*** Augener, 1918
- Hooded hooks uni-, and bidentate . . . . . ***M. spinosa*** Blake, 1996
13. Chaetiger 1 with notochaetae 14
- Chaetiger 1 without notochaetae 15
14. Branchiae long and straplike; branchiae present on chaetigers 8–11; up to eight hooded hooks per fascicle. . . . . ***M. profunda*** Maciolek, 1990
- Branchiae short, elliptical, densely packed with large elongate glands; branchiae missing on chaetigers 8–11; up to four hooded hooks per fascicle . . . . . ***M. ariena*** Maciolek & Blake, 2021
15. Occipital papilla present; notopodial lamellae on chaetiger 1 with two lobes. . . . . ***M. granulata*** Blake & Kudenov, 1978
- Occipital papilla absent; notopodial lamellae on chaetiger 1 with one lobe 16
16. Caruncle present 17
- Caruncle absent or indistinct 18
17. Notopodial lamellae on chaetiger 1 present; hooded hooks multidentate; anterior hirsute capillaries . . . . . ***M. microcera*** (Dorsey, 1977)

- Notopodial lamellae on chaetiger 1 absent; hooded hooks bidentate; anterior not hirsute capillaries. . . . . ***M. fischeri*** Maciolek & Blake, 2021

18. Neuropodial lamellae on chaetiger 1 absent. 19
- Neuropodial lamellae on chaetiger 1 present 20

19. Prostomium bifid (deeply incised along anterior margin, producing two widely diverging lobes); thickened capillaries arranged in a U shape on chaetigers 4–5 and notched notopodial capillaries present. . . . . ***M. paradoxa*** Blake, 1983
- Prostomium bilobed on anterior margin; thickened capillaries arranged in a U shape on chaetigers 4–5 and notched notopodial capillaries absent . . . . . ***M. minuta*** (Hartmann-Schröder, 1962)

20. Prostomium slightly incised on anterior margin; hooded hooks beginning on chaetiger 11; pygidium with two cirri . . . . . ***M. mecznikowiana*** (Claparède, 1869)
- Prostomium bilobed on anterior margin; hooded hooks beginning on chaetiger 9; pygidium with four cirri. . . . . ***M. atlantica*** (Langerhans, 1880)

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