Latin American Journal of Aquatic Research, 51(3): 351-362, 2023
DOI: 10.3856/vol51-issue3-fulltext-2946

Research Article

Biochemical indicators of contamination in the coastal area of Callao, Peru

Lorena Alvariño[1], Gloria Saez[2], Thiago Machado da Silva Acioly[3] Diego Carvalho Viana[4] & José Iannacone[1,5]
[1]Laboratorio de Ecología y Biodiversidad Animal (LEBA), Facultad de Ciencias Naturales y Matemática Universidad Nacional Federico Villarreal, El Agustino, Lima, Perú
[2]Laboratorio de Parasitología, Facultad de Ciencias Naturales y Matemática Universidad Nacional Federico Villarreal, Lima, Perú
[3]Casa Familiar Rural de Coquelândia, Programa de Pós-graduação em Ciência Animal da Universidade Estadual do Maranhão (PPGCA/UEMA), Imperatriz, Brasil
[4]Universidade Estadual da Região Tocantina do Maranhão (UEMASUL) y Programa de Pós-Graduação em Ciência Animal de la Universidade Estadual do Maranhão (PPGCA/UEMA), Imperatriz, Brasil
[5]Laboratorio de Ingeniería Ambiental, Facultad de Ciencias Ambientales, COEPERU - Coastal Ecosystems of Peru Research Group, Universidad Científica del Sur, Lima, Perú
Corresponding author: José Iannacone (joseiannacone@gmail.com)

ABSTRACT. Callao Bay is strategically important for the industrial and tourist sector, serving as a recreational space for a large part of the population of Chalaca. However, even so, it permanently receives discharges of effluents, chemical products, and residues from oil refineries, hydrocarbons, and domestic and agricultural residues. The objective of this research is to evaluate biochemical indicators in fish against contaminants in the coastal area of Callao, Peru. The seasonal criterion (collections in autumn, spring, summer, and winter) and the spatial criterion (collections at four points along the coastal zone of Callao) were considered. *Isacia conceptionis* and *Odontesthes regia* showed relatively high concentration values of the enzyme alanine aminotransferase (ALT). Specifically, ALT is an enzyme used to assess liver function. *Sciaena deliciosa* showed high levels of alkaline phosphatase (ALP) in the liver but low values of aspartate aminotransferase (AST) and ALT. In addition, ALP is an indicator of renal impairment; in saltwater fish, its increase may be related to the increase in water salinity. The fish with the highest trophic level among the marine fish analyzed was *O. regia*. This fish presented the lowest values of ALP in the liver and muscle ALP and AChE (acetylcholinesterase) in the liver and the highest values for AST. *S. deliciosa* and *O. regia* are species that can be used in environmental biomonitoring, showing variation to the biomarkers studied because they have shown changes in the values of biochemical markers, mainly AChE of the brain and muscle, and in the protein concentrations of muscle, brain, and liver between seasons and sampling points in Callao Bay. It is concluded that the marine fish models evaluated are very useful in assessing contamination in Callao Bay.

Keywords: heavy metals; enzymes; biomonitoring; xenobiotics; environmental quality; coastal zone

INTRODUCTION

The seas and oceans, which cover 70% of the world's surface, are one of man's great hopes for future food supplies. However, pollution is one of the most visible threats, and the nature of pollutants varies according to the productive and urban activities that occur near aquatic areas. These activities strongly pressure natural ecosystems, significantly affecting community abundance and structure (Cabral et al. 2001), leading to habitat loss, resource overexploitation, and the introduction of exotic species.

Corresponding editor: Alejandra Volpedo

Organic contaminants and heavy metals are fundamentals part of human sources from domestic, agricultural, and industrial waste, dangerous to humans, marine biota, and the environment. These are chemical elements of environmental interest, mainly due to the repercussions of their presence in different environmental compartments (Iannacone et al. 2022). In particular, organotin compounds (COEs) is one of the most relevant contaminants that cause endocrine disruption in marine organisms (Huaquín 2002). Primarily, tributyltin (TBT) and triphenyltin (TPT) contained in antifouling paints and used to prevent fouling have been reported as the principal contaminant of marine organisms on the hulls of ships, boats, buoys, and other submerged surfaces (Stanisavljevic 2019). Globally, there is a history of the effects that the presence of these compounds in water has on populations of non-white marine organisms and the entire food chain (Tanabe 1999, Contreras-Almazo & González-Rentería 2022). While for heavy metals, several authors point out that they can affect ecosystems and jeopardize fish consumption, as well as other levels of the food chain, causing cytotoxic, mutagenic, and carcinogenic effects in both animals and humans by consumption of contaminated fish (Storelli et al. 2005, Qiao-Qiao et al. 2007, Zapata-Rivera et al. 2018). Fishes also can bioaccumulate and biomagnify throughout the aquatic food chain (Ramírez-Morales et al. 2019).

Most of the effects of these contaminants on marine organisms include morphological changes; for example, it may affect secondary sexual characteristics in marine gastropod mollusks due to the high absorption of COEs (Miglioranza 2021, Ñaña-Reyes & Villanueva 2021), the low ability to metabolize this compound and the slow elimination of the xenobiotic through excretory organs. Masculinization of females in marine mollusks, imposex, is defined as developing male characteristics in females under the influence of very low concentrations of TBT and TPT (0.4 ng L^{-1}) (Romero et al. 2019).

In recent decades, environmental deterioration has increased in Peru, a situation that has become more evident in the coastal zone, where a third of the Peruvian population lives and where most domestic and industrial waste is disposed (Sánchez et al. 2008, Iannacone et al. 2022). These problems forced, from an early age, to monitor marine waters to determine the pollution produced. Monitoring the characteristics of aquatic ecosystems can be carried out in several ways, such as through evaluations through studies of the physicochemical characteristics of the different matrices associated with the receiving body (for example, water, sediments, and biota), as they provide valuable information related to the state and quality of these. However, these assessments do not provide insight into the effects of substances on the receptor body nor information about the bioavailability of potential stressors.

Callao Bay is located on the central coast of Peru, in the Mar de Grau. This region is strategically important from an industrial and tourist point of view, serving as a leisure space for a large part of the population of Chalaca. Unfortunately, it permanently receives wastewater discharges from various industries, including fish products, food for human consumption, and chemicals from a major oil refinery and one of the country's main seaports with different loading and unloading areas (minerals, hydrocarbons). Also, from domestic and agricultural collectors, discharges from the Chillón and Rímac rivers, which carry pesticide residues, minerals, and other products along their route, strongly impact the receiving environment (PNUMA 2005, Saez et al. 2018, Iannacone et al. 2022). As a result of these diverse activities, numerous discharges are emitted into the marine environment, in many cases altering the quality of the physical and chemical parameters of the environment (Saez et al. 2018, Romero et al. 2019, Iannacone et al. 2022, Osorio et al. 2022). A recent study provides specific information about potentially toxic elements in Callao Bay's surface water and fish muscle (*Thaisella chocolata*) (Iannacone et al. 2022). The authors reported that average concentrations of As, Cd, Cr, Hg, Pb, and Se in the study area exceeded the minimum limit concentration among the regulations for water in Perú. Also, they concluded that the consumption of *T. chocolata* from the bay of Callao would be a severe problem for people's health: the values in the water of Be <0.003 mg L^{-1}, Cs <0.0044 mg L^{-1}, Co <0.0028 mg L^{-1}, Sn <0.0138 mg L^{-1}, Hg <0.0001 mg L^{-1}, and Cd <0.0015 mg L^{-1} were found to be below the spectrophotometer limits of detection (LD) in most sampling points and seasons during 2015-2016. The metals with the highest mean values were: Sr (6.76-7.98 mg L^{-1}), Ba (6.76-7.98 mg L^{-1}), Si (6.76-7.98 mg L^{-1}), Li (0.20-0.23 mg L^{-1}), Fe (0.04-0.12 mg L^{-1}), Al (0.09-0.16 mg L^{-1}), zinc (0.01-0.09 mg L^{-1}), Pb (0.04-0.07 mg L^{-}), Se (0.01-0.05 mg L^{-1}), Ni (0.01-0.03 mg L^{-1}), As (0.01 - 0.02 mg L^{-1}) and Cr (0.00-0.01 mg L^{-1}).

In recent years, the search for innovative study methods to detect the impacts on aquatic ecosystems caused by pollution from human activities has intensified, intending to design and implement

preventive measures. Thus, the use and development of biomarkers have gained increasing interest to assess the risk (probability of producing adverse effects) of a substance or mixture of potentially toxic chemicals. In particular, effect biomarkers are valuable parameters that indicate the presence of exogenous substances or biological changes in response to different xenobiotics, such as pesticides (Bertrand et al. 2016), persistent organic pollutants (Bettinetti et al. 2012), or heavy metals (Villagran et al. 2019). The monitoring of the characteristics of aquatic ecosystems and the detection of xenobiotics can be carried out in different ways, for example, by monitoring the physicochemical characteristics (water, sediments, and biota) and quantifying the effect of these substances on the receiving body (living organism) (Iannacone et al. 2022).

The main types of biomarkers used in environmental biomonitoring are exposure, effect, and susceptibility. The exposure biomarker checks the amount absorbed or the internal dose, such as the quantification of enzymes in liver tissue. When the living organism is in a deleterious environment (with pollutants), there is a response in the form of oxidative stress; first, the xenobiotic will be metabolized by the action of first-phase and second-phase antioxidant enzymes, then the organisms will secrete the xenobiotic by specific organs (Lionetto et al. 2019).

Biomarkers used in integrated biomonitoring can contribute to a better understanding of the exposure routes and mechanisms underlying biota's adverse effects (Lionetto et al. 2019). Fish and shellfish have been widely used as bioindicators of metal contamination; their high protein and low saturated fat content are an important part of the human diet, and sufficient omega fatty acids are known to promote good health; therefore. Several studies have been adopted worldwide on the contamination of different fish species by heavy metals (Baqueiro-Cárdenas et al. 2007, Villagran et al. 2019, Tkaczyk et al. 2020, Urbina & Solano 2020). Fish muscle tissue is most used for laboratory analysis as it is an important metal storage tissue and is the main edible part of fish (Bhupander-Kumar et al. 2011, Flores et al. 2018). The toxic effects of pollutants on organisms may be specific to a group of pollutants or common to different classes of pollutants; therefore, samples from the liver (place of accumulation and metabolism of xenobiotics) and brain (by the number of contaminants that reach the central nervous system) must also be analyzed (Kumar et al. 2021).

Biomarkers can be specific or non-specific for the toxic substance (García & Arceo 2018, Tello-Vallejo 2018). In addition, environmental contamination can promote a redox imbalance in the exposed organism, resulting in oxidative damage (DNA damage, protein oxidation, lipid peroxidation), increased expression or activation of enzymes that generate oxidants and free radicals (catalase enzymes, superoxide dismutase, glutathione system, thioredoxin) in aquatic species (Lionetto et al. 2019).

The use of biomarkers in fish populations or communities could increase or decrease their levels to determine the toxicity of chemical substances is more relevant from an ecological point of view due to their sensitivity, specificity, and precision. That is why, currently, assessments on individuals at the molecular, cellular, and physiological levels are the most used, becoming powerful predictive tools that allow the development of protection and sanitation strategies before the damage associated with chemical contamination is irreversibly manifested in the ecosystem (Lionetto et al. 2019, Kumar et al. 2021).

It is hypothesized that fishes present in the marine ecosystem of Callao Bay are affected by activities of anthropic origin as heavy metals and other pollutants may present altered physiological and biochemical responses, such as increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) or decreased acetylcholinesterase (AChE) as the expression of biomarkers as enzymes and other biochemical indicators (Solé et al. 2009, Kumar et al. 2021). In this sense, this research aims to evaluate biochemical indicators in fish in the coastal area of Callao, Peru.

MATERIALS AND METHODS

Study area

The research was carried out in the coastal area of Callao Bay (Perú), which is strategically important from an industrial, tourist, and recreational point of view. Its coastal waters have contamination problems, mainly due to domestic, industrial, and agricultural collectors and mineral sediments resulting from the loading and unloading mineral concentrates. In addition, the discharges from the Chillón and Rímac rivers bring with them residues of pesticides, minerals, and other products from activities carried out along their entire course, which have a strong impact on the receiving environment (PNUMA 2005, Sáez et al. 2018, Iannacone et al. 2022, Osorio et al. 2022).

The evaluation was carried out from May 2015 to January 2016 with seasonal criteria in autumn 2015 (1), winter 2015 (2), spring 2015 (3), and summer 2016 (4). According to spatial criteria, four sampling stations-

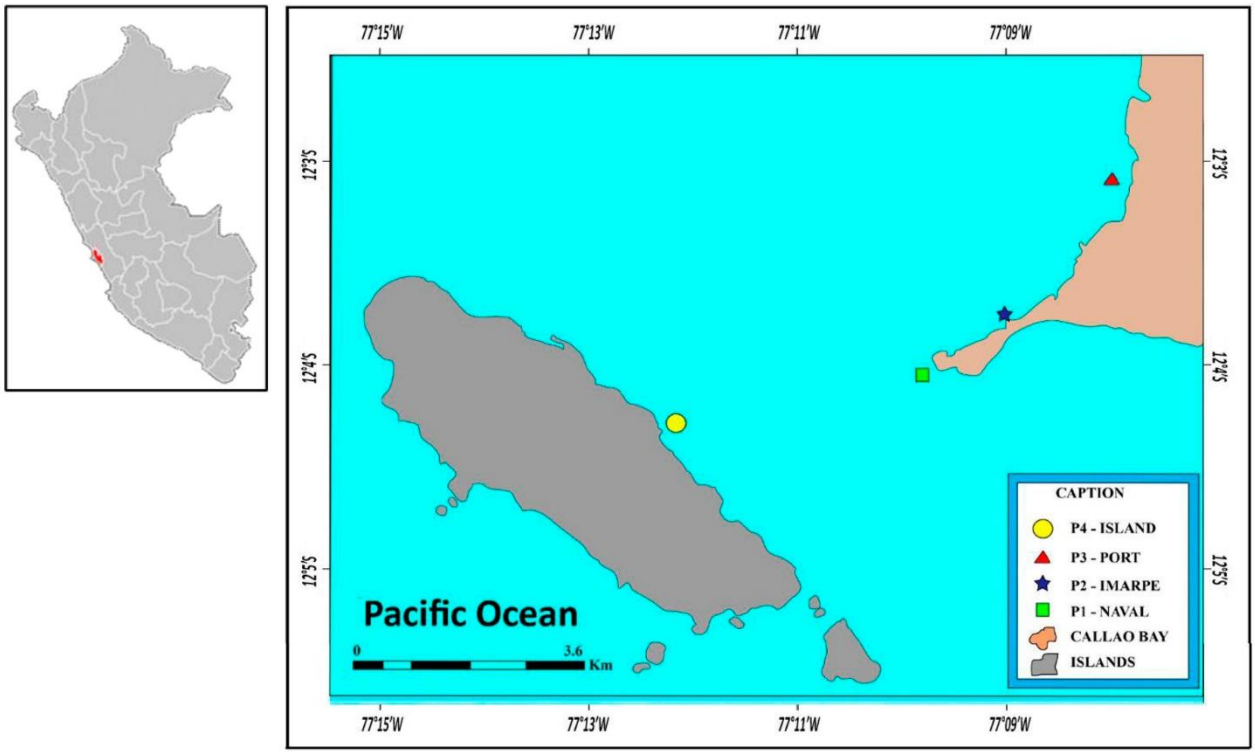

Figure 1. The geographic location of sampling stations-areas in the coastal zone of Callao, Peru. Modified from Pérez & Lleellish (2015).

areas were included, georeferenced with GPS and GARMIN 4215 probe model map (Fig. 1, Table 1). The first sampling point (P1) corresponds to the area in front of the naval school with industrial or sanitation activities. The second (P2) is located opposite the Instituto del Mar Peruano was in the area allocated for primary contact recreation; the third point (P3) corresponds to the area in front of the Callao Port Terminal, and finally, the last sampling point (P4) corresponds to the coastal zone of San Lorenzo Island. This island and its surroundings have a well-preserved biota due to the almost total absence of human activity for many years. Also, part of the island is devoted to the extraction and cultivation of mollusks (Osorio et al. 2022).

Iannacone et al. (2022) and Osorio et al. (2022) show the values by points and season for the physicochemical and heavy metal parameters of the bay of Callao, Peru, from May 2015 to January 2016. The physicochemical values were: sea surface temperature (SST) (°C), pH, electrical conductivity (mS cm^{-2}), salinity, total dissolved solids (TDS), dissolved oxygen (DO) (mg L^{-1}), transparency (m), turbidity (FTU), oxide reduction potential (mRV), ammonia (mg L^{-1}), and nitrates (mg L^{-1}), chlorophyll (ug L^{-1}) and phycocyanin (ug L^{-1}) obtained in each sampling area. The concentrations of 24 metals in surface water are Al, As, Ba, B, Cd, Ca, Co, Cu, Cr, Sr, P, Fe, Li, Mg, Mn, Ni, Ag, Pb, K, Se, Na, Ti, V and Zn.

Fish collection for biochemical analysis

A total of 67 live fish specimens were collected in the sampling points with the help of local fishermen in the coastal zone of Callao Bay from May 2015 to January 2016. Fish were captured with three lonera nets of 2 1/4 mesh, 40 threads, 60 m long by 6 m high. Evisceration was performed *in situ*, collecting tissues and organs from the fish; later, the samples were placed in polyethylene bags and frozen at -4°C for conservation and transfer; then, in the laboratory, they were stored at -20°C until further analysis.

For each sample, the following information was considered: total length (TL) ± standard deviation (SD); minimum and maximum in cm; weight (W) ± standard deviation (SD), minimum and maximum in g (Table 2). Nine selected fish species are the most common in the sampling areas, occupying different niches in the aquatic ecosystem. Fish were classified by feeding type: a 'primary consumer' who consumes mainly plant/detritus' (herbivores) with values of trophic level

Table 1. Geographic coordinates of fishing spots in Callao Bay.

Point	Location	Geographical coordinates	
		Latitude	Longitude
Point 1	In front of the Naval School	12°4'25"S	77°10'16"W
Point 2	In front of the Peruvian Sea Institute	12°3'56"S	77°9'36"W
Point 3	In front of Callao Pier	12°2'59"S	77°8'59"W
Point 4	Fronton and San Lorenzo Island	12°4'58"S	77°12'22"W

Table 2. Fish species collected at sampling points in Callao Bay. The values are shown as mean, and standard deviation (mean ± SD), minimum (Min), and maximum (Max), and the unit of each measurement is indicated between brackets. bp: benthopelagic, d: demersal, pn: pelagic-neritic, Trophic level: herbivores may have values of trophic level between 2.0 and 2.19; tc: consumers who consume 'mainly animals' (carnivores) may have trophic levels equal to or greater than 2.8; and fish which are partly herbivore and partly carnivore, i.e. omnivores which consume 'plants/detritus + animals' may have trophic levels between 2.2 and 2.79.

Specimens	Environment/ Trophic level	Number of fish collected	Total length (cm)		Weight (g)	
			Mean ± SD	Min-Max	Mean ± SD	Min-Max
Lorna drum (*Sciaena deliciosa*)	d / 3.6	34	21.26 ± 1.60	19-25	143.83 ± 31.08	96.05-208
Flathead grey mullet (*Mugil cephalus*)	bp / 2.5	7	26.89 ± 4.3	20-35.1	289.57 ± 104.09	115-478
Chilean silverside (*Odontesthes regia*)	pn/ 4.0	12	14.55 ± 1.11	12-16	21.42 ± 6.17	13-31
Peruvian morwong (*Cheilodactylus variegatus*)	bp / 3.7	3	18.06 ± 3.49	13-23	113.55 ± 36.25	46-172
Chalapo clinid (*Labrisomus philippii*)	d / 3.7	3	17.96 ± 3.17	13.1-21.5	98.8 ± 46.14	37-162
Minor stardrum (*Stellifer minor*)	d / 3.5	3	16.88 ± 2.39	13.5-19	100.38 ± 34.61	48.5-119
Pacific menhaden (*Ethmidium maculatum*)	pn / 2.1	3	26.17 ± 2.84	23-28.5	205.50 ± 56.37	143-252.5
Cabinza grunt (*Isacia conceptionis*)	bp / 2.9	1	18	18	89.6	89.6
Starry butterfish (*Stromateus stellatus*)	bp / 3.6	1	17.8	17.8	76	76

between 2.0 and 2.19; secondary and tertiary consumers who mainly consume animals (carnivores) and may have trophic levels equal to or greater than 2.8; and fishes which are partly herbivore and partly carnivore, i.e. omnivores which consume plants/detritus + animals and may have trophic levels between 2.2 and 2.79 (Jia et al. 2021). Also, considering their environment, fishes were classified as benthopelagic, demersal, and pelagic-neritic (Jia et al. 2021).

Biochemical analyzes (biomarkers)

Liver, brain, and muscle samples were treated as follows: 50 mg of each tissue was cut and placed in a 1.5 mL tube; then 0.2 mL of 1X PBS buffer was added, and the tissue was homogenized with a micro-homogenizer. The tubes were centrifuged at 5000 rpm for 10 min. The supernatant was separated into another tube and refrigerated (4°C) until further use, not longer than 48 h. Total proteins, acetylcholinesterase (AChE), aspartate aminotransferase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP), and oxidases were determined.

Determination of total proteins

The Bradford method was used to quantify total proteins, with a standard curve with bovine serum albumin (BSA) (10 mg mL^{-1}) generating dilutions from 0.01 mg to 10 mg mL^{-1}. After obtaining the standard curve, the samples were quantified (in duplicate) using the supernatant obtained from each sample previously diluted 10 times. The samples were analyzed by spectrophotometry at an absorbance of 595 nm. Absorbance values were plotted on the standard curve to calculate the total protein concentration of the samples.

Determination of acetylcholinesterase (AChE) in the brain, liver, and muscle of fish

The supernatant from each tissue was processed using an AChE determination kit Wiener®, following the manufacturer's instructions. Duplicate samples were read in a spectrophotometer at 405 nm at time zero and 10 min. The absorbance value T10-T0 was multiplied by 22.71 to obtain the value in international units (IU) per liter (L) (IU L^{-1}). The detection limit (DL) was 23

IU L^{-1}. Precision: coefficient of variation (CV) (%) = 2.59.

Determination of transaminases in fish liver

The supernatant of the liver samples was evaluated for the determination of AST and ALT. DL was 3.12 IU L^{-1} for AST and DL was 3.00 IU L^{-1} for ALT using Cypress diagnostics® kits developed for each enzyme and following the manufacturer's instructions. Duplicate samples were read in a spectrophotometer at 340 nm. Absorbance values were recorded at time zero, and every minute for 3 min, each reading was subtracted from the previous one, and then an average of the subtracted absorbances was obtained. Finally, the average value was multiplied by 3300 to get the value in IU L^{-1}. Sensitivity for AST and ALT: 1 IU L^{-1} = 0.00056 ΔA min^{-1} and precision: CV (%) = 1.32.

Determination of alkaline phosphatase in fish liver and muscle

The supernatant of each liver and muscle sample was processed using a kit to determine this enzyme ALP following the manufacturer's instructions. Duplicate samples were read in a spectrophotometer at 520 nm. The procedure to calculate the IU L^{-1} of the enzyme was the same as described for the determination of transaminases in fish liver. The detection limit was 1.31 IU L^{-1}, sensitivity: 1 IU L^{-1} = 0.0004 ΔA min^{-1}, and precision: CV (%) = 2.29.

For AChE, transaminases, and alkaline phosphatase, Biochemistry Normal and Pathological controls (HBC01, HBC02) were employed as controls. Also, dynamic range, linearity, quality control of the reagents, and monitoring of the assay procedures' performance were done following the manufacturer's instructions.

Determination of oxidases in fish liver

The supernatant of each liver sample was processed following the methodology described by Technical Standards Manual, Fiocruz-Brasil (Ministério da Saúde 2006) to quantify oxidase in fish liver. Duplicate samples were read in a spectrophotometer at 620 nm. The absorbance values obtained were plotted on a cytochrome standard curve B (0.1 mg mL^{-1} to calculate the concentration of cytochrome B, in micrograms) per milligram of protein (ug Cit. B mg^{-1} of protein).

Statistical analysis

The average values and standard deviation found for each season and sampling point are presented for the five enzymes and the protein concentrations in marine fish. For the three species of marine fish that presented the highest individual values collected, the biochemical indicators were compared between points and between seasons. In the case of more than three seasons/points, the analysis of variance (ANOVA) was used with subsequent Tukey's multiple comparison test, and in the case of two seasons/points, the Student's *t*-test was used. In the same way, the values of biochemical markers were compared among marine fish. In addition, the ALP values in the liver and muscle were contrasted, and the protein concentrations for each teleost fish were examined. The analyzes were performed with the PAST 4.0 statistical program at a significance level of 0.05.

RESULTS

The mean values of five enzymes (ALP, AST, ALT, AChE, and oxidases) in the muscle, liver, and brain of fish collected in the coastal zone of Callao are shown (Table 3). The fish *Odontesthes regia* and *Stromateus stellatus* showed relatively high concentration values of the enzyme AST with mean values of 69.82 and 40.1 IU, respectively (Table 3). The fish *Sciaena deliciosa* showed relatively high concentration values of the enzyme AST at season 2 with medium values of 45.42 IU (Table 3). In turn, *O. regia*, also in season 2, showed a variation in mean AST values from 2.6 IU L^{-1} (P1) to 115.9 IU L^{-1} (P2). In contrast, in season 3, the following averages were obtained: 75.1 IU L^{-1} (P2) and 85.1 IU L^{-1} (P3) (Table 3).

Liver, brain, and muscle oxidase values were relatively similar among the nine species evaluated (Table 3). *Labrisomus philippii*, *Isacia conceptionis*, *Cheilodactylus variegatus*, and *Mugil cephalus* showed the lowest values of the enzyme AST (<20 IU L^{-1}). Five species showed low values of ALT (<10 IU L^{-1}): *S. deliciosa*, *M. cephalus*, *C. variegatus*, *L. philippii*, and *S. stellatus*. Only *I. conceptionis* and *O. regia* showed positive values (>30 IU L^{-1}). *S. stellatus* showed a low value of AChE in the brain (4.6 IU L^{-1}) but the highest in the liver (24.2 IU L^{-1}) and muscle (28 IU L^{-1}).

At point P4, *S. deliciosa* has high levels of ALP in the liver (314.56 IU L^{-1}) and protein concentration (3220.37 mg mL^{-1}), with low values of AST (24.16 IU L^{-1}) and ALT (11.85 IU L^{-1}) (Table 4). The oxidase values in *S. deliciosa* were the same at points P1 and P3 (0.08 ug Cit. B mg^{-1} protein) (Table 4). The seasonal analysis of the biochemical indicators in *S. deliciosa* showed significant statistical differences for AST ($F = 6.57$, $P < 0.05$) between autumn 2015 and winter 2015 and between winter 2015 and spring 2015. The liver

Table 3. Mean values of five enzymes and proteins concentration in nine teleost fish species from Callao Bay, Peru. Season: 1: autumn 2015, 2: winter 2015, 3: spring 2015, 4: summer 2016.

Species	Season	Point	Samples (n)	Alkaline phosphatase (ALP) (IU L^{-1})		Aspartate aminotransferase (AST) (IU L^{-1})	Alanine aminotransferase (ALT) (IU L^{-1})	Acetylcholinesterase (AChE) (IU L^{-1})			Oxidases (ug Cit.B mg^{-1} protein)	Total proteins (mg mL^{-1})		
				Liver	Muscle	Liver	Liver	Liver	Brain	Muscle	Liver	Liver	Brain	Muscle
Sciaena deliciosa	1	1	6	141.7 ± 116.2	-	40 ± 27.2	3	16.1 ± 10.3	31.1 ± 5.1	-	0.09 ± 0.01	1597.1 ± 231.6	643.1 ± 666	-
		3	2	41.7 ± 15.4	-	6.3 ± 2	3	19.7 ± 4.6	34.3 ± 0.2	-	0.12	1184.65 ± 12.8	1091.2 ± 42.3	-
	2	1	3	283.0 ± 55	18.2 ± 12.8	45.1 ± 12.1	65.3 ± 42.9	11.5 ± 3.1	29.1 ± 1.4	28.2 ± 2.1	0.09 ± 0.01	1510.1 ± 228.5	1112.9 ± 74.9	1145.9 ± 113.7
		2	3	125.8 ± 36.4	61.7 ± 25.1	44.4 ± 27.2	25.6 ± 23.9	16.5 ± 6.3	27 ± 4.4	23.5 ± 3.5	0.09 ± 0.01	1462.4 ± 134	1177.2 ± 202.9	759 ± 21.1
	3	1	2	118.3 ± 35.3	379.4 ± 241.6	58.2 ± 0.5	3	8.9 ± 4.6	24.9 ± 0.2	7.7 ± 9.1	0.05 ± 0.01	2646.6 ± 298.2	2726.2 ± 114.2	2571.6 ± 143.4
		2	3	137	126.4 ± 35.4	77.9	3	6.4	10.5 ± 11.2	1 ± 0.6	0.05	2845.9	2823.9 ± 141.2	2741.4 ± 45
		4	3	327.3 ± 39.5	150.3 ± 88.8	4.4 ± 2.2	3	12.1 ± 2.8	11.4 ± 9.5	1.3	0.04 ± 0.01	3275.8 ± 416.1	2498.7 ± 151.2	2694.3 ± 31.5
	4	1	3	211.3 ± 130.3	180 ± 126	19.1 ± 13.7	3	11 ± 6.6	12.6 ± 13.3	1.3	0.05	3010.5 ± 215.1	2649.7 ± 81.5	2811.6 ± 22
		2	3	207.1 ± 13.4	94.8 ± 11.4	17.6 ± 18.8	74.7 ± 125	13.62 ± 6.8	25.5 ± 1.2	1.3	0.04	3231.4 ± 235.6	2583.2 ± 127.1	2752.9 ± 65.7
		3	3	283.1 ± 226.6	99.2 ± 47.8	25.1 ± 28.2	29.8 ± 46.4	5.5 ± 3.6	22.3 ± 4.1	4 ± 4.7	0.04 ± 0.01	3226.83 ± 551.2	2526.2 ± 292.3	2666.8 ± 214.5
		4	3	301.8 ± 213.9	108.7 ± 6.6	43.9 ± 21.7	20.7 ± 18.1	16.6 ± 9	25.2 ± 4	1.2 ± 0.2	0.04	3165 ± 89.3	2724.8 ± 160.1	2745.4 ± 33.3
Mugil cephalus	1	1	3	694.9 ± 200	-	19.8 ± 13.3	3	10.2 ± 8	34.1 ± 1.9	-	0.08	1699.2 ± 90.5	996.9 ± 162.7	-
		3	3	538.6 ± 271.6	-	16.9 ± 16.8	3	18.9 ± 14	22.3 ± 5.8	-	0.09 ± 0.02	1581.4 ± 360	645.6 ± 203.2	-
	4	3	1	150.5	108.41	5.2	3	3	5.6	1.3	0.04	3311.6	2895.6	2497.5
Stellifer minor	1	3	3	76.2 ± 50.7	-	34.1 ± 38.4	11.5 ± 14.7	19.5 ± 8.4	31.9 ± 8.9	-	0.08 ± 0.01	1768.1 ± 236.9	1252.4 ± 204.6	-
Odonthesthes regia	2	1	3	98.6 ± 40.5	9.3 ± 4.9	2.6 ± 2.8	43.3 ± 20.3	6.1 ± 1.9	19.7 ± 3.4	19 ± 13.3	0.09	1491 ± 102.2	1278.5 ± 22.5	1035.2 ± 131.3
		2	3	150.1 ± 20.2	12.9 ± 5.9	115.9 ± 51.2	51.7 ± 39.1	9.8 ± 1.2	28.5 ± 4.3	18.7 ± 1.9	0.1	1379.4 ± 67.9	860.2 ± 231.6	666.1 ± 167.3
	3	2	3	157.7 ± 36.9	29.5 ± 6.5	75.1 ± 26.3	21.7 ± 32.5	2.4 ± 1.5	1.3	10.1 ± 11.1	0.04	3183.9 ± 152.4	3251.2 ± 393.2	2745.4 ± 152.9
		3	3	122.8 ± 99.2	56.6 ± 20.18	85.1 ± 25.8	69.2 ± 56.2	1.1 ± 0.5	14.5 ± 6.2	1.3 ± 0.1	0.04	3460.2 ± 34.8	2836.1 ± 178.1	2840.5 ± 118.4
Etnmidium maculatus	2	2	3	1431.5 ± 485.9	5.4 ± 9.7	22 ± 16.7	15.6 ± 21.8	9 ± 3.3	18.8 ± 10.4	6 ± 1.5	0.1 ± 0.01	1488.4 ± 127.7	765.2 ± 17.4	853.9 ± 150.2
Cheilodactylus variegatus	3	4	3	16 ± 16.5	94.4 ± 52.3	18.5 ± 19.9	5.9 ± 5.4	8.8 ± 10.2	10.9 ± 9.4	8.7 ± 7.8	0.05	2916.7 ± 187	2881.9 ± 237.9	3056 ± 443.7
Isacia conceptionis	3	4	1	594.2	28.2	14.14	37.3	13.9	16,0	21.7	0.04	3303.6	2629.7	2908.2
Labrisomus philippii	3	4	3	74.3 ± 75.6	32.9 ± 28.3	18.2 ± 26.1	7.7 ± 4.1	0.9 ± 0,7	1.1 ± 0.4	13 ± 10.8	0.05	2924.3 ± 157.4	3079.3 ± 483.7	2272.9 ± 165.8
Stromateus stellatus	4	3	1	150.6	21.0	40.1	3	24.2	4.6	28.0	0.04	3467.9	2819.7	2070.3

Table 4. Mean values of some enzymes in *Sciaena deliciosa* according to Callao Bay, Peru sampling areas.

Enzymes	*Sciaena deliciosa*											
	Point 1			Point 2			Point 3			Point 4		
	Liver	Brain	Muscle	Liver	Brain	Muscle	Liver	Brain	Muscle	Liver	Brain	Muscle
ALP (IU L^{-1})	169.08	-	169.18	162.24	-	94.31	186.55	-	99.24	314.56	-	133.66
AST (IU L^{-1})	39.22	-	-	37.68	-	-	17.60	-	-	24.16	-	-
ALT (IU L^{-1})	16.34	-	-	43.42	-	-	19.09	-	-	11.85	-	-
AChE (IU L^{-1})	12.98	25.83	12.98	11.67	21.02	8.58	11.18	27.12	4.03	13.85	18.28	1.26
Oxidase (ug Cit.B mg^{-1} protein)	0.08	-	-	0.07	-	-	0.08	-	-	0.04	-	-
Protein concentration (mg mL^{-1})	2031.26	1467.50	2126.95	2418.18	2194.77	2084.45	2409.96	1952.24	2666.80	3220.37	2611.75	2714.71

protein showed seasonal differences (F = 20.03, $P < 0.05$) with high values for autumn and winter 2015 and low values for spring 2015 and summer 2016. The AChE in the brain showed seasonal differences between autumn 2015 with spring 2015 and between winter 2015 and spring 2015 (F = 8.48, $P < 0.05$). Seasonal oscillations were observed for brain proteins (F = 159.9, $P < 0.05$) among the four seasons evaluated. Muscle AChE and muscle proteins indicate seasonal variations (F = 61.82, $P < 0.05$) between winter 2015 with spring 2015 and summer 2016. The rest of the biochemical markers in *S. deliciosa* did not show seasonal differences (F = 0.47-3.26, $P > 0.05$). The absence of variation between sampling points was observed for all biochemical indicators in *S. deliciosa* (F = 0.15-2.36, $P > 0.05$). Liver protein was the only marker that showed differences between P1 and P4 (F = 3.23, $P < 0.05$).

In *M. cephalus* it was observed that the values of ALP (694.95 IU L^{-1}) and AST (19.76 IU L^{-1}) in the liver were higher at P1 (Table 5). However, muscle protein concentration was higher at P3 (2497.52 mg mL^{-1}). No changes were seen between sampling points P1 and P3 for the five enzymes and protein concentrations in *M. cephalus* (t = 0.17-1.21, $P > 0.05$). The AChE of the brain showed a higher value for P1 than P3 (t = 2.83, $P < 0.05$).

In *O. regia*, low levels of ALP and AST were observed in the liver at P1 (98.62 and 2.57 IU L^{-1}, respectively) and low values of ALT in the liver at P2 (36.57 IU L^{-1}) (Table 6). The protein concentration values in the liver were higher at P3 (3460.20 mg mL^{-1}) (Table 6). Finally, differences were noted between winter 2015 and spring 2015 for *O. regia*, with the highest values for liver AChE, liver oxidases, brain AChE, and muscle AChE in winter 2015 (t = 2.68-23.84, $P < 0.05$), and for liver, brain, and muscle protein concentration, muscle ALP, and muscle protein concentration, the highest levels for spring 2015 (t = 3.78-33.43, $P < 0.05$). No seasonal differences were seen for ALP, AST, and ALT (t = 0.08-0.70, $P > 0.05$). In *O. regia*, differences were observed between sampling points P1 and P2 and between P1 and P3 for AST (F = 7.81, $P < 0.05$). Liver protein concentration indicated changes between P1 and P3 (F = 5.37, $P < 0.05$) and for muscle ALP between P1 and P3, and between P2 and P3 (F = 11.84, $P < 0.05$). In the rest of the biochemical indicators in *O. regia*, there were no differences between the sampling points (F = 0.19-4.07, $P > 0.05$).

Table 5. Mean values of some enzymes in *Mugil cephalus* according to Callao Bay, Peru sampling areas. L: liver, B: brain, M: muscle.

Enzymes	*Mugil cephalus*					
	Point 1			Point 3		
	L	B	M	L	B	M
ALP (IU L^{-1})	694.95	-	-	441.59	-	108.41
AST (IU L^{-1})	19.76	-	-	14.01	-	-
ALT (IU L^{-1})	3	-	-	3	-	-
AChE (IU L^{-1})	10.24	34.51	-	13.61	18.14	1.31
Oxidase (ug Cit.B mg^{-1} protein)	0.08	-	-	0.08	-	-
Protein concentration (mg mL^{-1})	1699.20	996.86	-	2013.98	1208.14	2497.52

Table 6. Mean values of some enzymes in *Odontesthes regia* according to Callao Bay, Peru sampling areas. L: liver, B: brain, M: muscle.

Enzymes	*Odontesthes regia*								
	Point 1			Point 2			Point 3		
	L	B	M	L	B	M	L	B	M
ALP (IU L^{-1})	98.62	-	9.29	153.93	-	21.21	122.78	-	56.56
AST (IU L^{-1})	2.57	-	-	95.51	-	-	85.71	-	-
ALT (IU L^{-1})	43.27	-	-	36.72	-	-	69.25	-	-
AChE (IU)	6.06	19.68	19.02	6.10	14.88	14.38	1.04	14.52	1.26
Oxidase (ug Cit.B mg^{-1} protein)	0.09	-	-	0.07	-	-	0.04	-	-
Protein concentration (mg mL^{-1})	1490.99	1278.47	1035.19	2281.68	2055.70	1705.75	3460.20	2836.12	2840.55

When analyzing the biochemical parameters, higher ALP values were found in the liver in *E. maculatus* and *M. cephalus* compared to *C. variegatus*, *L. philippii*, *O. regia*, *S. deliciosa* and *S. stellatus* ($F = 33.92$, $P < 0.05$). The AST was lower in *M. cephalus* and statistically different from that observed in *O. regia* among the marine fish examined ($F = 3.27$, $P < 0.05$). The AChE in the liver was lower in *L. philippii* and *O. regia* compared to *S. deliciosa* and *S. stellatus* ($F=3.83$, $P < 0.05$). In contrast, AChE in the brain was statistically lower in *L. philippii* compared to *M. cephalus*, *S. deliciosa* and *S. stellatus* ($F = 4.25$, $P < 0.05$). Protein concentration in the brain was lower in *E. maculatus* and *M. cephalus* compared to *C. variegatus* and *L. philippii* ($F = 4.83$, $P < 0.05$). Muscle ALP was lowest in *O. regia vs. S. deliciosa*, which was highest ($F = 3.56$, $P < 0.05$). Muscle protein concentration was higher in *C. variegatus* and *S. deliciosa* than in *E. maculatus* ($F = 3.56$, $P < 0.05$). No differences were seen in ALT, oxidases, liver protein concentration, and AChE in muscle among the examined teleost marine fish ($F = 0.52$, $P > 0.05$).

No differences were found between ALP values in the liver and muscle for *C. variegatus* ($t = 2.46$, $P > 0.05$) and *L. philippii* ($t = 0.90$, $P > 0.05$). In contrast, higher ALP values in the liver than in muscle were observed in *E. maculatus* ($t = 5.07$, $P < 0.05$), *O. regia* ($t = 6.18$, $P < 0.05$), and *S. deliciosa* ($t = 2.17$, $P < 0.05$).

In *E. maculatus*, higher protein concentration values were observed in the liver than in the brain and muscle ($F = 35.74$, $P < 0.05$). In contrast, *L. philippii* was higher in the brain than in the muscle, but in the liver, it was the same as in the brain and muscle (5.75, $P < 0.05$). No differences in protein concentration were seen in *M. cephalus*, *O. regia* and *S. deliciosa* ($F = 0.87$-1.96, $P > 0.05$).

DISCUSSION

Fish are commonly used for biomonitoring of pollutants in aquatic ecosystems, as they are aquatic vertebrates that bioaccumulate toxic substances through ingesting contaminated sediments or food (Reis-Henriques et al. 2009). These animals have a wide distribution due to their diversity and importance in these environments, in addition to occupying different trophic levels and being in direct contact with pollutants (Osman et al. 2012).

Fishes have been shown to respond with great sensitivity to changes in the aquatic environment and low concentrations of environmental pollutants (Hafez 2009), in addition to being especially sensitive to

exogenous estrogens (Alamino & Silva 2021). As was previously reported, when the aquatic organism is in an environment with an excess of chemicals such as pesticides, industrial chemicals, and heavy metals, there is an increase in the production of oxidizing compounds and radicals (Kumar et al. 2021).

Heavy metal microparticles catalyze reactions, generating free radicals. They cause oxidative stress, mainly by forming hydroxyl groups that cause protein damage, lipid peroxidation, and DNA/RNA damage. In this sense, samples of the liver (the target organ of many pollutants, responsible for the metabolism of the pollutant), brain (by the number of pollutants that reach the central nervous system), and muscle (reflects the metabolic activity) are processed and isolated (Lionetto et al. 2019).

Transaminases are enzymes widely distributed in aquatic organisms. Serum activity under normal conditions is low or absent. Destruction of the tissues in which they are present leads to increased serum levels. Specifically, ALT is an enzyme used to assess liver function. According to Álvarez et al. (2012), a high concentration of ALT can be used with some specificity in diagnosing toxic liver injury. In the present work, no differences were observed between concentration values of the ALT in all the other teleost fish evaluated.

S. deliciosa and *O. regia* showed elevated levels of ALP in the liver compared to muscle. The enzyme alkaline phosphatase is found in all tissues. Tissues in which its concentration is very high are the liver, bile ducts, and bone cells (Sharifian et al. 2018). There is a wide variety of ALP enzymes called isoenzymes, with slightly different structures in different tissues. Because diseased or damaged tissues release enzymes into the blood, serum ALP measurements can differentiate bone from liver disease. In addition, ALP is an indicator of renal alteration; in saltwater fish, its increase may be related to the increase in water salinity.

All fish examined showed relatively similar and high values of total protein concentration in the liver but different in muscle and brain. However, *E. maculatus* showed low values in the brain and muscle but high values in the liver. Patterns of proteins in fish vary quantitatively and qualitatively under different stimuli. The main factors that produce variation are sex, age, seasonal changes, diseases, trophic levels, and toxins (Tilami & Sampels 2018).

The enzymatic biomarkers and protein concentration indicators of fish contamination did not show a specific pattern related to any of the four monitoring points in Callao Bay, Peru.

Trophic variables of marine teleost fish were significantly related to xenobiotic metabolism hepatic markers and especially high in benthic/supra benthic fish feeders (Solé et al. 2009). Significant interspecies differences were evidenced, although each biochemical marker varied independently. The fish with the highest trophic level among the marine fish analyzed was *O. regia*. This pelagic-neritic fish presented the lowest values of ALP in the liver and muscle and AChE in the liver and the highest values for AST. However, further research is needed to consider pollution gradients in Callao Bay to establish which fish species is the best adequacy as sentinel.

CONCLUSIONS

Variations in biochemical markers in the liver, brain, and muscle in fish can be used as bioindicators of exposure to heavy metals in monitoring water quality in Callao Bay, Peru. *I. conceptionis* and *O. regia* had a higher liver function, which may indicate toxic liver injury or a high degree of sensitivity to the contaminant. *S. deliciosa* and *O. regia* are species that can be used in environmental biomonitoring, showing variation to the biomarkers studied because they have shown changes in the values of biochemical markers, mainly AChE of the brain and muscle, and in the protein concentrations of muscle, brain, and liver between seasons and sampling points in Callao Bay.

REFERENCES

Alamino, D.A. & Silva, D.C. 2021. Riscos ambientais e para saúde humana da liberação de compostos estrogênicos residuais em ambientes aquáticos. Recurso Água, 60: 60-109.

Alvarez, M., Gimenez, I.T., Saitua, H., Enriz, R.D. & Giannini, F.A. 2012. Toxicidad en peces de herbicidas formulados con glifosato. Acta Toxicológica Argentina, 20: 5-13.

Baqueiro-Cárdenas, E.R., Borabe, L., Goldaracena-Islas, C.G. & Rodríguez-Navarro, J. 2007. Los moluscos y la contaminación. Una revisión. Revista Mexicana de Biodiversidad, 78: 1-7.

Bertrand, L., Monferrán, M.V., Mouneyrac, C., Bonansea, R.I., Asis, R. & Amé, M.V. 2016. Sensitive biomarker responses of the shrimp *Palaemonetes argentinus* exposed to chlorpyrifos at environmental concentrations: roles of alpha-tocopherol and metallothioneins. Aquatic Toxicology, 179: 72-81. doi: 10.1016/j.aquatox.2016.08.014

Bettinetti, R., Garibaldi, L., Leoni, B., Quadroni, S. & Galassi, S. 2012. Zooplankton as an early warning system of persistent organic pollutants contamination in a deep lake (Lake Iseo, northern Italy). Journal of Limnology, 71. doi: 10.4081/jlimnol.2012.e36

Bhupander, K., Mukherjee, D.P., Sanjay, K., Meenu, M., Dev, P., Singh, S.K. & Sharma, C.S. 2011. Bioaccumulation of heavy metals in muscle tissue of fishes from selected aquaculture ponds in east Kolkata wetlands. Annals of Biological Research, 2: 125-134.

Cabral, H.N., Costa, M.J. & Salgado, J.P. 2001. Does the Tagus estuary fish community reflect environmental changes? Climate Research, 18: 119-126. doi: 10.3354/cr018119

Contreras-Almazo, I.A.E. & González-Rentería, M. 2022. Bivalvos, organismos modelo en el biomonitoreo del riesgo ecotoxicológico de los antinflamatorios no esteroideos (AINE) para los ecosistemas acuáticos. Ecosistemas, 31: 2167. doi: 10.7818/ECOS.2167

Flores, E., Pozo, W., Pernía, B. & Sánchez, W. 2018. Niveles de cadmio en atún fresco y enlatado para consumo humano en Ecuador. Maskana, 9: 35-40. doi: 10.18537/mskn.09.02.05

García, J.D.D. & Arceo, E. 2018. Daño renal asociado a metales pesados: trabajo de revisión. Revista Colombiana de Nefrología, 5: 43-53. doi: 10.22265/acnef.5.2.254

Hafez, A.M. 2009. *Mugil cephalus* genome: a sensitive monitor for genotoxicity and cytotoxicity in aquatic environment. Australian Journal of Basic and Applied Sciences, 3: 2176-2187.

Huaquín, L. 2002. Alteraciones del sexo en poblaciones de moluscos gastrópodos marinos. Tecnovet, Santiago. [http://www.tecnovet.uchile.cl/CDA/tecnovet_articulo/0,1409,SCID%253D17129%2526ISID%253D626,00.html]. Reviewed: April 13, 2022.

Iannacone, J., Romero, S., Rosas, J., Guabloche, A., Alvariño, L., Castañeda, L. & Carrasco, L. 2022. Metals in *Thaisella chocolata* from the Callao Bay, Perú. International Journal of Mechanical Engineering, 7: 1356-1364.

Jia, Y., Jiang, Y., Liu, Y., Sui, X., Feng, X., Zhu, R. & Chen, Y. 2021. Understanding trophic structure variation in fish assemblages of subtropical shallow lakes: combined effects of ecosystem size, productivity, and disturbance. Ecological Indicators, 129: 107924. doi: 10.1016/j.ecolind.2021.107924

Kumar, N., Bhushan, S., Kumar-Gupta, S., Kumar, P., Kumar-Chandan, N., Kumar-Singh, D. & Kumar, P. 2021. Metal determination and biochemical status of marine fishes facilitate the biomonitoring of marine pollution. Marine Pollution Bulletin, 170: 112682. doi: 10.1016/j.marpolbul.2021.11268

Lionetto, M.G., Caricato, R. & Giordano, M.E. 2019. Pollution biomarkers in environmental and human biomonitoring. Open Biomarkers Journal, 9: 1-9. doi: 10.2174/1875318301909010001

Miglioranza, K.S.B. 2021. Agroquímicos. Informes de revisión. CONICET, Mar del Plata. [https://mardelplata-conicet.gob.ar/wp-content/uploads/2021/02/Informe-de-revision-Agroquimicos-1.pdf]. Reviewed: April 13, 2022.

Ministério da Saúde. 2006. Metodologia para qualificação de atividades de enzimas relacionados com a resistência a inseticidas em *Aedes aegypti*. Fundação Oswaldo Cruz. Ministério da Saúde, Brasília.

Ñaña-Reyes, M.L. & Villanueva-De La Cruz, R. 2021. Estado de contaminación del medio marino por contaminantes orgánicos persistentes. Revisión sistemática. Tesis de Ingeniería Ambiental, Universidad César Vallejo, Lima.

Osman, A.G.M., Abuel-Fadl, K.Y. & Y Kloas, W. 2012. *In situ* evaluation of the genotoxic potential of the river Nile: II. Detection of DNA strand-breakage and apoptosis in *Oreochromis niloticus niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822). Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 747: 14-21. doi: 10.1016/j.mrgentox.2012.02.013

Osorio, M., Romero, S., Guabloche, A., Alvariño-Flores, L., Ayala-Sulca, Y., Carrasco-Badajoz, C., et al. 2022. Use of *Brachionus plicatilis* (Rotifera) to assess the quality of marine water in Callao Bay, Peru. Journal of Integrated Coastal Zone Management, 22: 103-116. doi: 10.5894/rgci-n441

Pérez, J. & Lleellish, M. 2015. Reptiles terrestres de la isla San Lorenzo, Lima, Perú. Revista Peruana de Biología, 22: 119-122. doi: 10.15381/rpb.v22i1.11130

Programa de las Naciones Unidas para el Medio Ambiente (PNUMA). 2005. Perspectivas del medio ambiente urbano: GEO Lima y Callao. PNUMA-GEA, Lima.

Qiao-Qiao, C., Guangwei, Z. & Langdon, A. 2007. Bioaccumulation of heavy metals in fishes from Taihu Lake, China. Journal of Environmental Science, 19: 1500-1504. doi: 10.1016/S1001-0742(07)60244-7

Ramírez-Morales, D., Rodríguez-Artavia, B., Sáenz-Vargas, W., Sánchez-Gutiérrez, R., Villalobos-González, W. & Mora-Barrantes, J.C. 2019. Minerías artesanales para la extracción de oro mediante el uso de mercurio: estado del arte del impacto ambiental en

los medios agua, aire y suelo. Revista Tecnología en Marcha, 32: 3-11. doi: 10.18845/tm.v32i2.4475

Reis-Henriques, M.A., Ferreira, M., Coimbra, A.M., D'Silva, C., Costa, J. & Shailaja, M.S. 2009. Efectos del fenantreno y el nitrito en juveniles del róbalo, *Dicentrarchus labrax*, mediante el uso de enzimas hepáticas biotransformadoras, fluorescencia biliar y micronúcleos como biomarcadores. Ciencias Marinas, 35: 29-40.

Romero, S.E., Guabloche, A., Tuesta, E., Iannacone, J. & Braga-Castro, I. 2019. Imposex responses in *Thaisella chocolata* and *Xanthochorus buxeus* from Callao harbor, Peru. Regional Studies in Marine Science, 26: 100510. doi: 10.1016/j.rsma.2019.100510

Sánchez, G., Orozco, R. & Guzmán, M. 2008. Bahía del Callao, Perú. Evaluación ambiental en marzo 2002. Unidad de Monitoreo y Gestión Marino Costera. DIAGCAC. IMARPE, Lima, pp. 27-40.

Sáez, G., Chero, J., Cruces, C., Minaya, D., Rodriguez, C., Suyo, B., et al. 2018. Parámetros hematológicos y de bioquímica sanguínea en diez especies de peces marinos capturados por pesquería artesanal en la Bahía del Callao, Perú. Revista de Investigaciones Veterinarias del Perú, 29: 1161-1177. doi: 10.15381/rivep.v29i4.15204

Sharifian, S., Homaei, A., Kim, S.-K. & Satari, M. 2018. Production of newfound alkaline phosphatases from marine organisms with potential functions and industrial applications. Process Biochemistry, 64: 103-115. doi: 10.1016/j.procbio.2017.10.005

Solé, M., Rodríguez, S., Papiol, V., Maynou, F. & Cartes, J.E. 2009. Xenobiotic metabolism markers in marine fish with different trophic strategies and their relationship to ecological variables. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology, 149: 83-89. doi: 10.1016/j.cbpc.2008.07.008

Stanisavljevic, V.B. 2019. Pinturas y sus aplicaciones en el ámbito marítimo. Trabajo de Grado, Escuela Politécnica Superior de Ingeniería, Tenerife.

Storelli, M.M., Storelli, A., Giacominelli-Stuffler, R. & Marcotrigiano, G.O. 2005. Mercury speciation in the muscle of two commercially important fish, hake (*Merluccius merluccius*) and striped mullet (*Mullus barbatus*) from the Mediterranean Sea: estimated weekly intake. Food Chemistry, 89: 295-300. doi: 10.1016/j.foodchem.2004.02.036

Tanabe, S. 1999. Butyltin contamination in marine mammals - A review. Marine Pollution Bulletin, 39: 62-72.

Tello-Vallejo, Á.C. 2018. Biomarcadores genotóxicos en tilapia nilótica *Oreochromis niloticus* como indicadores de contaminación de aguas por metales pesados. Universidad Nacional Abierta y a Distancia, Bogotá.

Tilami, S.K. & Sampels, S. 2018. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Reviews in Fisheries Science & Aquaculture, 26: 243-253. doi: 10.1080/23308249.2017.1399104

Tkaczyk, A., Mitrowska, K. & Posyniak, A. 2020. Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: a review. Science of the Total Environment, 717: 137222. doi: 10.1016/j.scitotenv.2020.137222

Urbina, J.A.J. & Solano, J.A.V. 2020. Los contaminantes emergentes de las aguas residuales de la industria farmacéutica y su tratamiento por medio de la ozonización. Informador Técnico, 84: 249-263. doi: 10.23850/22565035.2305

Villagran, D.M., Frernández-Severini, M.D., Biancalana, F., Spetter, C.V., Fernández, E.M. & Marcovecchio, J.E. 2019. Bioaccumulation of heavy metals in mesozooplankton from a human-impacted southwestern Atlantic estuary (Argentina). Journal of Marine Research, 77: 217-241. doi: 10.1357/002224019826887362

Wheeler, J.R., Gimeno, S., López-Juez, E. & Morritt, D. 2005. Vitellogenin: a review of analytical methods to detect (anti) estrogenic activity in fish. Toxicology Mechanisms and Methods, 15: 293-306. doi: 10.1080/15376520590968789

Zapata-Rivera, A.M., Méndez, F., Páez. M.I., Chow, N.A., Ordoñez, J. & Artunduaga, Y.P. 2018. Estudio exploratorio de evaluación de riesgo en la salud de madres lactantes por consumo de pescado contaminado del río Cauca, en el Valle del Cauca (Colombia). Ambiente y Desarrollo, 22. doi: 10.11144/Javeriana.ayd22-43.eeer

Received: July 11, 2022; Accepted: January 29, 2023