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BIOACCUMULATION AND BIOMAGNIFICATION OF TRACE ELEMENTS IN TISSUES OF WHALE SHARK (*Rhincodon typus*) FROM THE GULF OF CALIFORNIA

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PRESENTA

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Dedication

...all’Oceano e a tutte le meravigliose creature che vivono in lui, soprattutto gli squali balena...

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- Whitehead, D.A., Petatán-Ramírez, D., Olivier, D., González-Armas, R., Pancaldi, F., and Galván-Magaña, F. 2019. Seasonal trends in whale sharks sightings in an established tourism site in the Gulf of California, Mexico. *Journal of Fish Biology*, 95: 982–984.
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ABSTRACT

Essential (Cu, Se and Zn) and non-essential (As, Cd, Hg, and Pb) trace elements (TEs) were determined in 130 dermal biopsies of whale sharks (*Rhincodon typus*) and 25 samples of zooplankton collected in two seasons (S1 (2016-2017) and S2 (2017-2018)) in Bahía de los Angeles (BLA) and Bahía de La Paz (LAP), Gulf of California (GC), Mexico. In biopsies, Zn occurred at the highest concentration (BLA: S1 = 298 ± 406 and S2 = 1959 ± 2545; LAP: S1 = 595 ± 554 and S2 = 2642 ± 1261; ng/g ww) and Cd the lowest (BLA: T1 = 17 ± 14 and T2 = 13 ± 10; LAP: T1 = 3 ± 3 and T2 = 4.0 ± 3.0; ng/g ww) relative to other TEs. Significant differences ($p<0.05$) in the concentration of TEs between site and season were noted. Sex was not significant for any element, except Pb in LAP S1 and Cu in LAP S2 ($p <0.05$). TE concentrations showed a negative correlation with total length in the males and positive relationship in the females, which could indicate differences in prey preferences and feeding grounds. Arsenic concentrations in the sharks from La Paz suggested that the area is enriched with this element. In the zooplankton, Zn and Hg were the most and least concentrated TE, respectively, in the two seasons, with significant differences ($p<0.05$) by season. Copepods and chaetognaths were identified as the main groups of zooplankton while sharks were feeding. Essential TEs were not biomagnified probably due to homeostatic mechanisms, while Hg and Pb were biomagnified in both seasons through zooplankton. Livers from two stranded whale sharks presented high As (24200 and 33700 ng/g) and Cd (5130 and 17500 ng/g ww) concentrations. High Cd levels were also found in filtering pads (1480 and 2240) of both sharks. In one stranded shark, high concentrations of Pb were found in skeletal muscle (13700 ng/g ww) and epidermis (6770 ng/g ww). In both sharks, molar ratio Se:Hg was >1 for all sampled tissues. TEs were not uniformly distributed in the lobes of the liver; Cd, As and Zn were found in higher concentrations in both right and left lobe while Hg and Pb were found in lower concentrations. The proximal area of the left lobe presented the lowest concentrations of Zn, As and Cd and the highest concentrations of Se. A molar excess of Se over Hg was found in both seasons and both sites in the biopsies, the zooplankton and the livers of the stranded whale sharks. Molar ratios suggested that Zn could improve Cd detoxification better than Se in the epidermis and the liver.

RESUMEN

Los oligoelementos (TEs) esenciales Cu, Se y Zn, y no-esenciales As, Cd, Hg y Pb, fueron determinados en 130 biopsias de tiburones ballena (*Rhincodon typus*) y 25 muestras de zooplancton recolectados durante dos temporadas (S1 y S2) en Bahía de Los Ángeles (BLA) y Bahía de La Paz (LAP), Golfo de California, México. En las biopsias, el Zn fue el TE más concentrado (BLA: S1 = 298±406 y S2 = 1959±2545; LAP: S1 = 595±554 y S2 = 2642±1261; ng/g ph) y Cd el menos (BLA: T1 = 17±14 y T2 = 13±10; LAP: T1 = 3±3 y T2 = 4.0±3.0; ng/g ph), encontrando diferencias significativas ($p < 0.05$) en la concentración promedio de TEs entre sitio y temporada. El sexo no influyó significativamente para ningún elemento, excepto Pb en LAP S1 y Cu en LAP S2 ($p<0.05$). Las concentraciones de TEs mostraron una correlación negativa con la longitud total en los machos y positiva en las hembras. Las concentraciones de As en los tiburones de LAP sugieren que el área está enriquecida con este elemento. El zooplancton acumula TEs siendo Zn el más concentrado y Hg el menos en las dos temporadas, con diferencias significativas ($p<0.05$) por temporada. Los copépodos y los quetognatos fueron identificados como las principales presas de zooplancton. El Hg y Pb se biomagnificaron a través del zooplancton. Los hígados de dos tiburones varados presentaron altas concentraciones de As (24200 ng/g ph y 33700 ng/g ph) y Cd (5130 ng/g ph y 17500 ng/g ph). También se encontraron altos niveles de Cd en los pads filtradores de ambos tiburones (1480 ng/g ph y 2240 ng/g ph). En un tiburón varado, se encontraron altas concentraciones de Pb en el músculo esquelético (13700 ng/g ph) y la epidermis (6770 ng/g ph). En ambos tiburones, la relación molar Se:Hg fue > 1 para todos los tejidos muestreados. Los TEs no se distribuyeron uniformemente en los lóbulos del hígado; Cd, As y Zn se encontraron en altas concentraciones tanto en el lóbulo derecho como en el izquierdo, mientras que Hg y Pb se encontraron en concentraciones más bajas. El área proximal del lóbulo izquierdo presentó las concentraciones más bajas de Zn, As y Cd y las concentraciones más altas de Se. Se encontró un exceso molar de Se sobre Hg en ambas estaciones y en ambos sitios en las biopsias, el zooplancton y los hígados de los tiburones ballena varados. Las relaciones molares sugirieron que Zn podría mejorar la desintoxicación de Cd mejor que Se en la epidermis y el hígado.

CHAPTER 1:

GENERAL INTRODUCTION

1.1. The whale shark: history, biology and ecology

By being the largest fish of the planet, the whale shark (*Rhincodon typus*) (Fig. 1.1) is a cosmopolitan species; it reaches 18-20 m of total length (Coleman, 1997) and is found in epipelagic and neritic waters, tropical and subtropical regions between 30º N and 35º S (Compagno, 1984) (Fig. 1.2). It seems to prefer waters with temperatures of 26.8 °C and 30.2 °C, with an average of 28 °C and salinities between 34.5 ‰ and 35.5 ‰ (Compagno, 1984).

The species was firstly described in 1828 by Andrew Smith from a 4.6 m individual harpooned in South Africa. Since then, records of whale shark have been mainly focused on captures, stranding and sightings principally based on feeding activities. The increase of the diving activity in the early 1990' has led to discover new aggregations of whale shark all over the world which had allowed to increase marine conservation and ecotourism activities together with scientific studies.

Periodic observations of feeding aggregations allowed to link whale shark presence to productivity events in Australia (Norman & Stevens 2007), Indonesia (Stacey et al., 2008), Philippines (Araujo et al., 2014), Maldives (Riley *et al.,* 2010), Seychelles (Rowat *et al.,* 2009), Saudi Arabia (Cochran *et al.,* 2016), India (Pavin, 2000), Kenya (Beckley *et al.,* 1997), Tanzania (Cochran, 2014), Saint Helena (Clingham *et al.,* 2016) and Belize (Heiman *et al.,* 2001). In Mexico, feeding aggregations have been described in the Mexican Caribbean (Motta *et al.,* 2010), Bahía de Los Angeles (Ramírez-Macías *et al.,* 2012), Bahía de La Paz (Ketchum *et al.,* 2013; Whitehead *et al.,* 2019) and Nayarit (Ramírez-Macías *et al.,* 2016). Feeding is based on zooplankton communities principally composed by copepods (Clark & Nelson, 1997; Hacohen-Domené *et al.,* 2006; Whitehead *et al.,* 2020) and chaetognats (Pancaldi *et al.,* 2019a; Whitehead *et al.,* 2020) but also cladocerans (Lavaniegos *et al.,* 2012), decapods (Whitehead *et al.,* 2020) and euphasiids (Hacohen-Domené *et al.,* 2006: Whitehead *et al.,* 2020).

Fig. 1.1 Whale shark (*Rhincodon typus*). Source: https://endlessocean.fandom.com

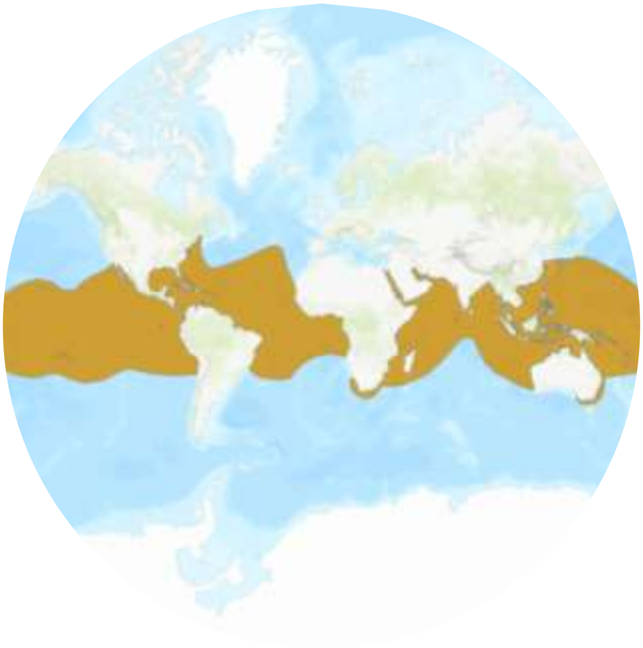

Fig. 1.2 Distribution map of the whale shark. Source: IUCN Red List https://www.iucnredlist.org/species/19488/2365291

Based on flow speed and mouth area it has been established that a 4 m whale shark feeds an average of 7-8 hours per day on plankton patches, corresponding to 1467 g of zooplankton per hour (Motta *et al.,* 2010).

Reproductive system of the whale shark has been under discussion from 1910 with several conjectures on the possibility of viviparity (Gudger, 1915, 1935) and oviparity (Gudger, 1952). In 1953, an egg case containing an embryo was found in the Gulf of Mexico, off Texas leading to the already existing idea that the species is oviparous. Nevertheless, due to the egg characteristics (egg case too thin respect to other oviparous shark species) the theory could not be confirmed, and whale shark reproductive strategy remained under discussion. Speculations ended in 1995 when a 10.6 m female was harpooned in Taiwan. The female was pregnant and had 304 embryos in different development classes. Embryos develop in brown horny egg cases and hatch in the uterus proving that the species is a lecithotrophic livebearer (Joung *et al.,* 1996).

According to several authors, sexual maturity in this species is reached at 9 m of length in females and around 8 m in males (Norman & Stevens, 2007). Nevertheless, observations of mature animals below these sizes has been reported by Wintner (2000). Information on growth is scarce and mainly reported from animals in captivity; pups hatch at around 60 cm in length and grow a mean of 28 cm per year during the first 3 years (Hsu *et al.,* 2007) with this rate decreasing by time.

Natural mortality in this species is rarely investigated but appears to be higher in very juvenile animals and pups due to natural predation (Rowat & Brooks, 2012) by the blue marlin *Makaira nigricans* (Colman, 1997) and the blue shark *Prionace glauca* (Kukuyev, 1995). Attacks to larger individuals by killer whales *Orcinus orca* (O'Sullivan & Mitchell, 2000) and other larger sharks (Fitzpatrick *et al.,* 2006) have been described, although it is not known how frequently this occurs.

Stranding has been observed in very few areas such as the Indian Ocean coast of South Africa (Beckley *et al.,* 1997) and coasts of Australia (Speed *et al.,* 2009). In the Gulf of California, 14 whale shark stranding have been recorded from 2000 to 2018 (Whitehead *et al.,* 2018). The causes of the stranding are usually unknown but regional topography, pollution, fishery pressure and disease have been speculated.

Whale shark docile behaviour towards humans and their predictability linked to feeding events rendered them vulnerable to captures. Surface swimming habits make them vulnerable to propeller injuries; death caused by boat collision have been reported (Rowat *et al.,* 2009) and continues mainly in high touristic areas (Fig. 1.3). Historically, mortality of *R. typus* has been linked to fishery activities especially in Asian countries where flesh (typically called "tofu shark") and fins were used to be consumed (Chen *et al.,* 1997; Chen & Phipps, 2002; Hanfee, 2007).

Bycatch from tuna purse-seine and illegal fishing still represent a conservation threat to whale sharks. In 2000, *R. typus* was classified by the International Union for the Conservation of Nature as vulnerable in the Red List of Threatened Animals. It was also included in the Appendix II of the Convention of Migratory Species of Wild Animals (CMS) in 1999 (CMS, 1999) and to Appendix II of CITES, the Convention of Trade in Endangered Species, in 2002 (CITES, 2002).

In 2016, *R. typus* classification in the Red List was upgraded to “endangered” from the IUCN. This decision was taken based on a report published by Pierce and Norman (2016; IUCN 2016) which state a global decline of whale shark population of around 50%.

Fig. 1.3 Injuries caused by propeller strike on a whale shark in the South Ari Atoll, Maldives. Source: Francesca Pancaldi

1.2. Trace elements (TEs)

Pollution in the marine environment has become a worldwide problem due to the increasing levels of toxic elements and their obvious impacts on biota and human health (Achary *et al.,* 2017). Pollutants in the marine environment can occur naturally as a result of natural biogeochemical processes (erosion, rock wear, volcanic eruptions and hydrothermal vents) or they can come from human sources (mining, industry, incineration, agriculture and aquaculture) (Páez-Osuna *et al.,* 2017). Some substances can be extremely toxic to living because their persistence and ability to accumulate in the trophic web, representing a serious threat to diversity, species abundance and human health (Naser, 2013). The biological availability and toxicity of various elements depends on their chemical form in the environment, which can be affected by water quality characteristics such as pH and salinity (Atchison *et al.,* 1987).

Essential trace elements (TEs) are those that are needed in very small quantities for growth, development, and physiology of the organism. They are persistent in the environment where they are generally found in concentrations below ppm (Newman & Unger, 2002), are potentially reactive and can bioaccumulate in the trophic web (Prasad *et al.,* 2006). Some of them, such as heavy metals, can cause severe problems for the health of organisms.

Trace elements (TEs) are classified in essential and not essential depending on the role that play in the organisms; essential TEs are those that are required to perform vital metabolic activities in organisms, and include iron (Fe) (haemoglobin), copper (Cu) (respiratory pigments), cobalt (Co) (Vitamin B12), manganese (Mn) and zinc (Zn) (enzymes) (Soto-Jiménez, 2011). An element can be considered essential when it has the following characteristics: 1. It is present in all tissues, 2. It is maintained at a constant concentration, 3. It is capable of producing physiological abnormalities if it is excluded from the organism, and 4. It is capable of remedy these conditions if it is reinstated in the body (Soto-Jiménez, 2011). Although essential, these elements become toxic in high concentrations. Non-essential elements, such as heavy metals, certain metals and metalloids have no biological functions and cause toxic effects even at low concentrations. Some of these substances like mercury (Hg), lead (Pb) and cadmium (Cd) are considered the most polluting in the environment (Chen *et al.,* 2008).

The sources of pollutants in the ocean are classified as direct, resulting from activities immediately adjacent to coastal waters (municipal effluents, industrial discharges, agricultural wastewater, aquaculture, hydrothermal vents) and diffuse, which provide materials resulting from activities carried out in relatively remote areas to the coast (Páez-Osuna *et al.,* 2017). Both types of sources reflect both natural distributions, as part of geological and biological cycles, as well as anthropogenic contributions due to industrial and urban activities.

TEs are contributed from the continent to the sea by natural processes as a result of the weathering of the continental crust (Rodríguez-Figueroa, 2004). The elements are mobilized as solid particles suspended and dissolved by rivers, groundwater, and coastal erosion, melting of the Arctic and Antarctic ice and by atmospheric route (Salomons & Förstner, 1984). Once at sea, the TEs are integrated into the suspended particulate matter of the water column by adsorption and absorption processes, participate in marine biogeochemical cycles and control part of the ocean's chemistry.

River discharges are mostly responsible for TE's contribution to the ocean (Rodríguez-Figueroa, 2004) in coastal zone. Element flows from rivers are initially incorporated into lagoons and estuaries, and finally into marine biogeochemical processes. Calculating the concentration of these flows is complicated because some elements exhibit non-conservative behaviour with respect to others. However, the concentration of TEs in seawater is typically low (<1 mg/kg) (Prasad *et al.,* 2006). The atmospheric contribution of TEs depends mainly on regional wind patterns and dust storms, periodically generated in the great deserts of the Sahara and East China (the two largest sources worldwide). The dust generated from these storms is transported thousands of kilometres offshore and precipitates through dry or wet deposition processes (Goudie, 2009). According to the world health organization, volcanic discharges reflect the entry of sediment and trace elements into the sea; this activity is the largest natural source of Cd to the atmosphere with a contribution of between 100-500 ton / year (WHO, 1992).

Underwater hydrothermal vents are associated with all biologically important TEs (Baross & Hoffman, 1988); mercury (Hg) enrichment has been observed in rocks of underwater hydrothermal systems in the Okinawa Channel, Japan (Halbach *et al.,* 1993), Plenty Bay, New Zealand (Stoffers *et al.,* 1999), and in the underwater hydrothermal system from Punta Mita, Mexico (Prol-Ledesma *et al.,* 2002). These vents provide abundant sulfur, pyrite, arsenic, antimony, and cinnabar (mineral constituted by mercury sulphide) resulting from great volcanic activity (Hedenquist & Lowenstern, 1994). As observed by Segovia-Zavala et al. (2004), upwellings transport dissolved and particulate metals, especially Cd, from deep areas to more coastal waters, and therefore represent another important natural source of this metal to coastal areas.

The high anthropogenic activities represent a source of toxic substances for the marine ecosystem. Potential sources of pollutants include urban tributaries, residual oils derived from maritime transport, industrial discharges and mining activity (Páez-Osuna *et al.,* 2017). Anthropogenic activities contribute around 7300 ton/year of cadmium (Cd) worldwide and the main route of entry into the environment is atmospheric emissions from mining, refining and smelting of minerals, the use of fossil fuels and the use of phosphorus fertilizers in intensive agriculture (Martelli *et al.,* 2006).

Lead (Pb), one of the most common chemical pollutants in the environment, is used in a wide range of products and, due to its resistance and ductility it has been used since ancient times accompanying man in his economic growth (Frías-Espericueta *et al.,* 2010). The atmospheric contribution, mainly due to gasoline, is the main source of Pb for both the terrestrial and marine environment, however, other human activities can cause serious impacts, among which the use of residual paints and use of automotive batteries (Frías-Espericueta *et al.,* 2010).

Used in various industries, gold mining, refinery, combustion, carbon and thermoelectric plants and oil refineries (Páez-Osuna *et al.,* 2017), Hg is considered one of the most toxic pollutants in the world. Its compounds are used as fungicides for seed treatments and growth inhibition in numerous industries such as cellulose. It is an excellent industrial catalyst and, because it is liquid at room temperature, it is used as a component in electrolysis. It can be released into the environment as a residue of laboratory chemicals, batteries, fungicides and pharmaceuticals and as a compound in sewage effluents. The elemental mercury (Hg 0), a predominant species of Hg in the air, has a time of residence in the atmosphere of up to 2 years, consequently its distribution is global, being even in areas considered pristine as the Arctic (Dehn *et al.,* 2006).

Selenium (Se), used in the production of electronics, crystals and pigments is also generated by the gold, cobalt, nickel mining industry and is also found in coal ashes. The main entrance to the atmosphere comes from the combustion and burning of vegetation (Newman & Unger, 2002). Arsenic (As) and its derivatives are used in many products such as metal alloys, pesticides, herbicides and wood preservatives (Páez-Osuna *et al.,* 2017). The presence of this metalloid is also associated with gold and lead mining. Arsenic derivatives are volatile and highly toxic and considered carcinogenic.

It is evident that regardless of the sources of these pollutants, the final receptors are the atmosphere, the aquatic ecosystem and the biota, so their use must be carefully regulated.

1.2.1 Bioaccumulation and biomagnification

Due to the progressive pollution of the seas and coastal ecosystems of the world and the potential health risks they represent, the study of the trophic transfer of TEs is becoming increasingly important worldwide. The bioaccumulation of a contaminant is defined as the total accumulation of a substance in the organism, through all possible sources of exposure (water, food and sediments) (Newman & Unger, 2002). Therefore, bioaccumulation depends on processes of adsorption and dermal absorption, inhalation, food intake and in addition to the excretion capacity and accumulation pathways, and sometimes the growth of organisms. The diet is recognized as the main source of TEs in aquatic organisms (Rand *et al.,* 1995) and is an important route for its transfer to marine trophic networks (Escobar-Sánchez *et al.,* 2010; Maz-Courrau *et al.,* 2012) and for humans. Once the element is in the body, biotransformation mechanisms influence the kidnapping and elimination of the contaminant. Due to their lipophilic characteristics, some substances such as Hg, As and Cd are reabsorbed through different organs (kidneys, brain and liver) so that their elimination is slow and their presence in the body can persist for years (Newman & Unger, 2002).

All TEs are transferred in the trophic web and eventually increase in organisms at the top of the trophic chain, an event that is defined as biomagnification (Wang, 2002). They can become toxic when the levels of accumulation are faster than the capacity to eliminate them from the organism (Fisher & Hook, 2002). The transfer and bioaccumulation of TEs in aquatic systems depends on the physicochemical properties of the elements, their concentration and bioavailability, geochemical fractionation and chemical speciation (Hattum *et al.,* 1991; Soto-Jiménez & Páez-Osuna, 2008). They also influence biotic factors (eating habits, habitat, age, sex, state of health of organisms) and abiotic (temperature, pH, salinity, redox reactions and synergism between the elements). In addition, the transfer of TEs depends on the detoxification and elimination mechanisms available to each species that makes up the chain or plot (Soto-Jiménez, 2011).

1.2.2 Toxicity in marine organisms

The excessive accumulation of TEs in aquatic animals can cause toxicity at different levels: physiological, cellular, and behavioural. The harmful effects depend mainly on the chemical species and its concentration in the most labile phase (Cheng *et al.,* 2008). Hg for example is considered one of the most toxic heavy metals for the health of aquatic and human organisms. However, the greatest ecological and health risk is in methylmercury (MeHg), the result of the transformation of inorganic Hg by some microorganisms in the aquatic systems (Cheng *et al.,* 2008). Because of its bioavailability and ease of absorption, this organic form of Hg is considered the most dangerous for marine organisms. In fish, Hg can cause alterations in the branchial and dermal epithelia, reduction in the absorption of amino acids and sugars in the intestine, and inhibition of intestinal enzymes (Berntssen *et al.,* 2004).

Arsenic dissolved or/and suspended in the water is absorbed by the gills, by the gastrointestinal tract and by the skin. It is distributed in liver, kidney, skin and scales (due to the affinity of the arsenites for keratin), gills and muscle, where the inorganic As is transformed into lipo and water soluble organic form (Prieto-García *et al.,* 2016). This metalloid has a genotoxic effect and in zebrafish it causes teratogenic damage including malformations of fetuses, increase in eggs and non-viable eggs, decrease in hatching percentage and survival of juveniles (Prieto-García *et al.,* 2016). In addition, it causes neuromotor damage and liver and kidney damage in trout (Kotsanis & Iliopoulou, 1999).

Zinc (Zn) causes degenerative changes in muscle and brain of fish embryos, such as: mitochondrial swelling, cytoplasmic vacuolization, swelling of the endoplasmic or sarcoplasmic reticulum, and other degenerative changes associated with cell death (Prieto-García *et al.,* 2016). Among the physiological alterations that can cause excessive accumulation of TEs include necrosis in hepatopancreas, loss of the regular structure of the gill and gastrointestinal tissue, as well as muscular atrophy in crustaceans (Jakimska *et al.,* 2011).

In fish, hypertrophy (increase in muscle fiber size), hyperplasia (increased number of cells), fusion of the second gill lamella, aneurysm, haemorrhage, vascular congestion, hyperaemia (increased irrigation to an organ or tissue) have been recorded and deformity of the branchial arches due to exposure to high concentrations (Hoffman, 2002).

The concentration of essential and non-essential trace elements can induce the formation of reactive oxygen species (ROS), modification of the antioxidant enzymatic activity, oxidative damage in lipids, proteins and DNA (Barrera-García *et al.,* 2012). At the cellular level, mitochondria are one of the main cellular targets of the effects of some TEs (especially metals). It has been proposed that some oxidizing species of As and Hg disturb the structure of the mitochondrial inner membrane favouring the production of ROS. The resulting adverse effects include an imbalance between prooxidant events (increase in free radicals, reactive oxygen and nitrogen species) and antioxidants (enzymatic and non-enzymatic) (Konisgberg, 2008).

Other indicators of oxidative stress include damage to total soluble proteins, production of superoxide radicals and protein damage (Barrera-García *et al.,* 2012). For example, exposure to iron (Fe) increases the levels of carbonyl proteins and TBARS (thiobarbituric acid reactive substances) but decreases the activity of some antioxidant enzymes such as glutathione S-transferase (GST), glutathione reductase (GR) and catalase (CAT). Exposure to copper (Cu) decreases the activity of CAT and glutathione peroxidase (GPx) (Barrera García *et al.,* 2012). Exposure to As increases lipid peroxidasi and CAT, superoxidase dismutase (SOD) and GPx activity in the goldfish Carassius auratus (Lushchak, 2012).

Berntssen et al (2004) found that exposure to organic Hg can increase the concentration of TBARS and decrease the activity of SOD and GPx in Atlantic salmon (*Salmo salar*). The presence of non-essential TEs and free radicals induces DNA damage and replication, nucleic acid synthesis and damage to nuclear chromatin. An example of genotoxic events and chromosomal instability is the formation of micronuclei, small nuclei that form when a chromosome or a fragment of a chromosome is not incorporated into one of the secondary nuclei during cell division (Yadav & Trivedi, 2009). Micronucleus formation has been confirmed in teleost fish exposed to lethal doses of Hg, As and Cu (Yadav & Trivedi, 2009).

The effects of the accumulation of toxic TEs on behaviour are related to abnormal behaviours, breathing out of water, reduced motility and erratic swimming (Atchinson *et al.,* 1987). Several authors have reported behavioural changes including locomotor and respiratory abnormalities in teleost fish exposed to Cd, Cu Zn, Hg, Pb and Se (Atchinson *et al.,* 1987). Studies of these effects on elasmobranch species are probably less common, however, Evans and Weingarten (1990) report vascular anomalies in the canyon shark, *Squalus acanthias*, related to the presence of Cd, Cu Zn, Hg, and Pb (Evans & Weingarten, 1990).

1.2.3 Toxicity in human health

Due to the commercial importance of some species of pelagic fish for consumption, the concern about the harmful effects of TEs on human health is a global problem. The consumption doses, the age of the person, and the chemical forms of the elements are some of the main factors that determine the occurrence and severity of adverse effects on human health (Zuluaga-Rodríguez *et al.,* 2015).

In general, the demonstrated effects on human health include damage to different organs and tissues, including the liver and kidneys, as well as learning difficulties, headaches, and damage to the central nervous system (Ki-Hyun *et al.,* 2016). The Official Mexican Standard (NOM-242-SSA1-2009) have established maximum concentration limits for some potentially toxic trace elements in products of commercial interest. Some of these limits are represented in Table 1.1.

The transport of Hg through the body is facilitated by a cysteine complex (R-SH) that can transfer Hg to the placental and blood-brain barrier. Therefore, the developing brain and fetus are considered the main biological targets of Hg toxicity, especially its more toxic form, methylmercury, ($[CH_3Hg]^+$), in vertebrates (Harley *et al.,* 2015). Among the main reported consequences of methylmercury are degeneration of the occipital cortex and cerebellum, causing paraesthesia (abnormal sensations of tickling, heat or cold on the skin), ataxia (lack of coordination), sensory damage and memory loss, (Dietz *et al.,* 2013). In new-borns, the accumulation of Hg can cause mental retardation, cerebral palsy, and low birth weight. In the 1950s, the city of Minamata, Japan was the centre of an outbreak of methylmercury poisoning (known today as "Minamata disease") where approximately 45 people died (Harada, 1995). Several years after the outbreak, the Japanese government announced that the cause of the disease had been the ingestion of Hg-contaminated fish and shellfish caused by spills from a petrochemical company in the area.

In the case of Se, the variation between diet deficiencies and toxicity is determined in a very low range of 0.1-1.0 µg/g, which makes this essential element the object of study (Chang *et al.,* 2016; Huang *et al.,* 2014;). Selenosis, a disease caused by the high concentration of Se in human body causes nausea, vomiting, hair loss, brittle nails, irritability, fatigue and abnormalities in the nervous system (Ralston *et al.,* 2008). Skorupa *et al.* (1996) suggest in the case of humans, a limited consumption of fish and other marine organisms with concentrations of Se ≥ 2 µg/g ww and a total ban on concentrations ≥ 5 µg/g ww, to avoid any risk of poisoning. On the other hand, Se deficiency (< 5 µg/kg per day) in human provoke severe heart pathologies and the Kashin-Beck disease which produce rheumatism and malformations in bones (Navarro-Alarcon & Cabrera-Vique 2008).

The consumption of contaminated organisms is not the only cause of disease for the population; Itai-Itai disease was the first documented case of massive cadmium poisoning. The disease, registered in 1912 in Toyama Prefecture, Japan, was caused by high mining activity that contaminated rivers and rice fields. Cadmium poisoning causes osteomalacia (demineralization of the bones) and kidney failure causing severe pain in the joints and spine (Harada, 1995).

In 2009, Haefliger *et al.,* (2009) investigated the death of 18 children who died of unknown causes in a community involved in recycling used lead batteries in the suburbs of Dakar, Senegal. What they found were values of up to 613.9 µg/dL of Pb in children's blood, compared to the maximum limit accepted by the Environmental Protection Agency which is 10 µg/dL.

It is evident that heavy metal contamination in humans has been a problem for centuries and it is necessary to implement constant environmental monitoring that includes air, water, sediments and organisms, as well as blood monitoring for populations exposed to these contaminants.

Table 1.1 Trace elements maximum limits (µg/g wet weight) allowed in different marine products established in Mexico. Sources: NOM-242-SSA1-2009.

Element	Product	Maximum limit
As	Crustaceans and bivalve molluscs	80
Cd	Molluscs	2
	Others	0.5
Hg	Fish	1
$[CH_3Hg]^+$	Fish, tuna, marlin	1
	Others	0.5
Pb	Fish and crustaceans	0.5
	Molluscs	1

1.3. Defence mechanisms

1.3.1 Enzymatic response

The study on the physiological responses of elasmobranchs to toxic substances is scarce, however, organisms, including fish, have mechanisms to prevent damage caused by high concentrations of TEs. Antioxidant systems including vitamins, carotenoids and low molecular weight enzymes such as glutathione and some amino acids containing sulfhydryl groups (R-SH) represent the main defence responses for organisms against oxidative stress induced by environmental factors and substances of anthropogenic origin (Rudneva, 1997). Antioxidant enzymes contribute to prevent oxidative damage of tissues by minimizing the production of ROS and their interaction with other molecules (Halliwell & Gutteridge, 2007). It has been shown that antioxidant enzyme activity is related not only to metabolism but also to behaviour including diet and mobility; ROS production is in effect, related to the degree of physical activity, especially in muscle, as a consequence of the flow of oxygen in the mitochondria (Copper *et al.,* 2002). Differences in oxidative stress between sexes have been found in several studies. Estrogens (Borras *et al.,* 2007), vitamin E and glutathione (Goldfarb *et al.,* 2007) in females have been shown to prevent oxidative damage.

1.3.2 Metallothioneins (MTs)

Metallothioneins (MTs), a group of metal-binding proteins, can serve as useful indicators to detect physiological responses to metal exposure in fish and other aquatic organisms (Nordberg & Nordberg, 2009). MTs are intracellular proteins, rich in cysteine and low molecular weight (6-7 kDa) that are present in many species of invertebrates and vertebrates and appear to play a role in homeostasis and detoxification of metal ions by joining and sequestering various metals such as Cu, Zn, Cd, Hg and Pb (Newman & Unger, 2002). MTs also seem to work in other important physiological processes, including the elimination of ROS and the regulation of cell proliferation and apoptosis (cell death) (Chiaverini & De Ley, 2010).

In general, the expression of MT increases in response to exposure to elevated metals, a property that has led to its widespread use as a biomarker to detect effects of toxic metals in human and wildlife populations. Since Hg has been shown to induce MT gene transcription and protein synthesis in various fish species (*e.g.*, *Scatophagus argus*, Sinaie *et al.,* 2010). Thus, MT is a potentially useful biomarker to explore whether ecologically relevant levels of Hg absorption in sharks are associated with physiological alterations.

A few laboratory studies have confirmed that MTs are present in elasmobranchs and can be induced by exposure to some toxic metals in certain species (Hidalgo & Flos, 1986). In the case of *Scyliorhinus canicula*, for example, high levels of hepatic MTs are induced by exposure to Cd (Hidalgo & Flos, 1986).

Although these studies corroborate the efficiency of the use of MTs as a specific indicator of exposure to metals in aquatic organisms, other studies question their suitability as a biomarker for Hg exposure due to the lack of positive correlations between levels of Hg and MT content in several species (Rotchell *et al.,* 2001). Despite the high levels of total Hg (THg) commonly observed in Florida's shovelhead shark (*Sphyrna tiburo*), there was no evidence of high amounts of MTs in such individuals and, in general, muscle THg concentrations and MT levels in muscle and liver were not positively correlated. Therefore, these results suggest that MTs are unlikely to be a useful biomarker for exposure to Hg in this species and perhaps in other sharks. As suggested in this study, this may be due to the chemical form of Hg; methylmercury, in fact, the most ecologically relevant and abundant Hg species found in wildlife seems to be less capable of inducing MTs than inorganic forms of Hg (Walker *et al.,* 2014).

1.3.3 Antagonism between TEs

It is known that the interaction between various elements influences the harmful and beneficial effects of these on organisms. Selenium, an essential element in organisms, is considered an element that opposes the toxicity of Hg (Cabañero *et al.,* 2007). These elements compete for the thiol (-SH) groups of the protein, and this produces an antagonism between them. The high affinity between these two elements allows the formation of the HgSe complex (mercury selenide) responsible for the protective effect of Se (Raleston *et al.,* 2008) that acts against the toxicity of Hg by compensating for the loss and sequestration of selenium by mercury. Further possible mechanisms of protection of Se against the toxicity of Hg include redistribution or excretion of Hg in the presence of Se, competition for binding sites between both elements, the conversion of toxic forms to less toxic Hg and its prevention of oxidative damage of Hg by Se, through the increase in glutathione peroxidase activity (Raymond & Ralston, 2004; Belzie *et al.,* 2005; Raleston *et al.,* 2008).

Considering that the protection of Se occurs through the capture or kidnapping of Hg, the molar ratio of Se and Hg in the tissues needs to approach or be greater than 1: 1 to be effective (Cabañero *et al.,* 2007). Therefore, the protection of Se is efficient only if there is enough free to support the normal synthesis of selenoproteins, responsible for the proper functioning of the brain, pituitary and thyroid (Correa *et al.,* 2015). An example of this trend was proposed by Kaneko and Ralston (2007), who observed in some species of pelagic fish caught in Hawaii a concentration of Se greater than that of Hg (Fig. 1.4), indicating sufficient availability of Se to counteract Hg (Kaneko & Ralston., 2007).

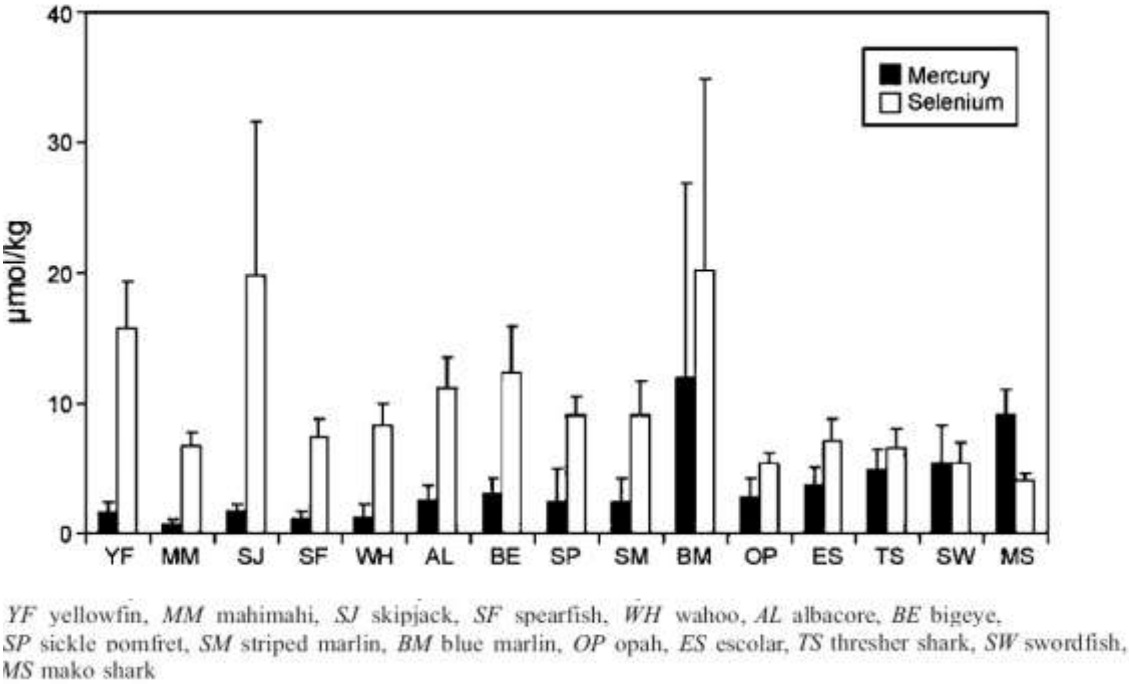

Fig. 1.4. Molar concentration of Hg and Se in pelagic fish of Hawaii. Data are expressed as mean ± standard deviation (Kaneko & Ralston, 2007).

1.4. Background

1.4.1 Trace elements and contamination in whale shark

Being a species with "k" life strategies such as slow growing, slow sexual maturation, large dimensions and extensive longevity, *R. typus* is a species vulnerable to exploitation (Fossi *et al.,* 2017). The greatest threats are represented by illegal fishing, bycatch, and incidents against boats, poor management of tourism and the growing human activities responsible for the increase of polluting substances in the sea (Fossi *et al.,* 2017).

Wang et al. (2014) evaluated the levels of copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), total mercury (THg) and inorganic arsenic (iAs) in different tissues (muscle, liver, epidermis and gill) of two whale sharks (TXT and PQ; Table. 1.2) found dead on the coast of East China. The results from this study showed that the average contents of Cu, Zn, Pb, Cd, Hg and iAs in the tissues were 1.69–22.04, 7.43–141.29, 0.30–4.46, 0.11–31.75, 0.00022–0.011, and 0.16–0.64 µg/g (wet weight), respectively. The six heavy metals contents in whale shark were in the order of Zn>Cu>Pb>Cd>iAs>Hg. Considering that whale shark meat is still consumed in China despite international regulation of protection and following the limits of the Food and Agricultural Organization of the United Nations

(FAO), Wang et al. (2014) suggested that fresh whale shark could represent a dangerous food based for consumers.

Tabla. 1.2 Summary of Cu, Zn, Cd, Pb, iAs and Hg in tissues of whale sharks from the coast of China. Concentrations are expressed in µg/g ww. Source: Wang *et al*., 2014.

Sample	Cu (µg/g ww)			
	Liver	Gill	Muscle	Epidermis
TXT	22.04±0.08	10.90±0.25	3.58±0.07	2.79±0.20
PQ		9.42±0.24	1.69±0.07	2.75±0.06
	Zn (µg/g ww)			
TXT	141.29±0.31	97.21±1.48	11.12±0.04	28.74±0.05
PQ		117.04±0.46	7.43±0.04	21.87±0.09
	Cd (µg/g ww)			
TXT	31.75±0.43	0.59±0.0008	0.12±0.0008	0.20±0.0008
PQ		0.53±0.0008	0.11±0.0008	0.16±0.0008
	Pb (µg/g ww)			
TXT	2.27±0.006	4.46±0.19	0.33±0.006	0.42±0.006
PQ		1.51±0.12	0.30±0.007	0.41±0.006
	iAs (µg/g ww)			
TXT	0.62±0.0028	0.64±0.0022	0.16±0.0002	0.26±0.0003
PQ		0.60±0.0024	0.17±0.0004	0.26±0.0004
	Hg (µg/g ww)			
TXT	0.0033±0.00001	0.0016±0.00001	0.0004±0.00001	0.00022±0.00001
PQ		0.011±0.00004	0.0023±0.00001	0.0012±0.00001
TXT: the samples came from the Taixitou village of Quingao city, Shangdon province; PQ: the samples came from the Puqi town of Wenzhou, Zhejiand province				

Results of THg concentrations from the muscle of three dead whale sharks from South Africa indicate an average of 0.26±0.08 mg/kg dw (McKinney *et al.,* 2016).

Trace element concentrations have been evaluated in twelve whale shark skin biopsies collected from an important feeding area located in the Gulf of Tadjoura, Dijibouti (Boldrocchi *et al.,* 2020). Results from this work show (mean concentrations in ww ± sd) 0.05±0.06 µg/g of Hg, 0.06±0.03 µg/g of Cd, 0.96±0.7 µg/g of As, 1.24±1.12 µg/g of Pb, 8.1±11 µg/g of Cu, 37.8±42.6 µg/g of Zn, 0.35±0.09 µg/g of Se and 18.4±19.7 µg/g of Cr. According to these results and to the Food and Agriculture Organization (FAO, 2003), whale sharks from this area are exposed to high concentrations of Pb probably resulting from human activities. The increasing human pressure in Dijibouti has given rise to ocean contamination from industrial activities, wastewater discharge, vessels, agriculture run-off and organic as well as solid waste (Ahmed *et al.,* 2017).

Fossi et al. (2017) found detectable concentrations of PCBs (polychlorinated biphenyl; mean 8.42 ng/g ww), DDTs (dichlorodiphenyltrichloroethane; mean 1.31 ng/g ww), PBDEs (polybrominated diphenyl ethers; mean 0.29 ng/g ww) and HCB (hexachlorobenzene; mean 0.19 ng/g ww) in whale shark biopsies collected in the Gulf of California. They also confirmed the action of the Cytochrome P450 1A (CYP1A), an enzyme produced from the cytochrome P450 genes that is involved in the formation (synthesis) and breakdown (metabolism) of various molecules and chemicals within cells. Cytochrome P450 enzymes play a role in the synthesis of molecules including hormones, certain fats (cholesterol and other fatty acids), and acids used to digest fats (bile acids).

1.4.2 Trace elements in zooplankton

One of the first study on metals in zooplankton was performed by Martin and Knauer (1973) who presented the concentration of TEs in zooplankton collected in the North Pacific along a transect from Hawaii to Monterrey Bay (USA). The authors affirmed that the elements could pass to higher levels of the food chain or can be redistributed in the marine environment by different routes, such as vertical migration of living planktonic organisms, feces or with the sinking of its exoskeletons.

Other study area included the Greenland Sea where Zn, Cu, Cd and Pb were analysed in different zooplankton taxa by Ritterhoff and Zauke, (1997). The authors found interspecific heterogeneity with low Cd concentrations in calanoid copepods (0.1–0.7 mg/kg, dry weight) and higher levels in the decapod *Hymenodora glacialis* (7–9 mg/kg) and in the amphipods *Themisto abyssorum* and *T. libellula* (24–34 mg/kg). They also found generally low levels of Pb (< 1 mg/kg).

Zauke (2006) evaluated the concentration of several metals in zooplankton from the Barent Sea and find interspecific heterogeneity of metals between groups, with lowest Cd concentrations in euphausiids and chaetognaths and highest ones in decapods and hyperiid amphipods; lowest Cu concentrations in chaetognaths and copepods and highest ones in euphausiids and decapods; and lowest Zn concentrations in euphausiids and decapods and highest ones in some copepods. On the other hand, Pb was often below or close to the limit of detection. Zauke (2006) also states that change in Cd concentrations in copepods from summer to winter/spring could be related either to changing accumulation strategies of the copepod species involved or to seasonally changing Cd absorption in copepods from food.

Concentration of TEs from zooplankton collected in the whale shark feeding area from Dijibouti show (mean ± sd) 1136 ± 1135 µg/g of Fe, 5110 ± 4858 µg/g of Sr, 231 ± 246 µg/g of Pb, 242 ± 155 µg/g of Zn, 1.33 ± 0.70 µg/g of Mo, 1.14 ± 1.65 µg/g of Hg, and 1.61 ± 0.93 µg/g of Co. According to this, biomagnification factor found in Dijibouti was 4.5 ± 2.9 for Cr ($p = 0.0014$), 1.9 ± 1.6 for Mn, 1.7 ± 1.3 for Co, 4.2 ± 2.3 for Ni ($p = 0.0006$), 1.4 ± 0.8 for As and 2.4 ± 1.8 for Mo ($p= 0.0197$) (Boldrocchi *et al.,* 2020).

Studies on trace elements in the zooplankton from the Gulf of California (GC) are scarce. Renteria-Cano (2011) investigated the concentrations of trace and major elements in the North and central area of the GC. Renteria-Cano (2011) concludes that the concentration and distribution of the elements in the zooplankton is affected by the resuspension of sediments by the mixing from tidal currents in the upper gulf, the summer cyclonic turn in the North of the GC and the effects of upwelling in the Ballena Channel.

In her PhD thesis, Renteria-Cano (2011) concludes that trace elements concentrations is not affected by zooplankton biomass; the concentrations of elements in the zooplankton of the GC, present a seasonal variability, as a result of the hydrological processes happening every seasons of the year. A combination of trace elements input sources is presented in the GC; however, resuspension is one of the most relevant mechanisms for enrichment in the northern region, while in the southern region of the archipelago it is believed to be the wind mechanism. Cadmium in the zooplankton showed high levels probably due to the high productivity of the GC associated with upwelling events. Finally, Reinteria-Cano (2011) found no significative differences in TEs concentrations from the zooplankton of the upper Gulf to the zooplankton of the central and southern Gulf, probably because of the intense water connectivity between these areas.

1.5. Justification

The uptake of trace elements in marine organisms may lead to bioaccumulation of toxic substances. Feeding strategy performed by organisms is a key factor in order to understand bioaccumulation mechanisms and very little is known about accumulation of toxics in filter feeder elasmobranchs such as the whale shark. This work aims to generate useful knowledge in a unique shark species whose feeding habit is different from most or almost all sharks. The results will allow contrasting the bioaccumulation pattern of TEs in filter feeder species with typically carnivorous sharks.

This species represents a source of benefit for local communities such as La Paz where touristic activities are carried out. The lack of information on whale shark ecotoxicology call to the need to invest in long term studies in order to establish the health state of this protected species. A better knowledge of their health state not only spread the general awareness about the species, but it is also fundamental to establish plans of environmental protection in the area of aggregation.

1.6. Research objectives

The main aim of this work is to evaluate the concentrations of essential (Cu, Se and Zn) and non-essential (As, Cd, Hg and Pb) elements in biopsies (epidermis) of whale sharks, *Rhincodon typus*, and zooplankton collected in Bahía de Los Angeles (BLA) and Bahía de La Paz (LAP), Gulf of California, Mexico, during two seasons (S1: 2016-2017; S2: 2017-2018).

1.7. Specific objectives

The objectives of this study are:

- To know and compare the concentrations of As, Cu, Cd, Hg, Pb, Se and Zn in the biopsies of whale sharks from two study areas and in the two seasons.
    - To know the bioaccumulation for size and sex.
    - To determinate the change in element' concentration in those animals that were sampled in both seasons.
    - To calculate the molar ratios of selected elements.

- To identify the main groups of zooplankton composing the diet of the sharks.
- To determinate the biomagnification of Se and Hg in the sharks.

- To evaluate the concentrations of As, Cu, Cd, Hg, Pb, Se and Zn in different tissues of two dead whale sharks, stranded in the Gulf of California.
- To quantify the concentrations of As, Cu, Cd, Hg, Pb, Se and Zn in the liver of two dead whale sharks, stranded in the Gulf of California.
  - To establish the distribution of TEs in the right and left lobe of the liver.
  - To calculate the molar ratios Se:Hg, Se:As, Zn:Cd and Zn:As in the right and left lobe of the liver.

1.8. Study area

This work was carried out in two study areas, both located in the lower Gulf of California, in sites adjacent to the Peninsula of Baja California, Mexico: Bahía de Los Angeles (BLA; 28.9519° N, 113.5624° W) (Baja California) and Bahía de La Paz (LAP; 24.0832° N, 110.1839° W) (Baja California Sur).

Different studies have shown that, in the two bays, young whale shark males are mainly sighted (Ramírez-Macías *et al.,* 2012). In La Paz Bay the same whale sharks are sighted up to 135 days with a 38% chance to see them again the following year (Ramirez-Macias *et al.,* 2012). This demonstrates the importance of this feeding site towards the species. As described by Ramírez-Macías *et al.,* (2012) between the two bays there is a coincidence of the same sharks of 13%. Whale sharks follow oceanographic patterns (physical and biological) within the Gulf of California that are favourable for food availability (Lavaniegos *et al.,* 2012). However, where they come from when they enter the bays and where they go when they leave them, they are questions still unanswered.

1.8.1 Bahía de Los Ángeles (BLA)

Bahía de Los Ángeles is located on the east coast of Baja California, Mexico (Fig. 1.5). It is an open bay towards the Gulf of California, 16 km long and

6.4 km in the widest part. It is a shallow bay that is mostly less than 40 m deep. The entrance to the bay measures approximately 10 km and is interrupted in the middle by seven islands and in the southern part by Horse Head and two more islands. In the northern part, the Coronado and Piojo Islands separate the Bay of the Whale Channel creating a narrow passage parallel to the entrance. The islands provide protection to the bay from east winds; however, strong winds can cause strong waves through the channels to the islands (Danemann & Ezcurra, 2008). The Whale Channel region is particularly rich in nutrients for phytoplankton growth (Millán-Núñez & Yentsch, 2000). The morphology, wind patterns that favour the mixing of the water column, the temperature and the high productivity of the Whale Channel, make the bay a habitat where whale sharks congregate seasonally (Ramírez-Macías *et al.,* 2012) for up to six months a year (from June to December with peaks in October and November) (Nelson & Eckert, 2007). In the bay, the distribution and abundance of these sharks coincides with the areas of higher abundance of the zooplankton (García-García, 2002). The most important sighting site is located south of the bay, in an area known as El Rincón, adjacent to a small coastal wetland that favours high productivity and high feed concentrations for whale shark aggregations. However, some sharks can be sighted in the northern part of the bay called La Gringa.

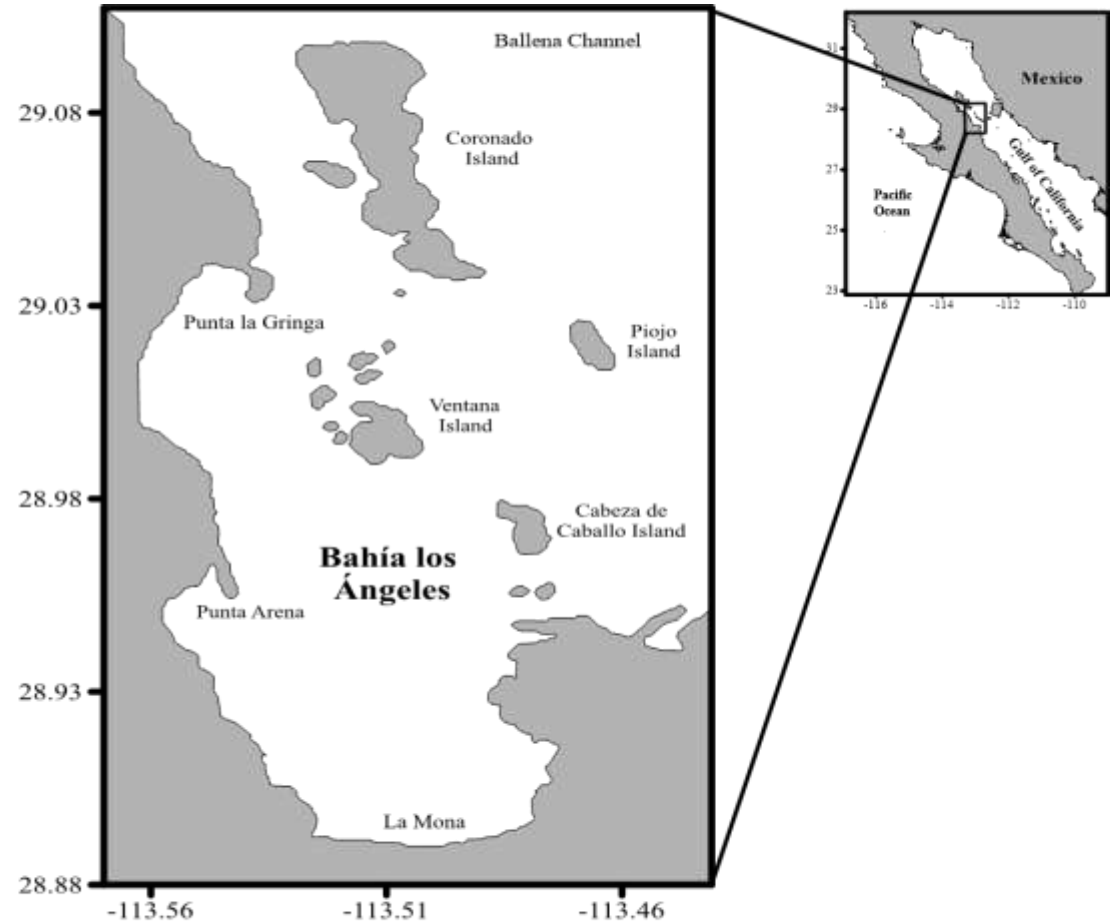

Fig. 1.5. Map of Bahía de Los Ángeles, BC, Mexico.

1.8.2 Bahía de La Paz (LAP)

Bahía de La Paz (Fig. 1.6) is the largest body of coastal water in the Gulf of California, with approximate dimensions of 80 km long, 35 km wide and an approximate area of 2,635 km^2 (Obeso-Nieblas *et al.,* 2007). It is an almost completely closed bay, semi-elliptical, located in the southern western part of the Gulf of California between geographical coordinates 24 ° 08'32 '' N 110 ° 18'39 '' W. It is bounded to the north by the San José Island, to the south by the Ensenada de La Paz and the sandy bar known as El Mogote and to the east by the Espiritu Santo and La Partida Islands. The connectivity of the bay and its circulation is mainly driven by the exchange of water with the Gulf of California and occurs in two steps; a channel to the northeast called Boca Grande and towards the southern part through the San Lorenzo Canal. The bay has a bathymetric gradient that identifies two areas delimited by the 200 m isobath: a deep zone (> 200 m deep) in the northern part with influences of oceanic water. Here, the greatest depth is reached in the Alfonso Basin (450 m deep). The coastal zone

(<200 m deep) is located in the west, southwest and Canal de San Lorenzo (Sánchez-Velasco *et al.,* 2006). Primary productivity in the Bay of La Paz is high and influenced by a seasonal winter-spring cycle (Sánchez-Velasco *et al.,* 2006). The upwellings and seasonal cyclonic spins in the centre of the bay enrich the surface layer of water and this favours secondary productivity especially in winter and spring (De Silva, 1997). Zooplankton biomass varies seasonally reaching its maximum volumes in spring and winter and its minimums in summer and autumn (De Silva, 1997).

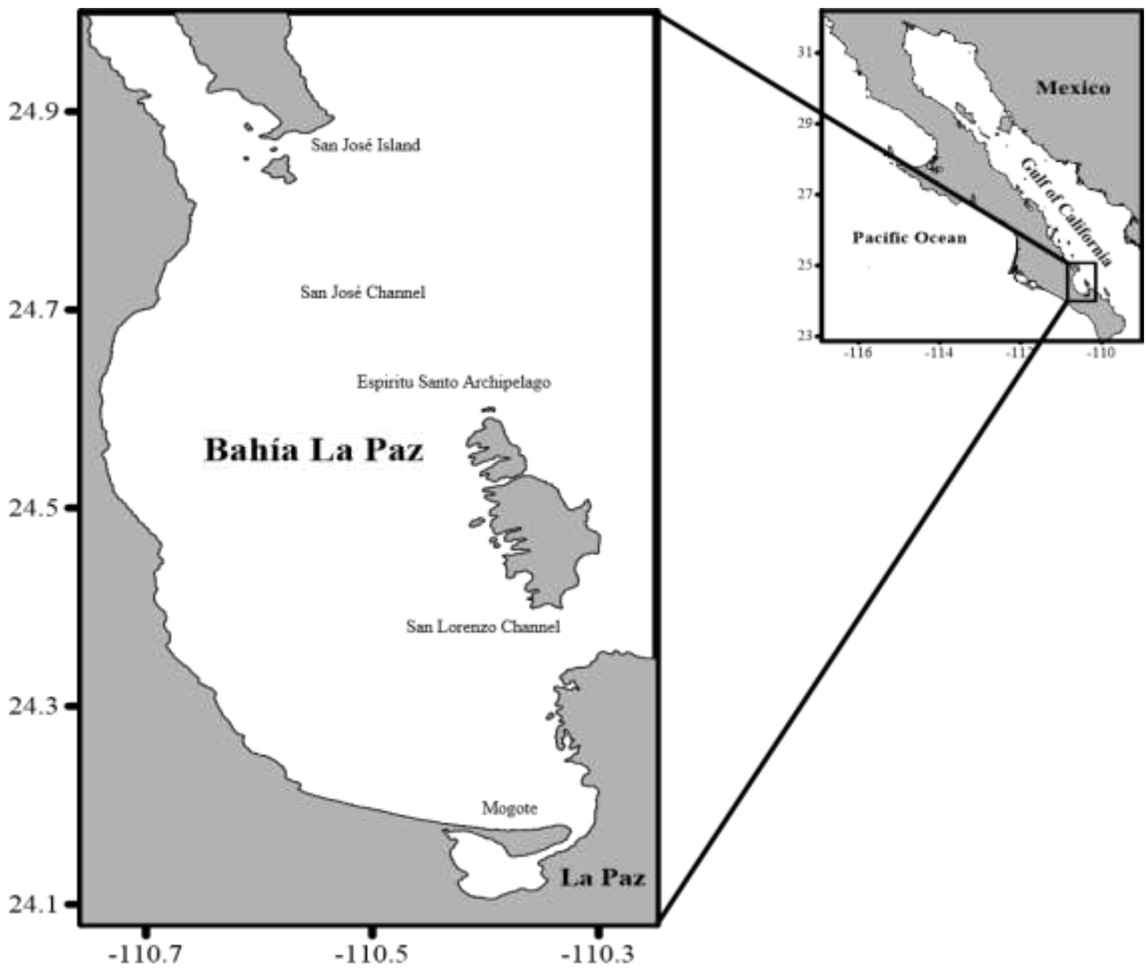

Fig. 1.6. Map of Bahía de La Paz, BCS, Mexico.

1.8.3 La Paz lagoon

The lagoon of La Paz is located inside the bay and it is separated from the bay by a peninsula called "Mogote" (Fig. 1.7). The lagoon has an extension of 45 km^2 and is a shallow area that reaches less than 10 m of depth (Del Monte-Luna *et al.,* 2005). It is constantly influenced by the exchange of waters with the bay and it presents areas with vegetations and mangroves.

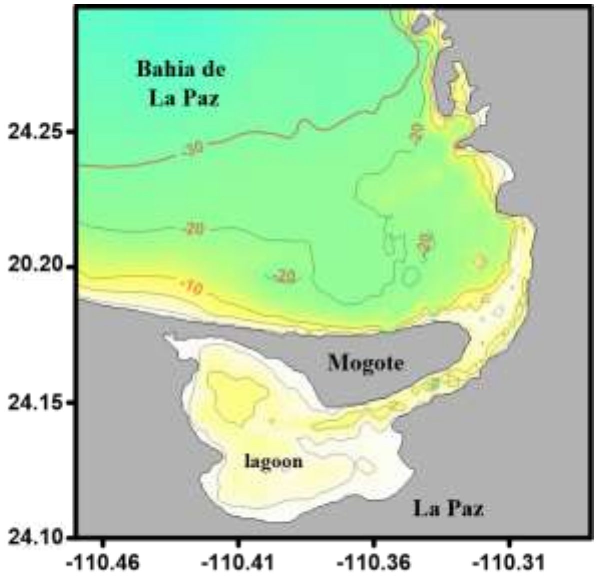

Fig. 1.7. Map of La Paz lagoon.

1.9. MATERIALS AND METHODS

1.9.1 Biopsy collection

Animals were approached using a boat like “panga”. Once a shark was observed, one person entered the water with snorkelling equipment and a digital subaquatic camera (GoPro™) to document: (i) estimated total length (TL); (ii) identification (ID) pictures (Fig. 1.8) by photographing key areas including the gills and the pectoral fin on the left side of the body (Van Tienhoven *et al.,* 2007). ID pictures allowed us to identify each shark and avoid repeating the biopsy of the same animal; and (iii) sex of the animal was determined by the presence of claspers on males. Clasper morphology distinguished juvenile from adult males. Claspers are shorter than pelvic fins, soft and smooth in immature males. Mature males, instead, present claspers extended beyond the pelvic fins with a cauliflower-like texture appearance (Norman & Stevens, 2007). Female maturity was determined using estimated TL > 9 m (Joung *et al.,* 1996). Biopsies were collected using a stainless-steel biopsy tip mounted on a pole of 200 cm length. The tip was disinfected (ethyl alcohol 70%) before and between each biopsy collection. Once collected, the biopsies were rinsed with distilled water, placed in a plastic

Eppendorf tube and stored on ice until returning to the laboratory. Once there, biopsies were weighed, placed in Eppendorf tubes and frozen at −40°C.

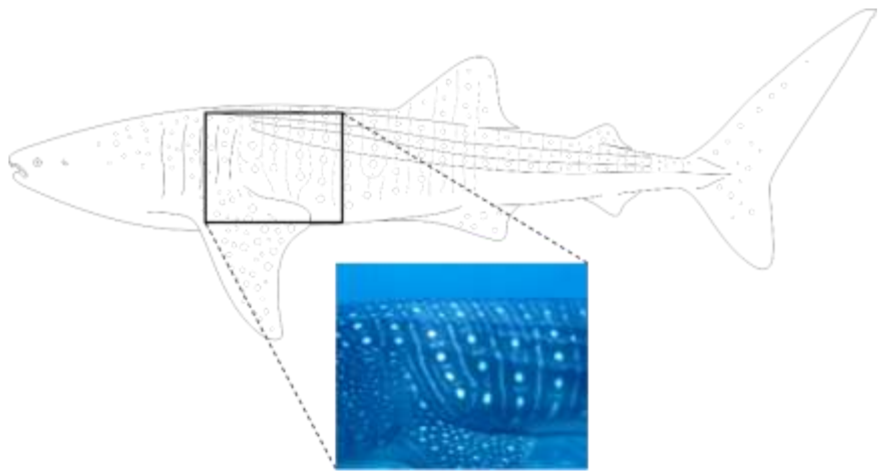

Fig. 1.8. Whale shark identification picture (ID).

1.9.2 Zooplankton collection

The collection of zooplankton was performed when sharks were observed feeding. A conical net (500 μm mesh, 60 cm diameter, 1.5 m in length) fitted with a flowmeter (2030, General Oceanics) was used. Plankton tows were performed in a different way according to the sharks' feeding behaviour (Fig. 1.9 a,b,c): (i) during horizontal active feeding, we performed linear tows for 5min, at 20–30 m distances from the shark; (ii) during horizontal passive feeding, one linear tow was carried out for 5 min with the net placed 20–30 m in front of the mouth of the shark attached to a 5 m long tow-line; and (iii) during vertical active feeding, circular tows were carried out for 5min around the shark at 20–30 m distance (Ketchum *et al.,* 2013).

During the trawls, the boat was kept at a distance of at least 20 m from the whale shark to avoid changing its behaviour. Before entering the net in the water, data on water temperature, longitude, latitude, flowmeter and time were recorded. Once the net was removed, the flowmeter and time data were recorded again. Zooplankton was filtered with a 333 μm mesh using ocean water from the feeding sites, then poured into a clean and sterilized plastic container.

The samples were left in a refrigerator with ice until the arrival at the laboratory. Once there, the zooplankton total wet biomass was determined by displaced volume and samples were examined with a dissecting microscope to identify zooplankton groups to phylum, subclass, or order (Beers, 1976). Total number of individuals for each group was counted. Each sample was divided into two parts based on mass: one half was used for the analysis of the total zooplankton, and the other half, was separated into main zooplankton groups.

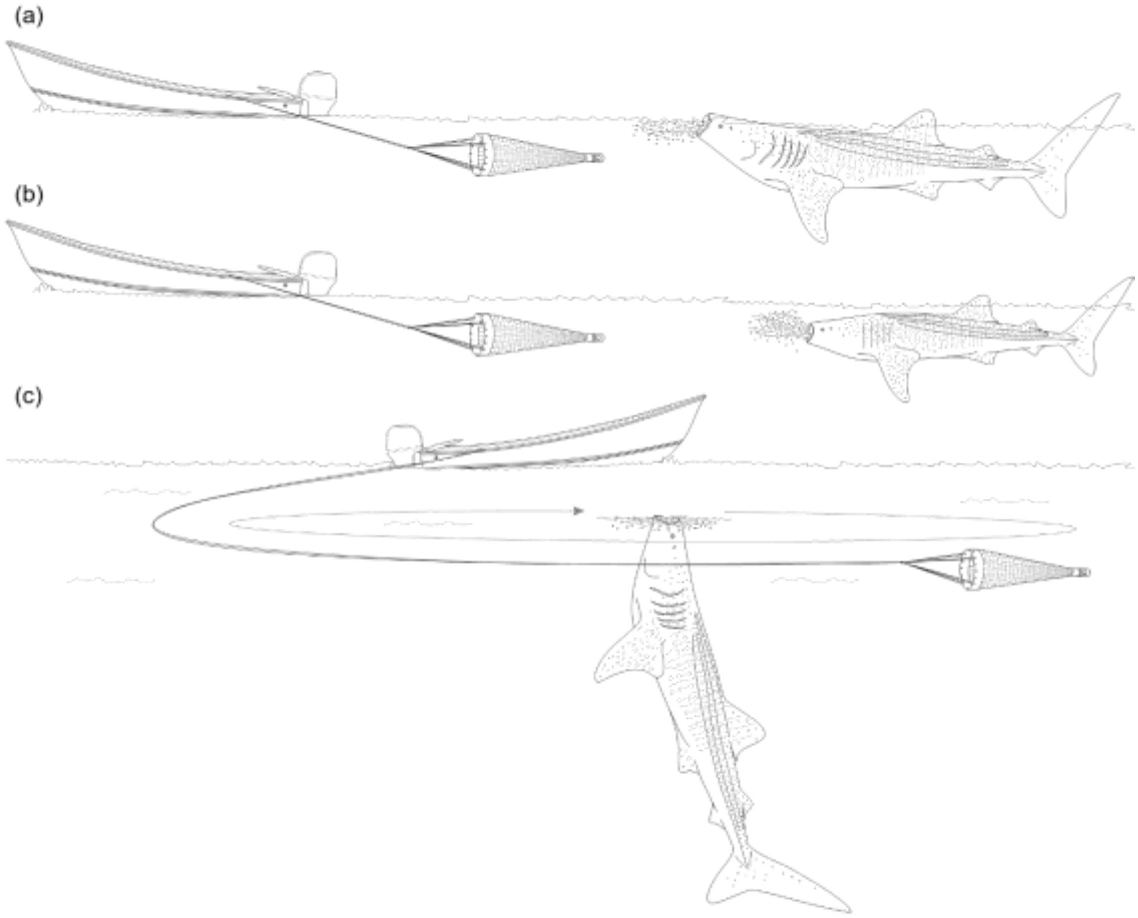

Fig. 1.9. Tows of zooplankton performed depending on whale shark feeding behaviour: active surface feeding (a); passive feeding (b); and vertical feeding (c).

1.9.3 Laboratory procedures

1.9.3.1 Drying and Homogenization

Zooplankton samples and biopsies frozen at -40 ° C were placed in a freeze dryer with three holes in the paraffin cover eppendorf tubes to ensure proper water removal. The samples were freeze dried (−50 °C and 137×10^{-3} mbar, 72 h). Once the samples were dried, the paraffin plug was removed from the eppendorf tubes and each sample was ground with the help of a porcelain mortar. Between each sample, the mortar was washed with a solution of 10% HCl and purified Milli-Q or three-distilled water.

1.9.3.2 Digestion

The digestion of the samples was carried out in the geochemistry and contamination laboratory of the Institute of Marine Sciences and Limnology of the National Autonomous University of Mexico UNAM, in Mazatlán (Sinaloa). The samples were deposited in 50 ml Teflon pumps (Savillex). The weight of each sample in each Teflon pump was noted. 1, 2, 3, 4 or 5 ml of 69% trace metal grade HNO_3 was added depending on whether the dry weight of the sample was 50, 100, 150, 200 or 250 mg, respectively. Teflon pumps were heated on heating plates at a maximum temperature of 130 ° C for 3 hours.

Once the digestion was finished, the Teflon pumps were allowed to cool for 3 hours. Digested samples of 50, 100 and 150 mg dry weight were received in 4.5 ml vials of polypropylene, Corning Incorporated brand. Samples of 200 and 250 mg dry weight were received in 15 ml polypropylene vials, Corning Incorporated brand. All samples were titrated with a solution based on In 115 containing HNO_3 and triple distilled water.

Aliquots of biopsies and zooplankton, blanks and reference material were digested in vials (Savillex) with HNO_3 (70%, trace metal analysis, J.T. Baker) to 130°C for 3 h (Bergés-Tiznado *et al.,* 2019). Aliquots of dried samples were gauged with a solution containing Indio (In 115), nitric acid and triple distilled water. Trace elements concentration was analysed using a high resolution inductively coupled plasma mass spectrometry (ICP-MS) Thermo Scientific Element 3XR. Hg was analysed by cold vapour atomic absorption spectrometry (SpectraAA 220, Varian VGA-110).

1.9.3.3 Validation of the method

Material used for digestion was cleaned with a double nitric acid bath for 48 h and rose with tridistilled water. The reference concentrations and the obtained by the recovery percentage after the digestion, is shown in Table 1.3 and Table 1.4. Procedural blanks analysis and reference material analysis was performed with no anomalies detected.

Table 1.3. Recoveries (%) of trace elements, and values certified and obtained for the reference material DORM-4 fish muscle during the season S1 (2016-2017) and season S2 (2017-2018). Concentrations are expressed in mg/kg per dry weight (dw).

DORM-4: fish muscle (mg/kg dw)					
	Certified values ± sd	Obtained values ± sd		Recovery %	
		S1	S2	S1	S2
As	6.87 ± 0.44	7.24 ± 1.38	6.90 ± 1.57	105.4	100.4
Cd	0.299 ± 0.018	0.311 ± 0.060	0.298 ± 0.071	104.0	99.7
Cu	15.7 ± 0.5	16.9 ± 2.6	16.6 ± 2.9	107.6	105.7
Hg	0.412 ± 0.036	0.409 ± 0.107	0.424 ± 0.126	99.3	102.9
Pb	0.404 ± 0.062	0.378 ± 0.057	0.441 ± 0.004	93.6	109.1
Se	3.45 ± 0.40	3.22 ± 0.07	3.57 ± 1.02	93.3	103.5
Zn	51.6 ± 2.8	55.7 ± 10.5	48.3 ± 10.5	107.9	93.6

Table 1.4. Recovery (%) of trace elements, and values certified and obtained for the reference material DORM-4 fish muscle during the season S1 (2016-2017) and season S2 (2017-2018). Concentrations are expressed in mg/kg per dry weight (dw).

DOLT-5: dogfish liver (mg/kg dw)			
	Obtained values ± sd	Certified values ± sd	Recovery %
As	31.8 ± 32.5	34.6 ± 2.4	92
Cd	14.5 ± 9.4	14.5 ± 0.6	100
Cu	39.3 ± 24.6	35.0 ± 2.4	112
Hg	0.46 ± 0.21	0.44 ± 0.18	106
Pb	0.163 ± 0.156	0.162 ± 0.032	100
Se	8.7 ± 7.0	8.3 ± 1.8	105
Zn	105.9 ± 98.7	105.3 ± 5.4	101

1.9.3.4 Calculation of molar ratios

The molar ratios Se:Hg, Zn:As, Zn:Cd, Cd:Pb, Cd:As, Se:Cd and Se:As were calculated from the individual results of each element and for each tissue divided by the molecular weight of each element: As = 74.922; Cd = 112.411; Hg = 200.59; Pb = 207.2; Se = 78.96; Zn = 65.39 (nmol/g wet weight). Finally, the molar ratio was obtained by dividing the concentration of one element in nmol/g with the other element in nmol/g. The accuracy was estimated using a certified material DORM-4 and DOLT-05 whose reference values are shown in Tables 1.3 and 1.4.

1.9.3.5 Calculation of the biomagnification (BMF)

Biomagnification factor (BMF) was calculated using the equation (Gray, 2002): BMF=CD/CP, where CD is the element concentration of the predator and CP is the element concentration of the prey, where it establishes the increase in concentration between trophic levels as long as the biomagnification factor is significantly > 1.0 (Bergés-Tiznado et al., 2019). Determining a biomagnification factor in this manner is based on the assumption that concentration has reached steady state in the biopsies of the sampled individuals.

1.9.3.6 Statistical analysis

Concentrations of TEs, and Se:Hg molar ratios were tested for normality using Shapiro's test. As these data were not normally distributed, non-parametric tests were used. The Grubb test was applied to detect outliers from each group of data; according to the test, statistical analyses were repeated excluding outliers to establish the effect of those individuals on the results. All statistical analyses were performed using R studio (Rstudio, Inc. R Core Team 2018).

We tested the effect of intrinsic (fish length) on variation in TEs and Se:Hg ratio, both among and within the collection sites. The influence of sex and the differences between TEs concentrations in the study areas were compared with a Mann-Whitney test. Spearman rank correlations were used to determine associations among metal levels and TL. Assuming that individuals < 4 m has a coastal habitat and that individuals > 4 m live in pelagic areas, animals' samples were separated in two groups according to their length (< 4 m and > 4 m) (Borrell *et al.,* 2011) to determine the correlation among BMF and TL for Hg and Se (see Chapter 2). All biomagnification factors were tested for significance using the t-test. Significance level used in these analyses was $p < 0.05$ (Zar, 2010). Concentrations below the limit of detection were treated following the EPA guidance for data quality assessment (USEPA, 2000). According with this guidance, procedures for analyzing data with non-detects depend on the amount of data below the detection limit. When non-detected data are <15%, data are replaced by the detection limit. When non-detected data are between 15% - 50% Cohen's adjustment is applied and when non-detected data are > 50% - 90% tests for proportions is used. Our data below the detection limit were adjusted using Cohen’s method which provides adjusted estimates of the sample mean and standard deviation that accounts for data below the detection level. The adjusted estimates are based on the statistical technique of maximum likelihood estimation of the mean and variance so that the fact that the non-detects are below the limit of detection but may not be zero is accounted for. The adjusted mean and standard deviation can then be used in the parametric tests.

CHAPTER 2

Hg AND Se BIOACCUMULATION AND BIOMAGNIFICATION IN EPIDERMIS OF WHALE SHARK DURING TWO SEASONS FROM TWO AREAS OF THE GULF OF CALIFORNIA, MEXICO

Chapter based partially on the paper: Pancaldi F., Galván-Magaña, F., González-Armas, R., Soto-Jiménez, M.F., Whitehead, D.A., O'Hara, T., Marmolejo-Rodríguez, A.J., Vázquez-Haikin, A., Páez-Osuna, F. (2019). Mercury and selenium in the filter–feeding whale shark (*Rhincodon typus*) from two areas of the Gulf of California, Mexico. Marine Pollution Bulletin. 146: 955-961.

2.1. ABSTRACT

Mercury and selenium were measured for first time in the endangered species whale shark (*Rhyncodon typus*) from two areas of the Gulf of California, Bahía de Los Angeles (BLA) and Bahía de La Paz (LAP) using dermal biopsies of 130 specimens and during two seasons (S1 and S2): S1= 2016-2017 and S2= 2017-2018. Additionally, in S1 nineteen zooplankton samples from LAP were analysed, while in S2 eleven zooplankton samples were analysed in BLA, and 14 zooplankton samples were analysed in LAP. During S1, concentrations (ng/g, wet weight (ww)) in biopsies of BLA ranged from 1 to 40 for Hg and 100 to 680 for Se; while in LAP varied from 1 to 9 for Hg and 11 to 850 for Se. A significative correlation was found for Hg in BLA males' biopsies with length. Mean±SD Hg and Se concentrations in the zooplankton from LAP were 1.6±1.8 and 770±930 ng/g, respectively. Hg biomagnification factor ranged from 0.8 to 5.3 in sharks. During S2, Hg concentrations in biopsies from males of BLA ranged from 21 to 497 ng/g ww and from 26 to 1833 for Se; while in LAP varied from 12 to 58 for Hg and from 124 to 1183 for Se. A significative and negative correlation was found for Hg and Se in BLA males' biopsies with length ($p<0.05$). Mean±SD Hg and Se concentrations in the zooplankton from LAP were 1.2±0.6 and 340.6±120.6 ng/g ww, respectively. In BLA, mean±SD Hg and Se concentrations in the zooplankton were 1.7±1.6 and 296.2±117.7 ng/g ww. Hg biomagnification factor was 19.9 in the whale sharks from LAP and 41.0 in the animals from BLA.

A molar excess of Se over Hg was found in both seasons and both sites in the biopsies and the zooplankton.

2.2. INTRODUCTION

The uptake of Hg and Se by marine organisms is mainly influenced by diet and trophic position, however, Hg accumulation is usually higher for fishes feeding at a higher trophic position (Storelli *et al.,* 2009). Therefore, top predators like some sharks tend to accumulate some chemicals due to their trophic position (Maz-Courrau *et al.,* 2012).

The Hg:Se ratio has been examined in diverse organisms such as elasmobranchs, cetaceans, pinnipeds and fish (e.g., Escobar-Sánchez *et al.,* 2010; Correa *et al.,* 2015; Bergés-Tiznado *et al.,* 2015; 2019). The protection mechanisms of selenium over mercury include redistribution or excretion of Hg in the presence of Se, competition for the binding sites between the two elements, formation of the Hg:Se complex, conversion of toxic forms to less toxic Hg and their inhibition of oxidative damage of Hg by Se, through the increase in the activity of glutathione peroxidase (Raymond & Ralston, 2004; Ralston *et al.,* 2008). Due to the high affinity between Hg and Se, the formation of an inorganic Hg:Se complex is proposed as the mechanism responsible for the protective effect of Se (Ralston *et al.,* 2008). Nevertheless, if Se protection occurs through Hg sequestration, the molar ratio of Se:Hg in the tissues needs to be >1:1 to be operative (Raymond & Ralston, 2004; Ralston *et al.,* 2008).

The whale shark (*Rhincodon typus*, Smith, 1828) is a filter feeder and a migratory pelagic long-lived species (Rowat & Brooks, 2012). It is a protected species, listed as endangered by the International Union for Conservation of Nature (IUCN) since 2016. Seasonally, this species aggregates in several areas of the Gulf of California and is regularly observed in Bahía de Los Angeles (BLA) and Bahía de La Paz (LAP), Mexico. Segregation by sex and size occurs in several areas; according to Borrell et al. (2011), whale sharks show an isotopic signature of $\delta^{13}C$ that indicates a change of habitat from the coastal zone to the pelagic areas, when the animals exceed the 4 m of length. The aims of this study in particular are: (i) evaluate the bioaccumulation of Hg and Se in whale shark skin biopsies collected from two feeding areas of the Gulf of California: BLA and

LAP; (ii) identify bioaccumulation differences for sex and size of the sharks; (iii) determine possible biomagnification of these elements through the main prey of zooplankton collected in the same study areas, and; (iv) determine the molar ratio of Se:Hg in dermal biopsies and zooplankton samples.

2.3. RESULTS

2.3.1 Sex and total length (TL) of the sharks

A total of 130 whale shark biopsies, were collected in two locations in the Gulf of California (Table 2.1). In Bahía de Los Angeles (BLA) (28.9519° N, 113.5624° W) samples were collected in September 2016 and 2017, while in Bahía de La Paz (LAP) (24.0832° N, 110.1839° W) samples were collected from September 2016 until March 2018 (Fig. 2.1).

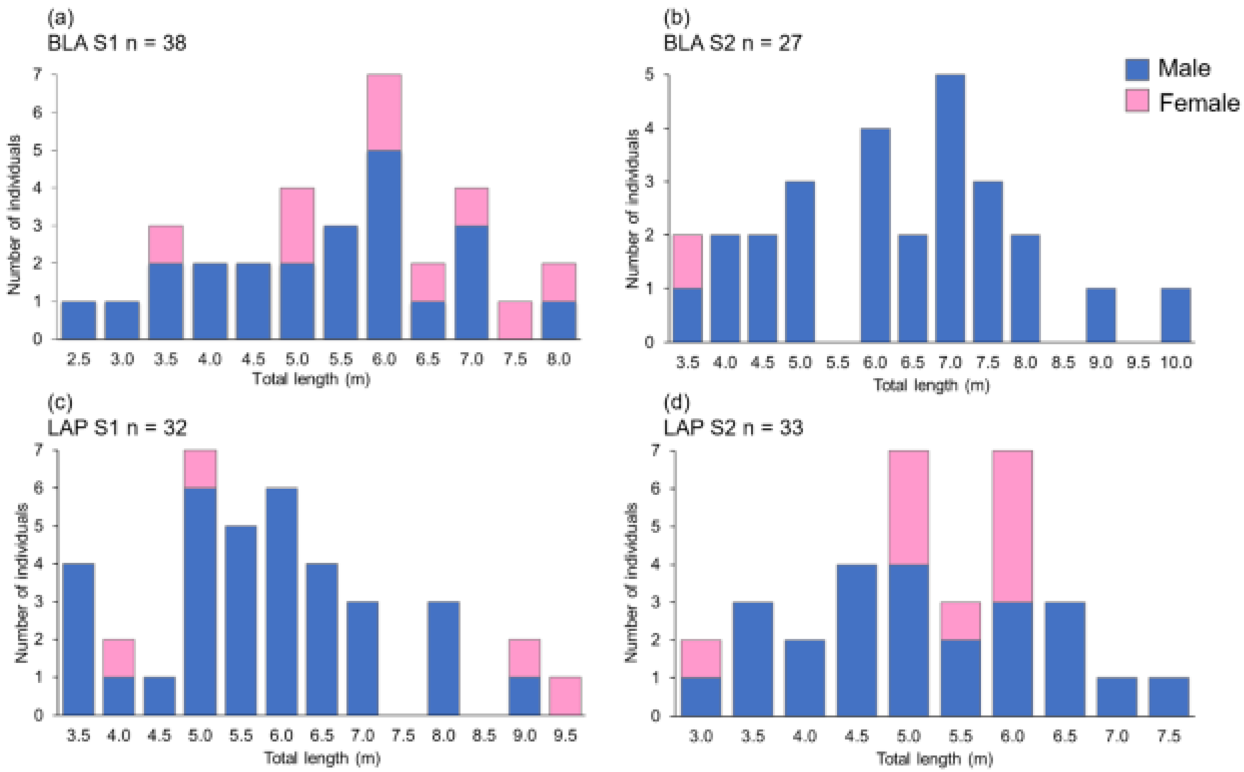

Fig. 2.1 Sex and size of whale sharks sampled in BLA (ab) and LAP (cd) in S1 and S2.

In S1 in BLA, 34 individuals were males (89%) and 4 were females (11%) with an estimated TL ranging between 3.5 and 9.5 m (Table 2.1). In LAP, 23 individuals were males (72%) and 9 were females (23%) with a TL ranging between 2.5 and 8.0 m. In S2 in BLA, 26 individuals were males (96%) and 1 was female (4%) with an estimated TL ranging between 3.5 and 10 m. In LAP, 24 individuals were males (73%) and 9 were females (27%) with a TL ranging between 3.5 and 7.0 m.

Considering both seasons, 82% (n=107) of the animal sampled were males and 18% (n=23) were females. Most of the animals (92%; n=120) were considered immature, and 8% of them (n=10) were recognized as mature.

Table 2.1. Number of biopsies collected in Bahía de Los Ángeles (BLA) and Bahía de La Paz (LAP) in S1 (2016-2017) and S2 (2017-2018).

	S1 (2016-2017)				**S2 (2017-2018)**					
	BLA		LAP		BLA		LAP		Total	
	n	%	n	%	n	%	n	%	n	%
♂	34	89	23	72	26	96	24	73	107	82
♀	4	11	9	23	1	4	9	27	23	18
Total n	38		32		27		33		**130**	

2.3.2 Season 1- Concentrations of Hg and Se in biopsies

2.3.2.1 Differences between sites

In biopsies of sharks sampled from BLA (n = 38), the Hg content (in ww) ranged between 1 and 40 ng/g with a mean ± sd of 9 ± 7 ng/g, while Se ranged between 100 and 680 ng/g with a mean ± sd of 230 ± 140 ng/g. The Hg in the biopsies of sharks from LAP (n = 32) ranged between 1 and 9 ng/g with a mean ± sd of 4 ± 2 ng/g and Se ranged between 11 and 850 ng/g with a mean ± sd of 280 ± 200 ng/g (Fig. 2.2). Biopsy water percentage ranged from 76% to 92% in BLA and from 83% and 96% in LAP (Table 2.2). Mann-Whitney test showed significant differences ($p < 0.05$) for both Hg (p=0.002) and Se (p=0.02) between the two areas.

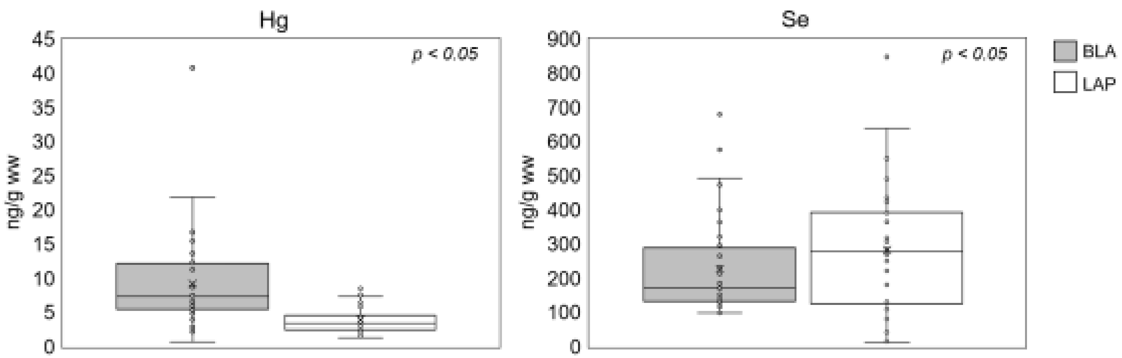

Fig. 2.2 Hg and Se concentration (ng/g ww) in biopsies of whale sharks collected in S1 in BLA and LAP.

Table 2.2 Mean ± standard deviation (min-max), concentrations (ng/g ww) of Hg and Se, and molar ratio Se:Hg in the biopsies of whale shark collected in two bays from the Gulf of California: Bahía de Los Ángeles (BLA) and La Paz (LAP) in 2016 and 2017.

	n	Hg ng/g	Se ng/g	Hg nmol/g	Se nmol/g	Molar ratio Se:Hg
BLA						
Male	34	9 ± 7	230 ± 150[1]	0.05 ± 0.04	2.9 ± 1.9	100 ± 115[1]
		(1-40)	(100-680)	(0.003-0.20)	(1.2-8.6)	(8.3-627)
Female	4	7 ± 4	180 ± 26[2]	2.25 ± 0.33	0.03 ± 0.02	96 ± 71[2]
		(2-11)	(160-210)	(0.01-0.06)	(2.0-2.7)	(40-193)
Total	38	9 ± 7[a]	230 ± 140[a]	0.05 ± 0.03	2.9 ± 1.8	100 ± 111[a]
		(1-40)	(100-680)	(0.003-0.20)	(1.2-8.6)	(8.3-627)
LAP						
Male	23	4 ± 2	260 ± 210[1]	0.02 ± 0.01	3.4 ± 2.6	188 ± 164[1]
		(1-9)	(11-850)	(0.04-0.01)	(0.1-10.7)	(5.2 -672)
Female	9	3 ± 1	320 ± 160[2]	0.02 ± 0.01	4.1 ± 2.0	295 ± 160[2]
		(1-6)	(80-550)	(0.03-0.01)	(1.0-7.0)	(55-519)
Total	32	4 ± 2[b]	280 ± 200[b]	0.02 ± 0.01	3.6 ± 2.5	219 ± 170[b]
		(1-9)	(11-850)	(0.04-0.01)	(0.1-10.7)	(5.2-672)
Total of 2 bays	70	7 ± 6	250 ± 170	0.03 ± 0.03	3.2 ± 2.2	154 ± 152
		(1-40)	(11-850)	(0.003-0.20)	(0.1-10.7)	(5.2-672)

Different superscript letter indicates significantly different ($p < 0.05$) mean concentrations between sites for the same element or Se:Hg molar ratio; different superscript number indicate significantly different ($p < 0.05$) mean concentrations between sexes for the same element or Se:Hg molar ratio. Statistical were performed by a Mann-Whitney test.

2.3.2.2 Differences between sex

- *Bahía de Los Ángeles*

Mean concentration of Hg in males from BLA (n = 24) was higher (9±7 ng/g ww) compared with females (n = 4; 7±4; Fig. 2.3). Hg ranged from 1 to 40 ng/g in males and from 2 to 11 ng/g in females. Mean concentration of Se in males from BLA was higher (230±150 ng/g ww) compared with females (180±26). Se ranged from 100 to 680 ng/g in males and from 160 to 210 ng/g in females. No statistical tests were applied to these data due to the low number of females from this site.

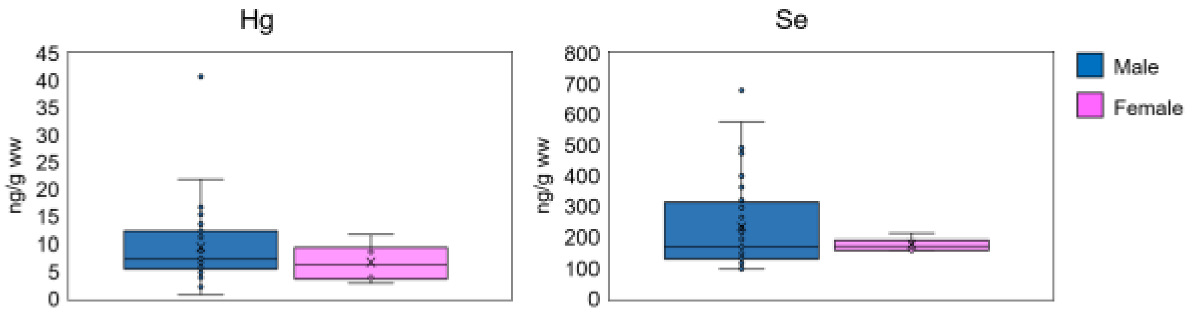

Fig. 2.3 Hg and Se concentrations in males and females from BLA in S1.

- *Bahía de La Paz*

Mean concentration of Hg in males from LAP (n = 23) was higher (4±2 ng/g ww) compared with females (n = 9; 3±1). Hg ranged from 1 to 9 ng/g in males and from 1 to 6 ng/g in females (Fig. 2.4). Mean concentration of Se in males from LAP was lower (260±210 ng/g ww) compared with females (320±160). Se ranged from 11 to 850 ng/g in males and from 80 to 550 ng/g in females. Mann-Whitney test shows no statistical differences ($p > 0.05$) of Hg and Se between sex in this study area (Table 2.2).

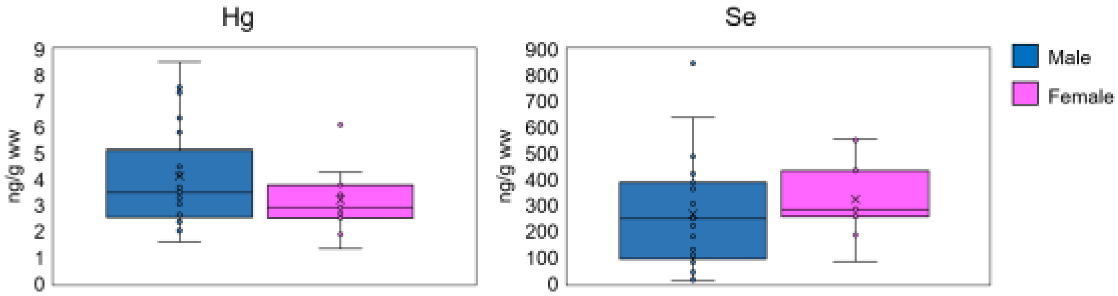

Fig. 2.4 Hg and Se concentrations in males and females from LAP in S1.

2.3.2.3. Relation with total length (TL)

➢ *Bahía de Los Ángeles*

Males from this study area showed a negative and significant correlation between Hg and TL (Spearman p value < 0.05; linear equation [Hg]=−2.16 TL+ 22.1) (Fig. 2.5) The same relationship showed a positive behaviour in females with no significant correlation ($p > 0.05$) probably due to the low number of females. Se exhibited a negative relationship but was not-significant ($p > 0.05$) with TL for both sexes (Fig. 2.5b).

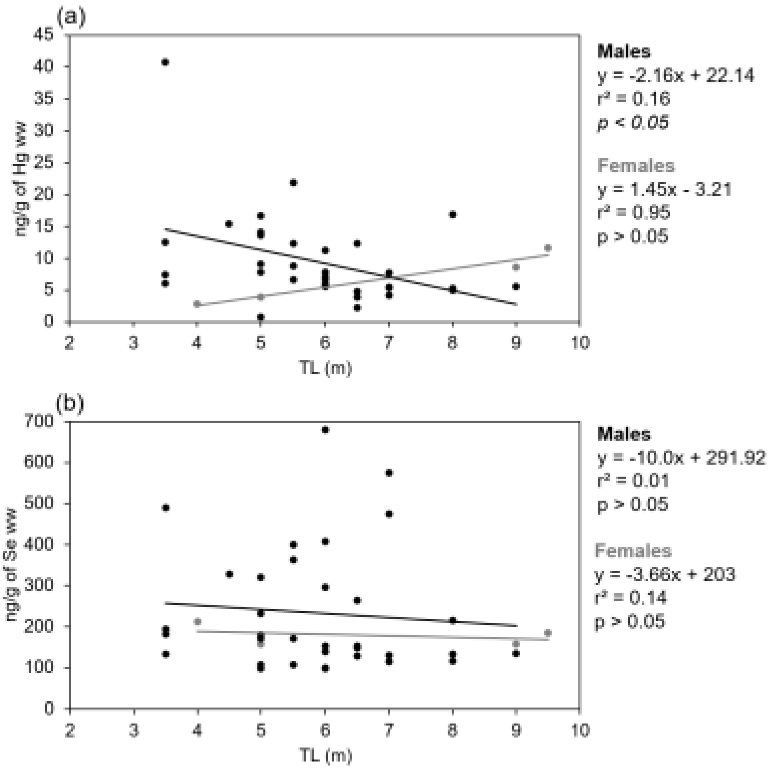

Fig. 2.5. Correlation between total length (m) with Hg (a) and Se (b) in males (black) and females (grey) from BLA in S1.

- *Bahía de La Paz*

Males from LAP showed a negative correlation between Hg and TL while females presented a positive behaviour; no significant correlation was found between these variables for both sexes ($p > 0.05$). Se exhibited a negative relationship but not-significant ($p > 0.05$) with TL for both sexes (Fig. 2.6).

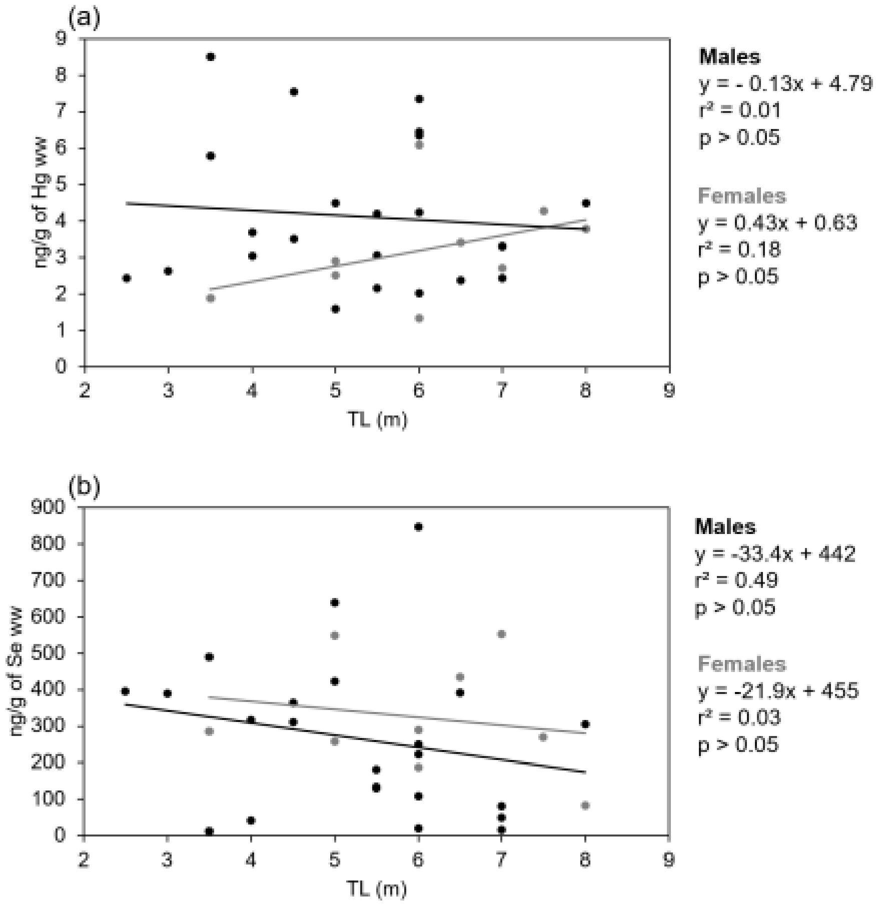

Fig. 2.6. Correlation between total length (m) with Hg (a) and Se (b) in males (black) and females (grey) from LAP in S1.

2.3.2.4 Molar ratio Se:Hg

The Se:Hg molar ratio varied widely among whale shark biopsies within each bay (Table 2.2) and ranged from 8.3 to 627 in BLA and from 5.2 to 672 in LAP. All values of the Se:Hg molar ratio always were significantly >1:1 ($p < 0.001$). Molar ratio Se:Hg in BLA was 100:1 and in LAP was 220:1 (Table 2.2). Biopsy concentrations of Hg (nmol/g) and Se (nmol/g) were not significantly correlated in both BLA and LAP. Molar ratio Se:Hg did not show any significan correlation with the TL in both study areas.

2.3.3 Season 1 - Concentrations of Hg and Se in the zooplankton

2.3.3.1 Zooplankton composition

A total of 19 samples of zooplankton were collected in LAP from September 2016 to March 2017 (during February no sampling was performed); 12 samples include zooplankton and 7 subsamples were obtained to measure the Hg and Se concentrations in the main groups of zooplankton.

Copepods and chaetognatha were the main groups of zooplankton collected while the sharks were feeding followed by decapods and euphausiids (Fig. 2.7). Concentrations of TEs were analysed only in copepods (n=2=, chaetognatha (n=3) and euphausiids (n=2).

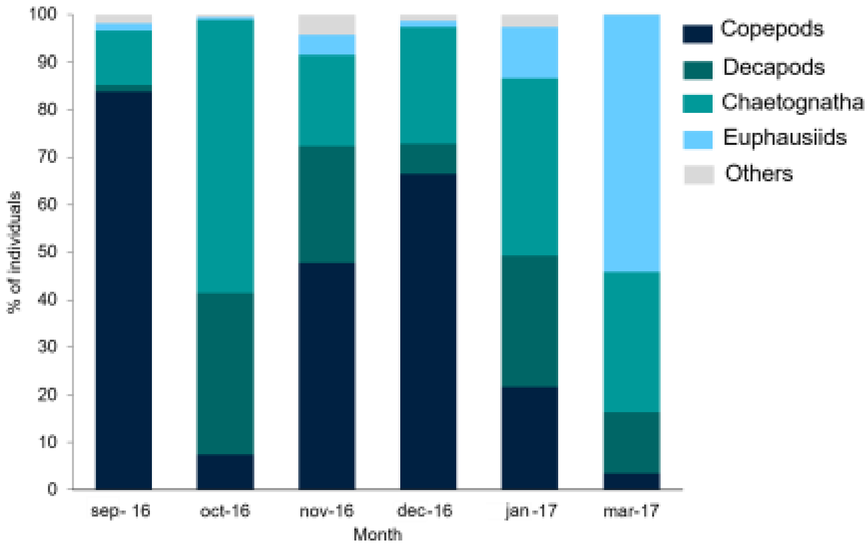

Fig. 2.7. Composition and abundance of zooplankton collected in LAP in S1.

2.3.3.2 Trace element concentrations

Zooplankton exhibited wide variations fluctuating from 0.1 to 5.5 ng/g (wet weight) for Hg and from 1 to 2830 ng/g for Se (Fig 2.8). Chaetognatha was the group with the highest concentration of Hg (4.5 ng/g) and copepods with the lowest (0.06 ng/g). In contrast, the highest level of Se was found in copepods (220 ng/g) and the lowest in chaetognatha (9.8 ng/g) (Fig. 2.9). Concentrations of Hg and Se, mean and SD in the zooplankton are shown in Table 2.3. Statistical tests were not applied due to low number of samples.

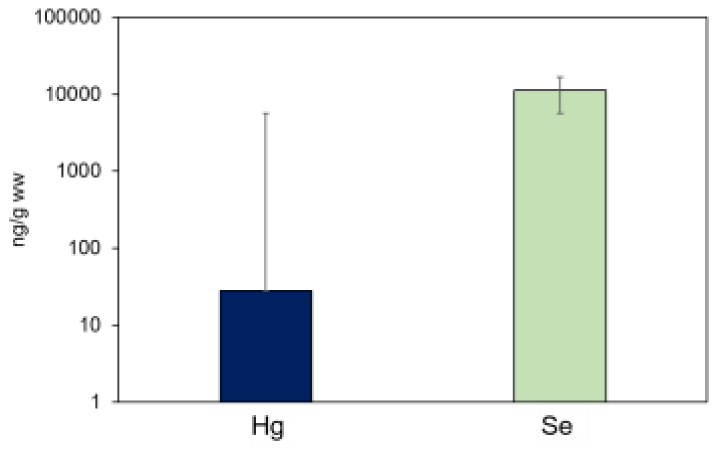

Fig. 2.8. Hg and Se concentration in the zooplankton collected in LAP in S1.

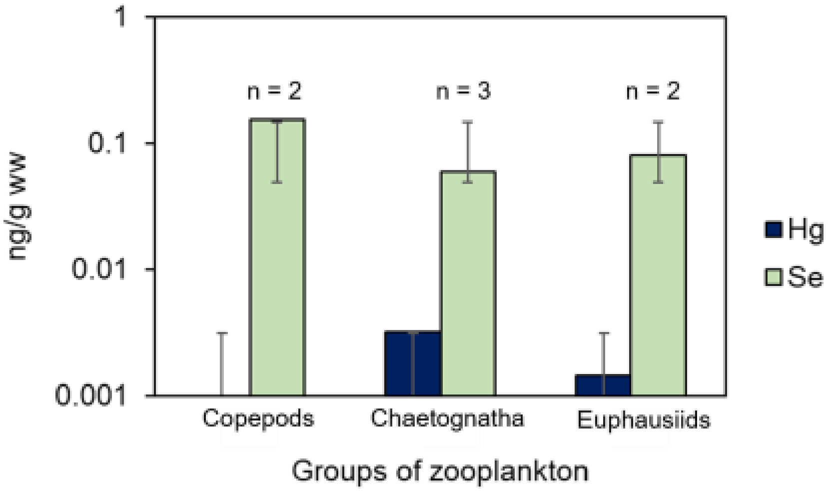

Fig. 2.9. Hg and Se concentrations in the main groups of zooplankton in LAP in S1.

Table 2.3. Concentrations of Hg and Se (on ww basis) and molar ratio Se:Hg in the zooplankton and main groups collected in 2016 and 2017 in Bahía La Paz (LAP), Gulf of California.

		Month	Year	Hg ng/g	Se ng/g	Hg nmol/g	Se nmol/g	Se:Hg
		Sept	2016	0.13	482	0.0006	6.1102	9567
		Oct	2016	0.01	1	0.0000	0.0145	454
		Oct	2016	4.49	87	0.0224	1.0966	49
		Oct	2016	5.51	129	0.0275	1.6397	60
		Nov	2016	2.55	198	0.0127	2.5051	197
		Nov	2016	1.90	509	0.0095	6.4435	678
Zooplankton		Dec	2016	2.53	902	0.0126	11.4231	905
		Dec	2016	0.30	2,825	0.0015	35.7838	24086
		Dec	2016	0.09	273	0.0004	3.4567	7633
		Jan	2017	0.21	1,398	0.0010	17.7048	17275
		Mar	2017	0.41	1,189	0.0021	15.0567	7292
		Mar	2017	1.05	2,272	0.0052	28.7749	5476
Average±SD				1.60±1.85	773±931	0.008±0.009	10.8±11.5	6139±7750
	Copepods	Sept	2016	0.28	220.3	0.0014	2.7896	2022
	Copepods	Dec	2016	0.06	85.7	0.0003	1.0849	3493
Main groups	Chaetognatha	Oct	2016	1.18	67.0	0.0059	0.8482	144
	Chaetognatha	Dec	2016	3.87	9.8	0.0193	0.1247	6.4
	Chaetognatha	Mar	2017	4.53	101.4	0.0226	1.2844	57
	Euphausiids	Jan	2017	2.05	98.7	0.0102	1.2496	122
	Euphausiids	Mar	2017	0.82	63.6	0.0041	0.8053	198

2.3.3.3 Biomagnification factor (BMF)

Average Hg BMF through total zooplankton was 2.4 and it was statistically >1.0 ($p < 0.001$). The implicit assumption with this calculation is that the concentrations found in the biopsies represent the entire organism which is obviously not known. All sharks below 4 m of total length (n=7) showed Hg (BMF) significantly >1 ($p < 0.001$) (Fig. 2.10). Hg BMF in these sharks ranged from 1.1 to 5.3 (average ± SD, 2.5 ± 1.5). Sharks of this category showed a positive but not significant (p Spearman > 0.005) relationship between Hg BMF and TL. Sharks above 4 m of TL (n=25) showed a negative tendency (not significant) between Hg BMF and TL; in this group, all sharks but two, showed Hg BMF significantly>1.0 ($p < 0.001$). Hg BMF in this category ranged from 0.8 to 4.7 (2.4 ± 1.0) (Fig. 2.10).

Average BMF of Se through total zooplankton was 0.3 in LAP and was significantly < 1.0 ($p < 0.001$). Se BMF ranged from 0.01 to 0.6 in sharks < 4 m ($p < 0.05$) and from 0.01 to 1.0 in sharks > 4 m ($p < 0.001$). Considering that the predominant diet of the whale shark is composed of copepods (Clark and Nelson, 1997; Hacohen-Domené et al., 2006), then Hg BMF ranged in LAP from 8 to 50 (23 ± 11). Hg BMF ranged from 11 to 50 (24 ± 14) in sharks < 4 m and from 8 to 44 (23 ± 10) in sharks > 4 m. On the other hand, the Se BMF ranged in LAP from 0.1 to 5.5 (1.7 ± 1.3); Se BMF ranged from 0.1 to 3.2 (1.8 ± 1.2) in sharks < 4 m and from 0.1 to 5.5 (1.9 ± 1.4) in sharks > 4 m. All Hg BMF calculated for copepods, chaetognatha and euphuasiids showed values significantly>1.0 ($p < 0.05$).

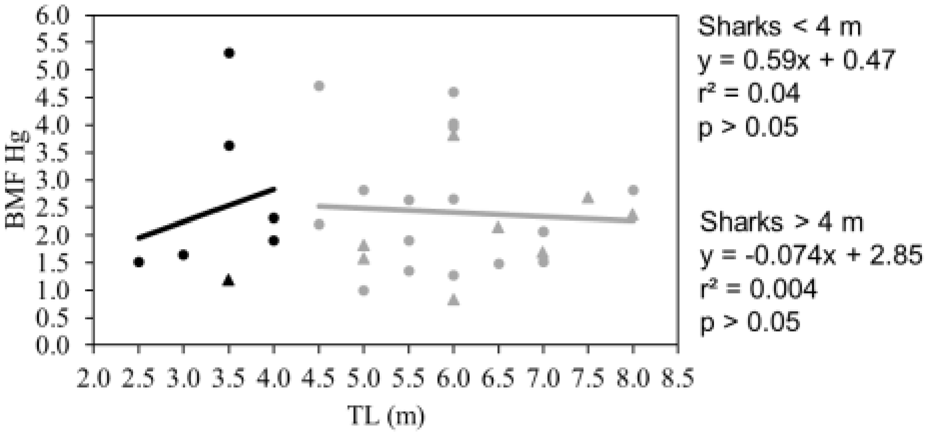

Fig. 2.10. Variation between Hg BMF (calculated from zooplankton and the biopsies) and total length of sharks below 4m (black circles) and above 4m of total length (grey circles) from Bahía de La Paz. Circles are males, and triangles are females.

2.3.4 Season 2: Concentrations of Hg and Se in biopsies

2.3.4.1 Differences between sites

In biopsies of sharks sampled from BLA (n = 27), the Hg content (in wet weight) ranged between 21 and 497 ng/g with a mean ± SD of 71 ± 109 ng/g, while Se ranged between 26 and 1833 ng/g with a mean ± SD of 263 ± 410 ng/g. The Hg in the biopsies of sharks from LAP (n = 33) ranged between 12 and 58 ng/g with a mean ± SD of 24 ± 11 ng/g and Se ranged between 89 and 1183 ng/g with a mean ± SD of 329 ± 194 ng/g (Table 2.4). In BLA, water percentage of the biopsies ranged from 68% to 94% while in LAP water percentage of the biopsies ranged from 65% to 94%. Mann-Whitney test showed significant differences ($p < 0.001$) for Hg between the two areas (Fig. 2.11).

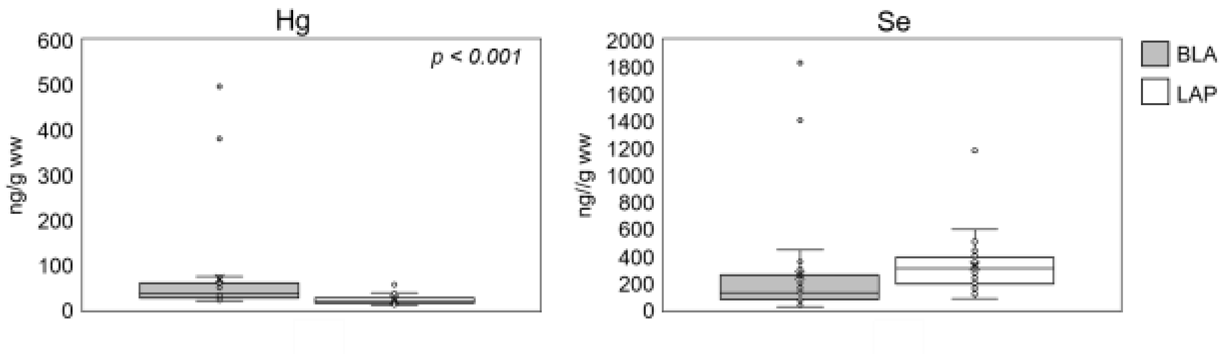

Fig. 2.11. Hg and Se concentration (ng/g ww) in biopsies of whale sharks collected in S2 in BLA and LAP.

Table 2.4. Mean ± standard deviation (min-max), concentrations (ng/g ww) of Hg and Se, and molar ratio Se:Hg in the biopsies of whale shark collected in two bays from the Gulf of California: Bahía de Los Ángeles (BLA) and La Paz (LAP) in S2.

	N	Hg ng/g	Se ng/g	Hg nmol/g	Se nmol/g	Molar ratio Se:Hg
BLA						
Male	26	72 ± 111	263 ± 418	0.4 ± 0.6	3.3 ± 5.3	9 ± 3[1]
		(21-497)	(26-1833)	(0.1-2.5)	(0.3-23.2)	(2.3-15.3)
Female	1	54	265	0.3	3.4	12.4
Total	27	71 ± 109[a]	263 ± 410	0.4 ± 0.5	3.3 ± 5.2	9 ± 3[a]

		(21-497)	(26-1833)	(0.1-2.5)	(0.3-23.2)	(2.3-15.3)
LAP						
Male	24	23 ± 11	340 ± 210	0.1 ± 0.1	4.3 ± 2.7	40 ± 18[1]
		(12-58)	(124-1183)	(0.1-0.3)	(1.6-15.0)	(15.2 -71.3)
Female	9	25 ± 11	300 ± 150	0.1 ± 0.1	3.8 ± 1.9	30 ± 8
		(15-45)	(89-601)	(0.1-0.2)	(1.1-7.6)	(14.6-41.7)
Total	33	24 ± 11[b]	329 ± 194	0.1 ± 0.1	4.2 ± 2.5	37 ± 17[a]
		(12-58)	(89-1183)	(0.1-0.3)	(1.1-15.0)	(14.6-71.3)
Total of 2 bays	60	46 ± 76	299 ± 309	0.2 ± 0.4	3.8 ± 3.9	24 ± 19
		(12-497)	(26-1833)	(0.1-1.5)	(0.3-23.2)	(2.3-71.3)

Different superscript letter indicates significantly different ($p < 0.05$) mean concentrations between sites for the same element or Se:Hg molar ratio; different superscript number indicate significantly different ($p < 0.05$) mean concentrations between sexes for the same element or Se:Hg molar ratio. Statistical were performed by a Mann-Whitney test.

2.3.4.2 Differences between sex

- *Bahía de Los Ángeles*

Hg concentration ranged from 21 to 497 ng/g in males from BLA with a mean ± SD of 72 ± 111. Mean concentration of Se in males from BLA was higher (263±418 ng/g ww) and ranged from 26 to 1833 ng/g. Hg concentration in the only female sampled in this study area during this season was ng/g while Se was 265 ng/g.

- *Bahía de La Paz*

Mean concentration of Hg in males from LAP (n = 24) was similar (23±11 ng/g ww) to concentration in females (n = 9; 25±11). Hg ranged from 12 to 58 ng/g in males and from 15 to 45 ng/g in females. Mean concentration of Se in males from LAP was higher (340±210 ng/g ww) compared with females (300±150). Se ranged from 124 to 1183 ng/g in males and from 89 to 601 ng/g in females. Mann-Whitney test shows no statistical differences ($p > 0.05$) of Hg and Se between sex in this study area (Fig. 2.12).

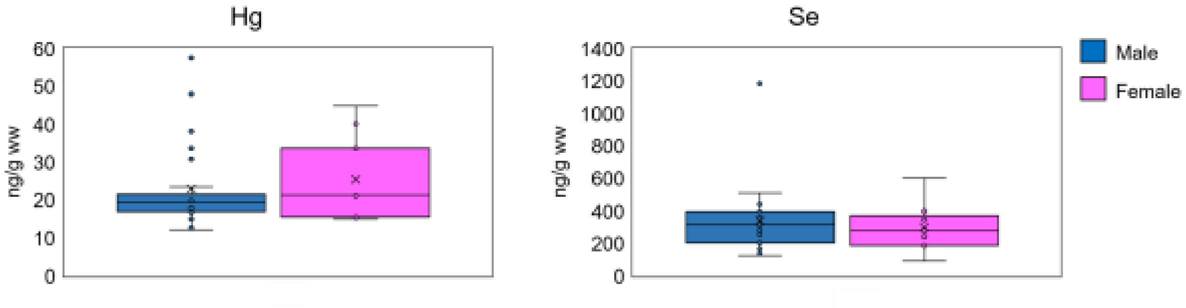

Fig. 2.12 Hg and Se concentrations in males and females from LAP in S2.

2.3.4.3 Relation with total length

- *Bahía de Los Ángeles*

In the organisms sampled in BLA, both Hg and Se showed a negative relationship with TL in males where Spearman correlation showed a significant factor ($p<0.05$) (Fig. 2.13 a and b.). In LAP (Fig. 2.14 a and b), males showed a negative relation with the TL while females showed a positive relation between TE concentration and TL, nevertheless, no significative statistical differences were found.

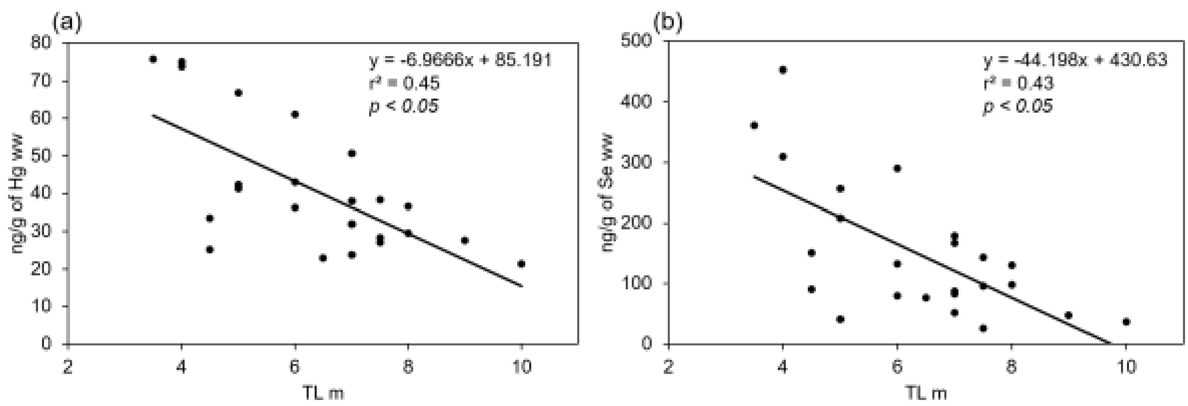

Fig. 2.13. Correlation between TL and concentrations of Hg (a) and Se (b) in males of BLA in S2.

- *Bahía de La Paz*

Males from LAP showed a negative correlation between Hg and TL, while females presented a positive behaviour; no significant correlation was found between these variables for both sexes ($p > 0.05$). Se exhibited a positive relationship but not-significant ($p > 0.05$) with TL for both sexes (Fig. 2.14 b).

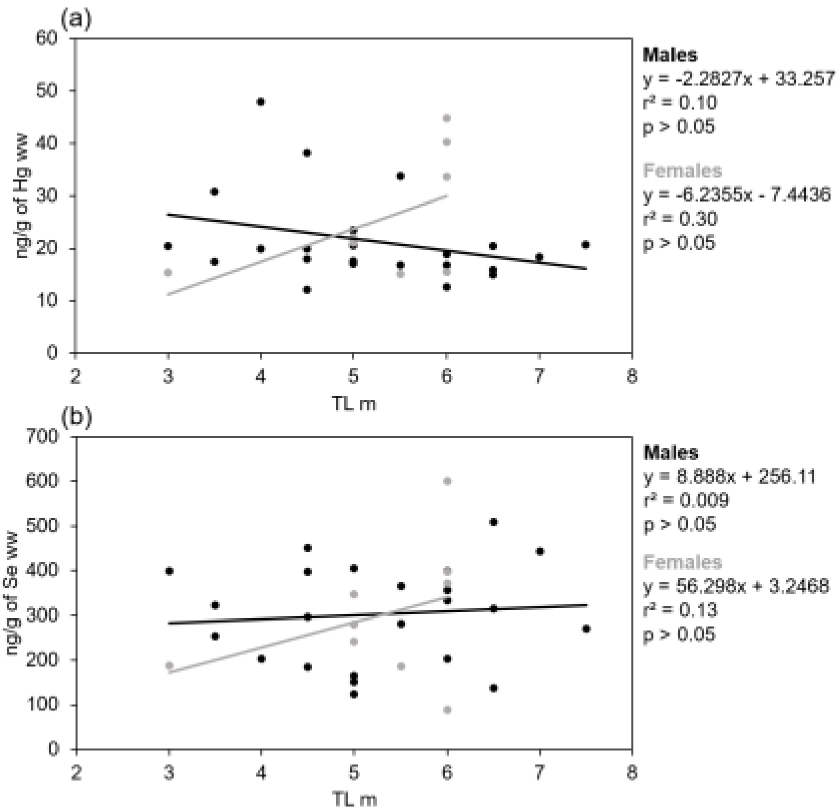

Fig. 2.14. Correlation between TL and concentrations of Hg (a) and Se (b) in males (black) and females (grey) whale sharks of LAP in S2.

2.3.4.4 Molar ratio Se:Hg

The Se:Hg molar ratio calculated from the individual concentrations varied widely among whale shark biopsies within each bay (Table 2.4) and ranged from 2.3 to 15.3 in BLA and from 14.6 to 71.3 in LAP. All values of the Se:Hg molar ratio always were significantly >1:1 ($p < 0.001$). Molar ratio Se:Hg in BLA was 9:1 and in LAP was 37:1 (Table 1). Biopsy concentrations of Hg (nmol/g) and Se (nmol/g) showed a positive and significant correlation in LAP (Spearman $p < 0.05$; Fig. 2.15) but not in BLA. Se:Hg molar ratio showed a negative and significative correlation ($p = 0.0007$) with the total length of the sharks from BLA (Fig. 2.16).

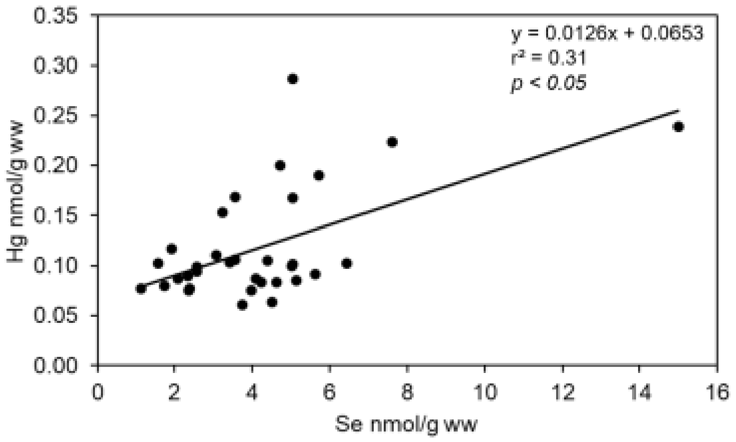

Fig. 2.15. Correlation between concentrations of Hg (nmol/g) and Se (nmol/g) from whale shark of LAP in S2.

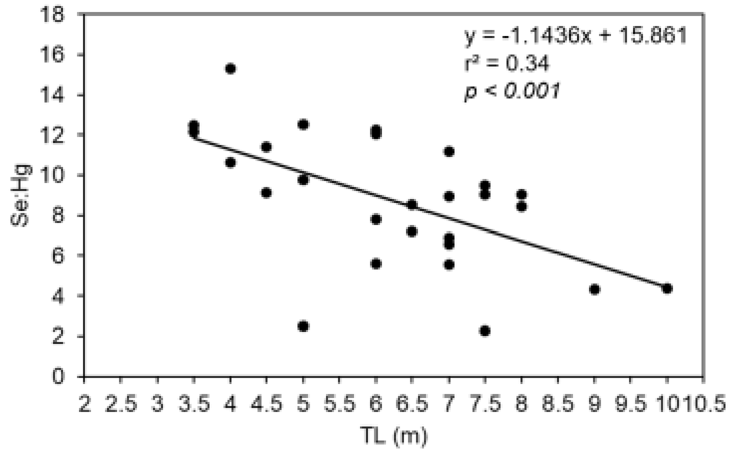

Fig.2.16. Correlation between Se:Hg molar ratio and TL from bopsies of whale shark of BLA in S2.

2.3.5 Season 2 - Concentrations of Hg and Se in zooplankton

A total of 25 samples of zooplankton were collected from September 2017 to February 2018; in LAP, 7 samples of zooplankton were collected, and in BLA 6 samples in September and October 2017. Twelve subsamples (5 in BLA and 7 in LAP) were obtained to measure the Hg and Se concentrations in the main groups of zooplankton.

2.3.5.1 Zooplankton composition

In BLA, copepods represent the main group of zooplankton (97% in September and 85% in October) followed by chaetognatha (2% in September and 8% in October 2017) and in minor percentage decapods and euphausiids (Fig. 2.17).

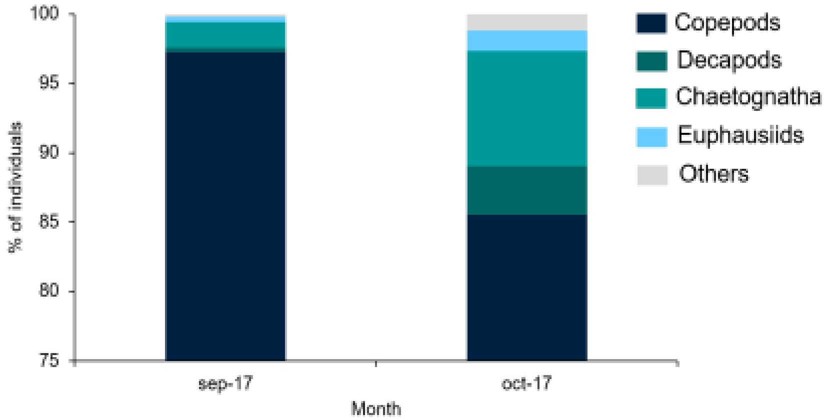

Fig. 2.17. Composition and abundance of zooplankton in BLA in September and October 2017.

In LAP, the copepods percentage ranged from 72% in January to 97% in February 2018. Chaetognatha was the second main group in this study area ranging from 4% in December 2017 to 13% in October 2017. Decapods and euphausiids were found in minor percentages and a peak of euphausiids abundance was registered in January 2018 (Fig. 2.18).

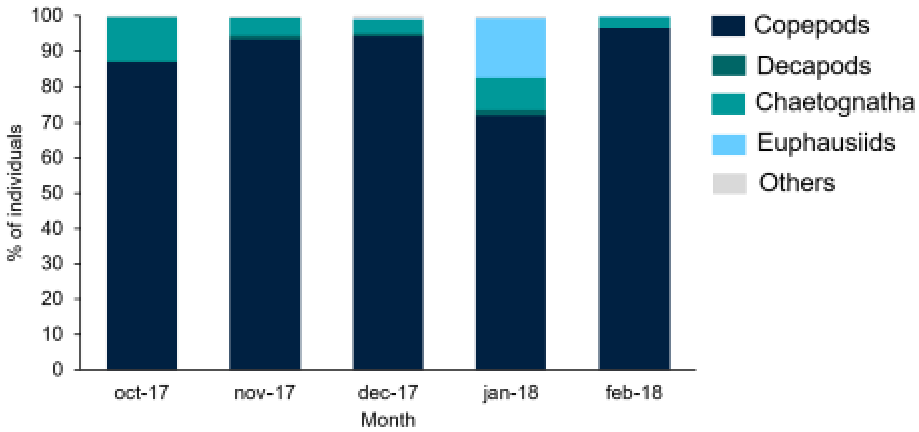

Fig. 2.18. Composition and abundance of zooplankton in LAP from October 2017 to February 2018.

2.3.5.2 Trace elements concentrations

Zooplankton exhibited wide variations fluctuating from 0.7 to 4.7 ng/g (wet weight) for Hg and from 178 to 593 ng/g for Se (Fig. 2.19). Mean ± SD of Hg in BLA was 1.7 ± 1.6 and in 1.2 ± 0.6. Mean ± SD of Se was 296 ± 118 in BLA and 341 ± 121 in LAP (Table 2.5). Despite these, no significant differences were found between sites.

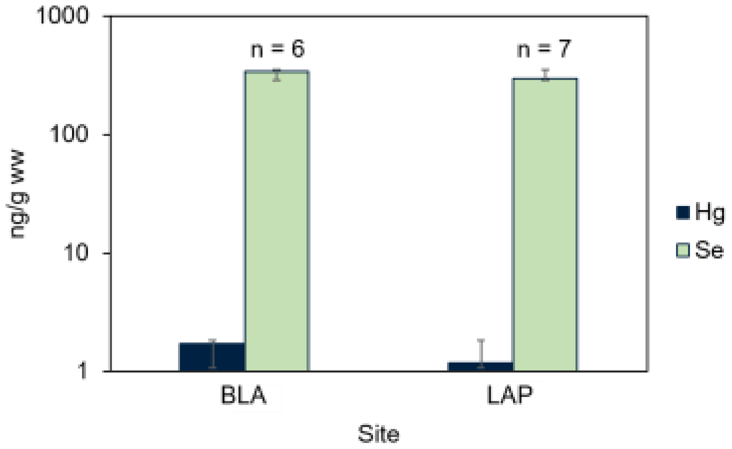

Fig. 2.19. Hg and Se concentration in zooplankton in BLA and LAP in S2.

As in the first season, chaetognatha was the group with the highest concentration of Hg (mean ± SD; 9.0 ± 2.7) (Fig. 2.20), nevertheless, due to the low sampling number (n = 2) no statistical tests were applied. Copepods displayed the lowest concentration of Hg in both sites (5.5 ± 6.5 ng/g in BLA and 1.2 ± 0.7 in LAP).

The highest level of Se was found in chaetognatha from LAP (1990 ± 802 ng/g) and the lowest one in copepods from BLA (531 ± 421 ng/g). Statistical tests were not applied due to low number of samples.

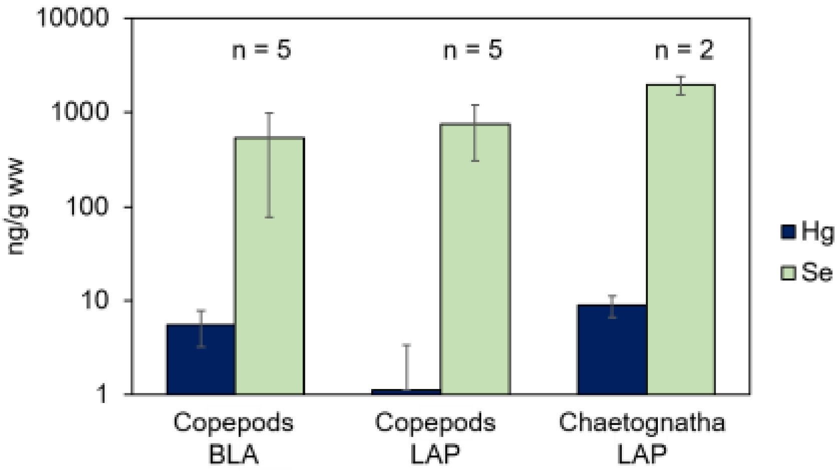

Fig. 2.20. Hg and Se concentrations found in the main groups of zooplankton in BLA and LAP during S2.

Molar ratio Se:Hg varied widely in the zooplankton within each bay (Table 2.5) and ranged from 108 to 1800 in BLA and from 264 to 1561 in LAP. Molar ratio Se:Hg in BLA was 757:1 and in LAP was 849:1.

Table 2.5 Concentrations of Hg and Se (ng/g on wet weight basis) and molar ratio Se:Hg in the zooplankton and main groups of zooplankton collected in 2017 and 2018 in Bahía de Los Angeles (BLA) and Bahía de La Paz (LAP), Gulf of California.

	Site		Month	Year	Hg ng/g	Se ng/g	Hg nmol/g	Se nmol/g	Se:Hg
Zooplankton	BLA	Mixt	Sept	2017	2.5	328	0.013	4.2	329
	BLA	Mixt	Sept	2017	4.7	198	0.023	2.5	108
	BLA	Mixt	Sept	2017	0.9	254	0.005	3.2	706
	BLA	Mixt	Sept	2017	0.7	502	0.004	6.4	1800
	BLA	Mixt	Oct	2017	0.7	178	0.004	2.3	611
	BLA	Mixt	Oct	2017	0.8	317	0.004	4.0	986
		Average±SD			1.7±1.6	296±118	0.009±0.008	3.8±1.5	757±595
	LAP	Mixt	Oct	2017	1.5	302	0.008	3.8	504
	LAP	Mixt	Nov	2017	1.0	593	0.005	7.5	1561
	LAP	Mixt	Nov	2017	0.9	277	0.005	3.5	748
	LAP	Mixt	Dec	2017	0.9	375	0.005	4.8	1025
	LAP	Mixt	Jan	2018	0.9	337	0.004	4.3	988
	LAP	Mixt	Jan	2018	2.3	242	0.012	3.1	264
	LAP	Mixt	Feb	2018	0.8	257	0.004	3.3	855
		Average±SD			1.2±0.6	341±121	0.006±0.003	4.3±1.5	849±414
Main groups	BLA	Copepods	Sept	2017	3.1	244	0.02	3.1	197

BLA	Copepods	Sept	2017	16.3	445	0.08	5.6	69.5
BLA	Copepods	Sept	2017	0.7	256	0.00	3.2	947
BLA	Copepods	Sept	2017	6.7	447	0.03	5.7	170
BLA	Copepods	Oct	2017	0.9	1262	0.00	16.0	3693
	Average±SD			5.5±6.5	531±421	0.03±0.03	6.7±5.3	1015±1537
LAP	Copepods	Oct	2017	0.9	1135	0.005	14.4	3159
LAP	Copepods	Nov	2017	0.7	611	0.003	7.7	2332
LAP	Copepods	Dec	2017	1.3	676	0.01	8.6	1277
LAP	Copepods	Jan	2018	0.8	641	0.004	8.1	2080
LAP	Copepods	Feb	2018	2.4	728	0.01	9.2	784
	Average±SD			1.2±0.7	758±215	0.01±0.003	9.6±2.7	1926±927
LAP	Chaetognats	Oct	2017	11.0	2557	0.05	32.4	593
LAP	Chaetognats	Nov	2017	7.1	1423	0.04	18.0	507
	Average±SD			9.0±2.7	1990±802	0.05±0.01	25.2±10.2	550±60

2.3.5.3 Biomagnification factor

In BLA, Hg BMF through total zooplankton ranged from 12.3 to 287. Average ± SD was 41.0 ± 62.7 and it was statistically >1.0 ($p < 0.001$). Due to the low number of animals with total length < 4 m we could not applied a relation between BMF and TL for this size category.

In LAP Hg BMF through total zooplankton was 20 and it was statistically >1.0 ($p < 0.001$). In LAP, sharks below 4 m of total length (n=6) showed Hg BMF significantly >1 ($p < 0.001$) (Fig. 2.21). Hg BMF in these sharks ranged from 13.0 to 40.4 (average ± SD, 21.3 ± 10.3). Sharks of this category showed a positive but not significant (p Spearman > 0.05) relationship between Hg BMF and TL. Sharks above 4 m of TL (n=27) did not show a significant correlation between Hg BMF and TL (Spearman p value > 0.05); in this group, all sharks showed Hg BMF significantly >1.0 ($p < 0.001$). Hg BMF in this category ranged from 10.2 to 48.5 (19.6 ± 9.2).

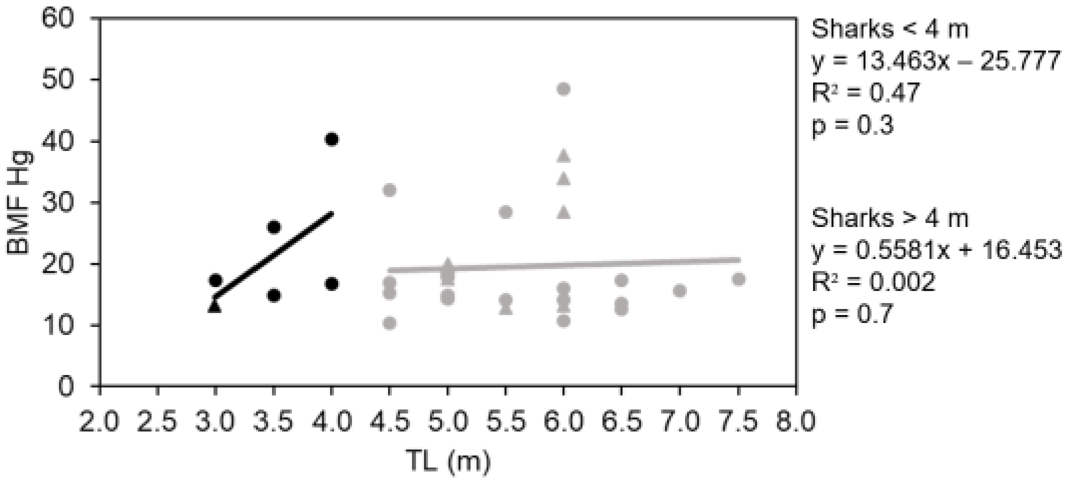

Fig. 2.21. Biomagnification factor calculated in whale sharks < and > of 4 m in LAP during S2.

In BLA average BMF of Se from zooplankton was 0.9 ranging from 0.008 to 6.1. In LAP average BMF of Se from zooplankton was 0.9 ranging from 0.2 to 3.4.

Considering that the predominant diet of the whale shark is composed of copepods (Clark and Nelson, 1997; Hacohen-Domené et al., 2006), then Hg BMF ranged in BLA from 4.0 to 90.0 (12.8 ± 19.6) (Fig. 2.22). In LAP, copepods Hg BMF ranged from 10.0 to 47.4 (19.4 ± 9.0) while chaetognaths, the second most common zooplankton group, exhibited a Hg BFM ranging from 1.3 to 6.3 (2.6 ± 1.2). All Hg BMF calculated for copepods and chaetognatha showed values significantly>1.0 ($p < 0.05$).

On the other hand, in BLA, the Se BMF of copepods ranged from 0.05 to 3.4 (0.5 ± 0.7). In LAP copepods Se BMF ranged from 0.1 to 1.5 (0.4 ± 0.2) and in chaetognatha Se BMF ranged from 0.04 to 0.6 (0.1 ± 0.09).

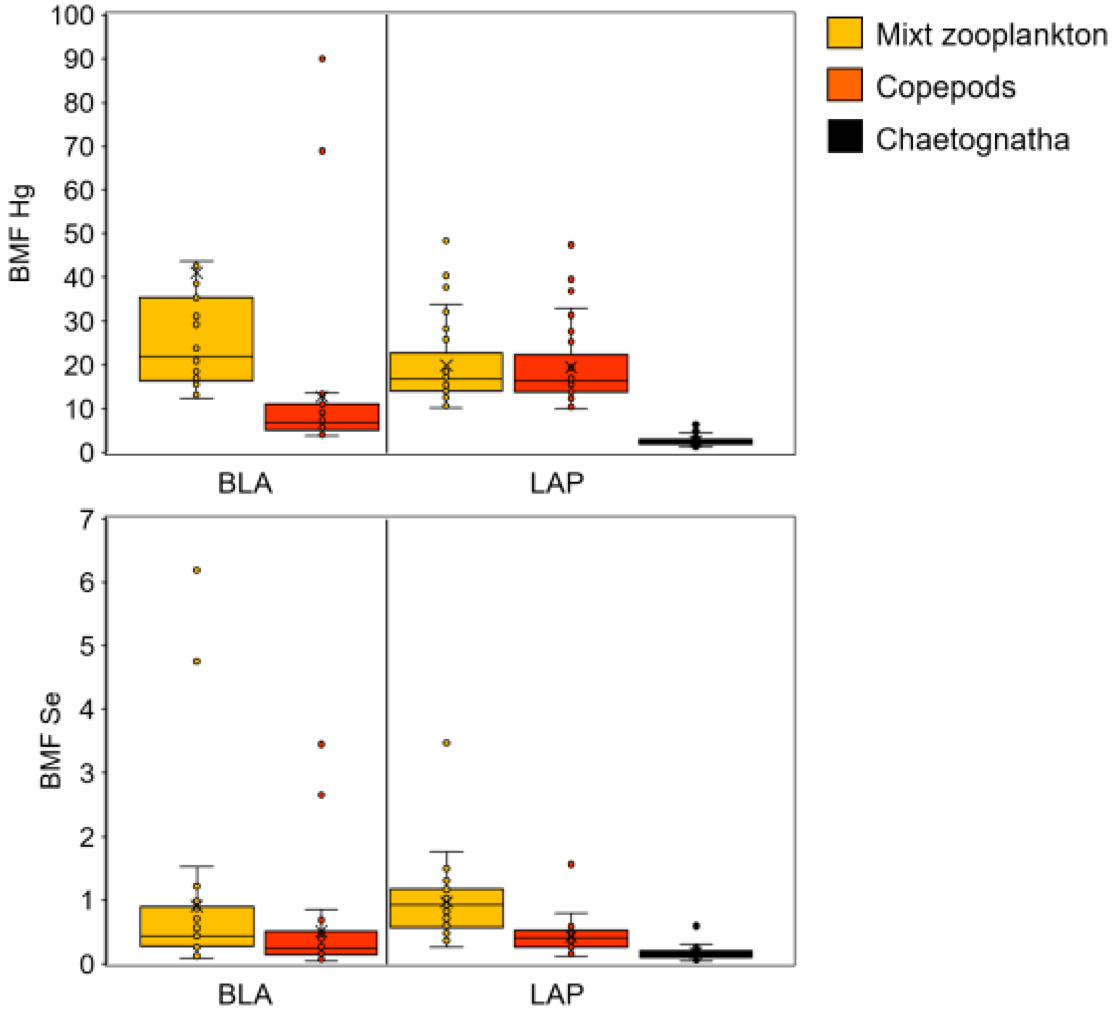

Fig. 2.22. BMF of Hg and Se calaculated using biopsies of whale sharks assuming that the zooplankton, copepods and chaetognatha constitute their main feeding.

2.3.6 Comparison of TEs concentration between seasons

- *In whale shark biopsies*

Hg and Se concentration increased in both areas from S1 to S2 (Fig. 2.23); Mann-Whitney test showed significant differences ($p < 0.001$) in the mean concentration of Hg between seasons in both areas. Se concentration showed p value < 0.05 only in BLA from S1 to S2. In LAP, Se showed no significant differences in concentration from one season to the other one.

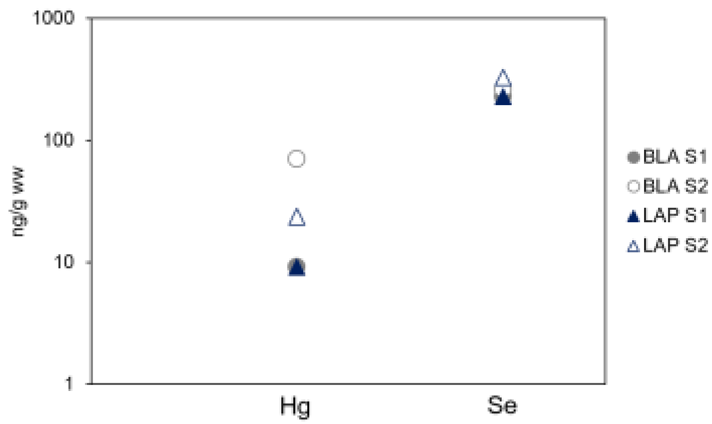

Fig. 2.23. Mean Hg and Se concentrations in biopsies of whale sharks collected in BLA and LAP in S1 and S2.

- *In the zooplankton*

Hg and Se concentrations in the zooplankton decrease from S1 to S2 in LAP with no significant difference found from one season to the other one (Fig. 2.24). On the other hand, concentrations of Hg and Se rose in copepods and chaetognatha from one season to the other one but statistical tests were not applied as the number of samples was too low.

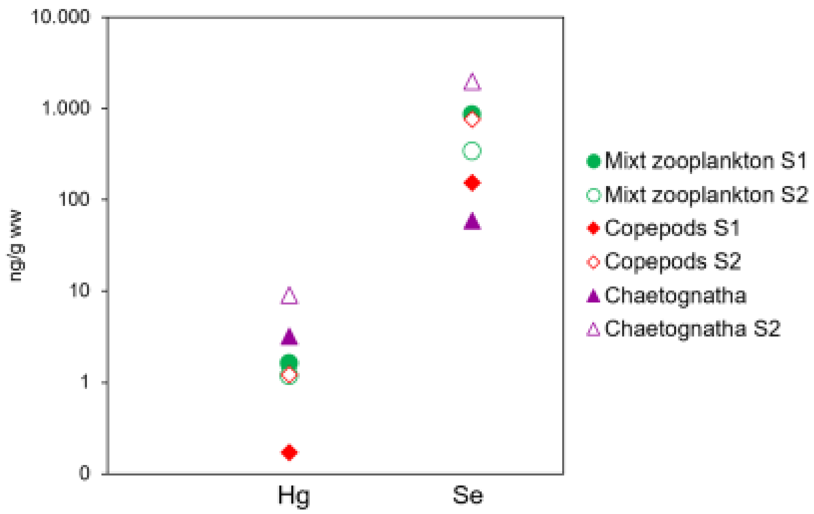

Fig. 2.24. Mean Hg and Se concentrations in zooplankton, copepods and chaetognatha collected in LAP in S1 and S2.

➢ *Variability in Hg and Se in the same individuals*

According with our results, in BLA, 6 sharks were spotted in both seasons, corresponding to a 25% of fidelity for this study area. In LAP, 9 sharks were spotted in both seasons corresponding to a 27% of fidelity for this site.

In BLA, Hg concentrations rose in all animals (Fig. 2.25) with a general mean ± SD of 107 ± 20.6 ng/g ww; WS4 showed the least increased (84 ng/g ww) while WS5 presented the highest one (143.2 ng/g ww). Se concentration also increased (mean ± SD; 230 ± 231) in all animal except in WS1, which presented a slight decrease of - 2 ng/g w.w (Fig. 2.25).

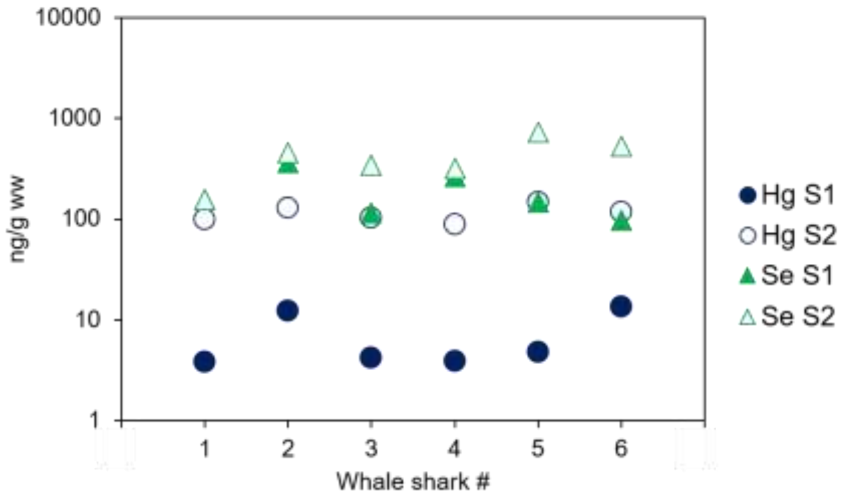

Fig. 2.25. Variability in Hg and Se concentrations in the six whale sharks spotted in S1 and S2 from BLA.

In LAP, Hg concentrations rose in all animals (Fig. 2.26) with a general mean ± SD of 83.2 ± 57.2 ng/g ww; WS8 showed the least increased (28.8 ng/g ww) while WS4 presented the highest one (188 ng/g). On the other hand, Se concentration decreased (mean ± SD; -1848.7 ± 2413) in all animal except in WS9, which presented an increase of 3342 ng/g (Fig. 2.26).

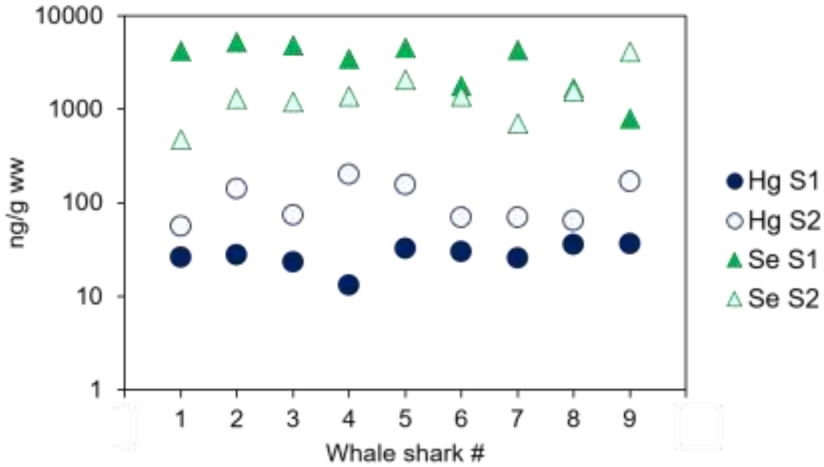

Fig. 2.26. Variability in Hg and Se concentrations in the nine whale sharks spotted in S1 and S2 from LAP.

2.4. DISCUSSION

2.4.1 Hg and Se concentrations in biopsies

Mercury concentrations in whale shark biopsies collected in the peninsular margin of the Gulf of California were higher (S1: 9 ± 7 in BLA and 4 ± 2 ng/g ww in LAP; S2: 71 ± 109 in BLA and 24 ± 11 ng/g ww in LAP) than those reported by Wang *et al.,* (2014), who found a mean of 0.22 and 1.2 ng/g (ww) in epidermis of two whale sharks caught from the coast of China. Hg concentrations from this work were lower compared to the one found in whale shark biopsies from Dijibouti (50 ± 60 ng/g ww; Boldrocchi *et al.,* 2020). Highly urbanized area such as Dijibouti could affect ocean contamination by increasing levels of heavy metals such as Hg. Trophic position is a key factor in Hg accumulation and, as a filter feeder, the whale shark exhibit lower Hg concentrations respect to carnivorous sharks. Diverse studies have found higher Hg levels in carnivorous sharks, including species from the Gulf of California region (Escobar-Sánchez *et al.,* 2010;

Hurtado-Banda *et al.,* 2012; Maz-Courrau *et al.,* 2012; Bergés-Tiznado *et al.,* 2015).

Food is the principal source of Hg in predators so feeding strategy differences can significantly affect the bioaccumulation of this element. In addition, it is widely recognized that Hg accumulates in specific tissues of marine biota. Numerous studies have reported that muscle and liver are biological targets for the accumulation of Hg in several vertebrate species (*e.g.*, Ruelas-Inzunza *et al.,* 2009; Taylor *et al.,* 2014). The muscle is a biological target for the accumulation of Hg, due to its high affinity towards the thiol groups of the proteins, and the liver, due to its importance in the capture, biotransformation and elimination of Hg (Taylor *et al.,* 2014).

During season S1 (2016-2017), Se mean concentrations in whale shark's biopsies were 230 ± 150 ng/g ww in BLA and 280 ± 200 ng/g ww in LAP, while in season S2 (2017-2018) Se mean concentrations were 234 ± 410 ng/g in BLA and 329 ± 194 ng/g in LAP. These findings suggest that there was enough Se in the epidermis to counter the actions of Hg. Se is an essential element with the capacity to bioaccumulate and biomagnify in marine trophic chains (Penglase *et al.,* 2014). These findings are in accordance with what found in Dijibouti, where Se mean concentration (± sd) in whale shark skin biopsies were 350±90 ng/g ww (Boldrocchi *et al.,* 2020). Se exposure for aquatic biota can be through water or/and diet. However, the latter is considered the main route of recruitment (Hamilton, 2004). The biological reason which could explain the moderated Hg concentrations and the high variability for Se in the biopsies is not clear due to *R. typus* physiology is still largely unknown. Still, one possibility may be related to temporary up-regulation of selenoproteins to meet physiological needs (Ralston & Raymond, 2010). Se is a relevant structural constituent in selenoproteins and, once the activity of these proteins is optimized, organisms regulate Se levels excreting the excess (Burk & Hill, 2015).

2.4.2 Comparison between sites

Concentrations of both Hg and Se in our study were significantly different between the two areas ($p < 0.05$). Higher levels of Hg in the northern-center (BLA) Gulf of California could possibly be associated to discharges from mining activities in Santa Rosalia, Baja California. In fact, Kot *et al.,* (2009) reported a halo dissipation enriched of various trace metals in the Santa Rosalia coastal region, which was linked to the copper mining of the District El Boleo; mining and smelting operations were strong during the period 1885-1985 and marine sediments were enriched by stream discharges.

Additionally, the source of Hg and other metals in the Gulf of California could be associated to hydrothermal vents active in the Guaymas basin, anthropogenic loads from metal impurities in raw materials, anthropogenic releases from production processes (*e.g.*, cement and metallic manufacture), and past anthropogenic emissions mobilized from soils, sediments and water (Páez-Osuna *et al.,* 2017).

Sharks tend to accumulate toxic substances, such as Hg, because of their trophic position and because they are long-lived animals (Storelli *et al.,* 2011). This trend is verified by the positive associations between length and Hg concentrations that have been observed for numerous species of sharks (*e.g.*, Storelli *et al.,* 2011; Hurtado-Banda *et al.,* 2012; Maz-Courrau *et al.,* 2012). Our findings only exhibit a correlation with the size for the case of those sampled in BLA which showed a significant ($p < 0.05$) negative correlation between Hg in males and total length.

2.4.3 Comparison between seasons

Hg concentration in the biopsies from both study areas presented an increase in concentration that was significatively different ($p < 0.001$) between the seasons. Se concentration in the biopsies, instead, showed significative differences ($p < 0.05$) only in BLA between both seasons. Even it is complicated to establish the reasons of this increase in Hg from S1 to S2, environmental factors between seasons might have affected the bioavailability of the elements.

2.4.4 Influence of sex and TL

Sex was not a significant factor in the accumulation of Hg and Se in the biopsies of *R. typus*; nevertheless, the number of samples for females in both study areas was too small to apply any statistical test.

Despite we found a negative and significant ($p < 0.05$) correlation between Hg and Se with TL in S2 in BLA, in general, we found a non-significant relationship of Hg and Se with sharks' length, which could indicate that these elements may not bioaccumulate in dermis, evidencing homeostatic regulation of Se.

The null or negative correlation of Hg with the size in the whale shark can be due partial or totally, to the feeding habit of this fish, which consumes zooplankton unlike most sharks that are carnivorous top of the food web. In addition, the high mobility of individuals from the coastal area to the oceanic area and vice versa may be another factor that affects the Hg bioaccumulation rates. It's been observed that juvenile and adult whale sharks exhibited differences in their movement into the Gulf of California (Ramírez-Macías *et al.,* 2017), where adults are usually found in oceanic waters and juveniles show high degree of fidelity in feeding grounds located in coastal areas (Ramírez-Macías *et al.,* 2012). The latter areas are typically influenced by pollution compared to oceanic waters. Nevertheless, whale sharks are highly migratory sharks, and then Hg and Se uptake could occur in organisms inhabiting the two areas as well.

2.4.5 Molar ratio

Molar ratio Se:Hg showed a significant correlation with the TL only in the sharks from BLA in S2 ($p = 0.0007$). In addition, Hg nmol/g presented a positive correlation (Spearman $p < 0.05$) with Se nmol/g in the sharks from LAP in S2. Correlations between molar Se and Hg are more commonly found in bony fish (Kaneko & Ralston, 2007) and the liver and kidney of marine mammals (Burger *et al.,* 2013); particularly, correlations have been shown to be stronger in predators, such as predatory fish (Yamashita *et al.,* 2011).

Selenium concentrations were in excess in the epidermis of *R. typus* as, also confirmed by the Se:Hg molar ratios found in this tissue, which were significantly >1.0, indicating that Se is incorporated in selenoproteins (Yang *et al.,* 2008). Due to the high affinity between Hg and Se, the formation of an inorganic HgSe complex is proposed as the mechanism responsible for the protective effect of Se (Ralston *et al.,* 2008). In S2, a strong and negative correlation ($p < 0.001$) was found between TL and Se:Hg molar ratio in sharks from BLA. As Hg concentrations increased, Se:Hg molar ratios tended to decrease in both areas, which also has been found in the shark *Sphyrna lewini* (Bergés-Tiznado *et al.,* 2015).

The Se:Hg ratios were significantly above 1.0 for the biopsies from both areas, reaching 100 in BLA and 219 in LAP in S1 (Table 2.2). These results are in accordance with what found in whale shark biospies from Dijibouti where the Se:Hg molar ratio ranged from 3.8 to 54.9 and exceeded 1 in all samples (Boldrocchi *et al.,* 2020). Molar excess of Se relative to Hg, as well as high variation in Se:Hg ratios is commonly found in Gulf of California marine species (Bergés-Tiznado *et al.,* 2015; 2019) and in fish species elsewhere (Burger *et al.,* 2001). These results suggest that *R. typus* have enough Se to prevent Hg toxicity and still maintain selenium-dependent metabolic functions (Ralston & Raymond, 2010).

Whale sharks are opportunistic filter feeders (Taylor, 1997) and according to previous studies they have been observed feeding in Bahía de La Paz on diffuse patches of euphausiids (Ketchum *et al.,* 2013) and copepods (Clark & Nelson, 1997; Hacohen-Domené *et al.,* 2006). Our findings are in accordance with the previous ones, although we also report chaetognatha as one of the main groups of zooplankton during feeding activity.

2.4.6 Elements in zooplankton

Hg and Se varied greatly in the zooplankton during the collection period with no significative differences detected between seasons. Hg concentrations from our study were similar to those found by Syderman & Jarman (1998) in the central coast of California (USA); and Se concentrations were similar to those found by Liu *et al.,* (1987) in the coast of China. The essentiality of Se for marine zooplankton has not been demonstrated. Still, the high assimilation efficiency of Se in marine zooplankton found by Fisher & Reinfelder (1991) indicates that this element enters the organic cycle via the marine zooplankton. Correlation between Hg and Se:Hg was significant ($p < 0.05$) in the zooplankton with a Hg tendency to decrease with the increasing of Se:Hg molar ratio. However, further studies are required to confirm these results, because our sample size was limited. Assuming that the collected zooplankton is representative of the diet of *R. typus*, it can be proposed that Hg biomagnification is exhibited by this fish.

2.4.7 Biomagnification factor

Despite the BMF did not show any significant correlation with the total length of the animals, sharks < 4 m showed a positive tendency with total length and sharks > 4 m exhibited a negative tendency with the total length, which could indicate a shift in the shark diet or metabolic changes once the animal reaches such size. Biomagnification factor found in Dijibouti was < 1:1 for Hg and Se (Boldrocchi *et al.,* 2020).

2.5. CONCLUSIONS

Our results show that Hg and Se are both present in whale sharks' biopsies from the two bays studied in the Gulf of California with significative differences found between sites and seasons. Sex is not a significant factor that influence the element concentrations in biopsies; nevertheless, the correlation of the elements with the total length of the sharks seems to be different depending on the sex; in males, trace elements seems to decrease with the size of the shark, instead in female, trace elements concentrations seems to increase with size. Despite so, the number of females sampled in both study areas and in both seasons were too low to establish a strong correlation. Molar ratio Se:Hg shows enough concentration of Se in order to detoxicate Hg in all animals.

Hg and Se were also detected in the zooplankton collected from both study areas; in particular, in the second season (2017-2018), zooplankton was more enriched in elements and this was reflected in the shark biopsies. Mercury is, apparently, captured by the shark mainly from chaetognatha and in minor quantity from copepods, which seems to be more decisive in the concentration of Se. Finally, our results show that Hg is biomagnified by the sharks while Se is not, probably because of its metabolic role in the organism.

CHAPTER 3

As, Cd, Cu, Pb, Zn BIOACCUMULATION AND BIOMAGNIFICATION IN EPIDERMIS OF WHALE SHARK DURING TWO SEASONS IN TWO AREAS OF THE GULF OF CALIFORNIA, MEXICO

3.1. ABSTRACT

Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb) and Zinc (Zn) were measured in the whale shark (*Rhincodon typus*) from two areas of the Gulf of California, Bahía de Los Angeles (BLA) and Bahía de La Paz (LAP) using dermal biopsies of 130 specimens and during two seasons (S1 and S2): S1= 2016-2017 and S2= 2017-2018. Additionally, in S1 nineteen zooplankton samples from LAP were analysed, while in S2, 11 zooplankton samples were analysed in BLA and 14 zooplankton samples were analysed in LAP. In both seasons and both study areas, Zn was the most abundant element. On the other hand, Cd and Pb showed the lowest concentrations in both areas and both seasons. Significative differences in TEs concentrations were found between sites and seasons. Arsenic concentrations found in the sharks from La Paz suggested that the area is enriched in this element. Essential elements and As were the elements that presented the most variability within animals sampled in both seasons while not essential element remained more constant from one season to the other one. In general, sex was not a significant and influential factor in trace element concentration; nevertheless, correlation between TEs and length of the animals suggested different behaviour depending on the sex (negative correlations in males, positive correlations in females) confirming that whale shark' feeding strategies implies segregation between sex and size. In S2 zooplankton showed enrichment in trace elements and this was reflected in the sharks as well. Essential TEs were not biomagnified by the sharks probably due to the metabolic use; on the contrary, Pb was apparently biomagnified in both seasons through zooplankton. Molar ratios suggested that in the epidermis Zn could improve Cd detoxification better than Se.

3.2. INTRODUCTION

In the environment, some elements are essential (Cu and Zn) and are necessary to guarantee a correct function of the metabolic processes; nevertheless, they can become toxic in high concentrations (Soto-Jiménez, 2011). Other elements, called not essential, such as As, Cd and Pb are toxic even at low concentrations.

As we discussed, the processes at which trace elements are accumulate in the organisms are different for each species and even can be different in different individuals of the same species. These processes depend on biotic and abiotic factors, metabolism and on detoxification mechanisms. Excretion of heavy metals can be slow; some of them, like Cd, have a life of over 10 years in terrestrial mammals and excretion depend to the bounding with MTs (Endo *et al.,* 2008).

Heavy metals like Cd and Pb are poorly soluble in water and they are adsorbed in the suspended particulate matter (Newman & Unger, 2002). Filter feeder animals such as the whale shark, *Rhincodon typus*, can be susceptible to those elements as they continuously filter the surface of the ocean where those toxic elements are present. Therefore, the study of metals concentration in species like *R. typus* is important in order to establish the health status of the species in a certain area.

The main objective of this chapter is to establish the concentrations of essential (Cu and Zn) and not-essential (As, Cd and Pb) elements in whale shark biopsies from two study areas during two seasons (S1 and S2). We assess key potential biological drivers of TEs concentrations including period (two consecutive years), site (location of shark), sex and total length. TEs concentration in zooplankton were determined in both study areas where sharks typically feed. We estimated biomagnification. Finally, we explored possible interactions between elements in the epidermis.

3.3. RESULTS

3.3.1 SEASON 1. TEs concentrations in biopsies

3.3.1.1 Differences between sites

In biopsies of sharks sampled from BLA (n = 38), the Zn content (in ww) ranged from 35 to 2107 ng/g with a mean ± SD of 298 ± 406 ng/g, As ranged from 18 to 1013 ng/g with a mean ± SD of 131 ± 161 ng/g, Cu ranged from 5 to 57 with a mean ± SD of 53 ± 99, Pb ranged from 1 to 36 with a mean ± SD 7 ± 7 and Cd ranged from 1 to 15 ng/g with a mean ± SD of 3 ± 3.

In biopsies of sharks sampled from LAP (n = 32), the Zn content (in ww) ranged from 35 to 1987 ng/g with a mean ± SD of 595 ± 554 ng/g, As ranged from 35 to 611 ng/g with a mean ± SD of 172 ± 150 ng/g, Cu ranged from 4 to 1070 with a mean ± SD of 244 ± 338, Pb ranged from 1 to 162 with a mean ± SD 15 ± 32 and Cd ranged from 0.5 to 12.0 ng/g with a mean ± SD of 4 ± 3.

Mann-Whitney test showed significant differences ($p < 0.001$) of Zn, As and Cu between the two areas (Fig. 3.1).

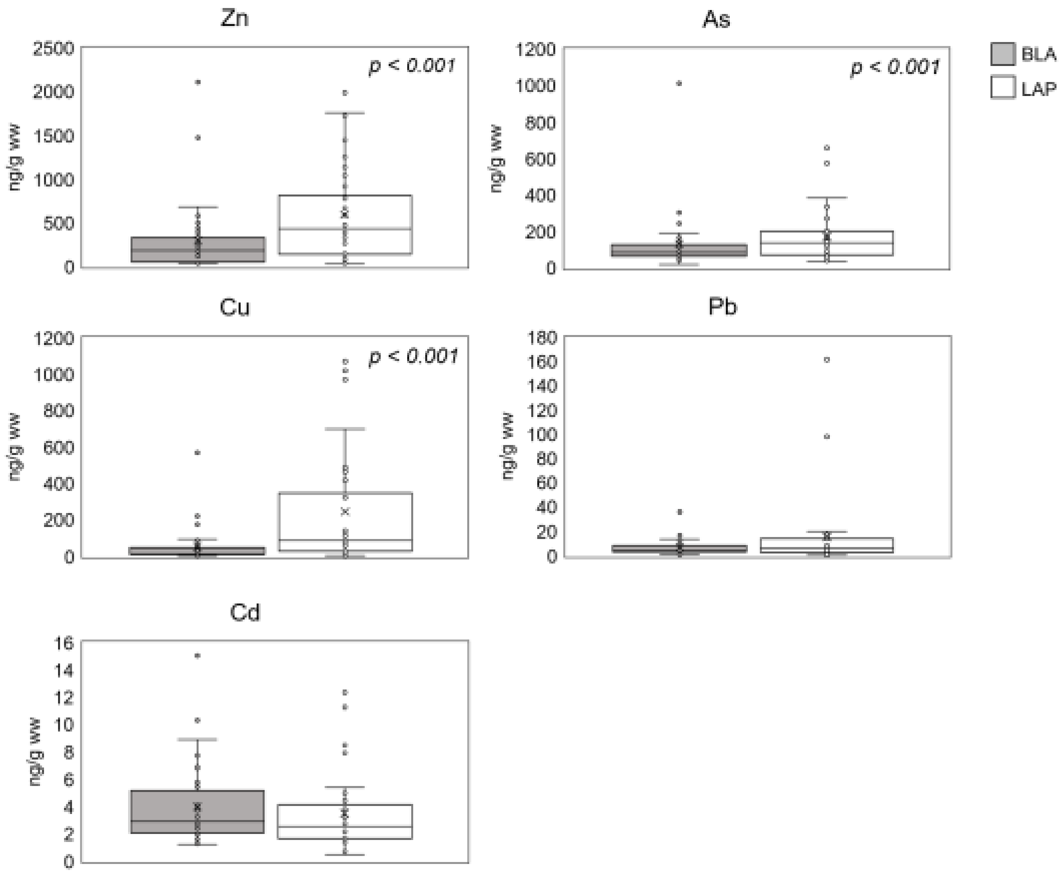

Fig. 3.1. Concentrations of Zn, As, Cu, Pb and Cd (ng/g ww) in biopsies of whale sharks collected in S1 in BLA and LAP.

Table 3.1. Concentrations (mean ± SD in ng/g ww) of Zn, As, Cu, Pb and Cd in males and females of whale shark samples in BLA and LAP in S1.

	N	Zn	As	Cu	Pb	Cd
BLA						
Male	34	318 ± 427	135 ± 170	58 ± 104	7.5 ± 6.8	4.2 ± 3.0
		(35-2107)	(18-1013)	(5-570)	(1.2-5.8)	(1.2-15.0)
Female	4	122 ± 97	96 ± 21	11 ± 9	3 ± 1	2 ± 1
		(43-258)	(75-126)	(5-25)	(2-5)	(2-3)
Total	38	298 ± 406^{a}	131 ± 161^{a}	53 ± 99^{a}	7 ± 7	3 ± 3
		(35-2107)	(18-1013)	(5-570)	(1-36)	(1-15)
LAP						
Male	23	725 ± 596	195 ± 169^{1}	255 ± 325	13 ± 19^{1}	4 ± 3
		(35-1987)	(35-661)	(4-1020)	(2-98)	(1 -12)
Female	9	265 ± 204	115 ± 62^{2}	215 ± 391	22 ± 53^{1}	2.0 ± 1.0
		(40-607)	(40-202)	(4-1070)	(1-162)	(0.5-3.0)
Total	32	595 ± 554^{b}	172 ± 150^{b}	244 ± 338^{b}	15 ± 32	4.0 ± 3.0
		(35-1987)	(35-611)	(4-1070)	(1-162)	(0.5-12.0)
Total of 2 bays	70	434 ± 499	150 ± 156	140 ± 257	11 ± 22	4 ± 3
		(35-2107)	(18-1013)	(4-1070)	(1-162)	(0.5-15.0)

Different superscript letter indicates significantly different ($p < 0.001$) mean concentrations between sites for the same element; different superscript number indicate significantly different ($p < 0.05$) mean concentrations between sexes for the same element. Statistical were performed by a Mann-Whitney test.

3.3.1.2 Differences between sex

- *Bahía de Los Ángeles*

In BLA, mean concentration of all elements was higher in males (n = 24) compared to females (n = 4). No statistical tests were applied to these data due to the low number of females (Fig. 3.2).

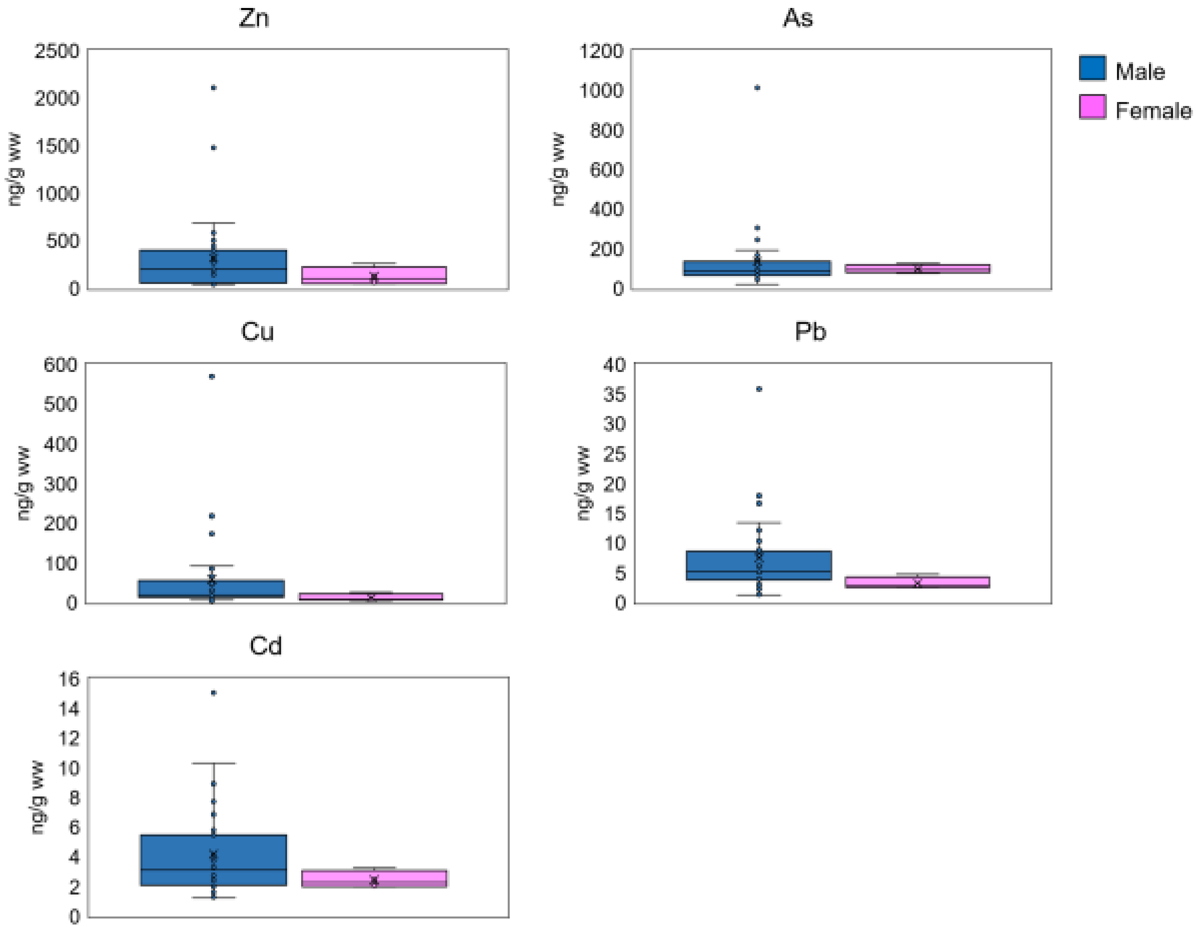

Fig. 3.2. TEs concentrations in males and females from BLA in S1. No statistical tests were applied due to the low number of females.

- *Bahía de La Paz*

In LAP, mean concentration of TEs were higher in males (n = 23) compared to females (n = 9); these differences were significant only in the concentration of Pb ($p < 0.05$; Fig. 3.3).

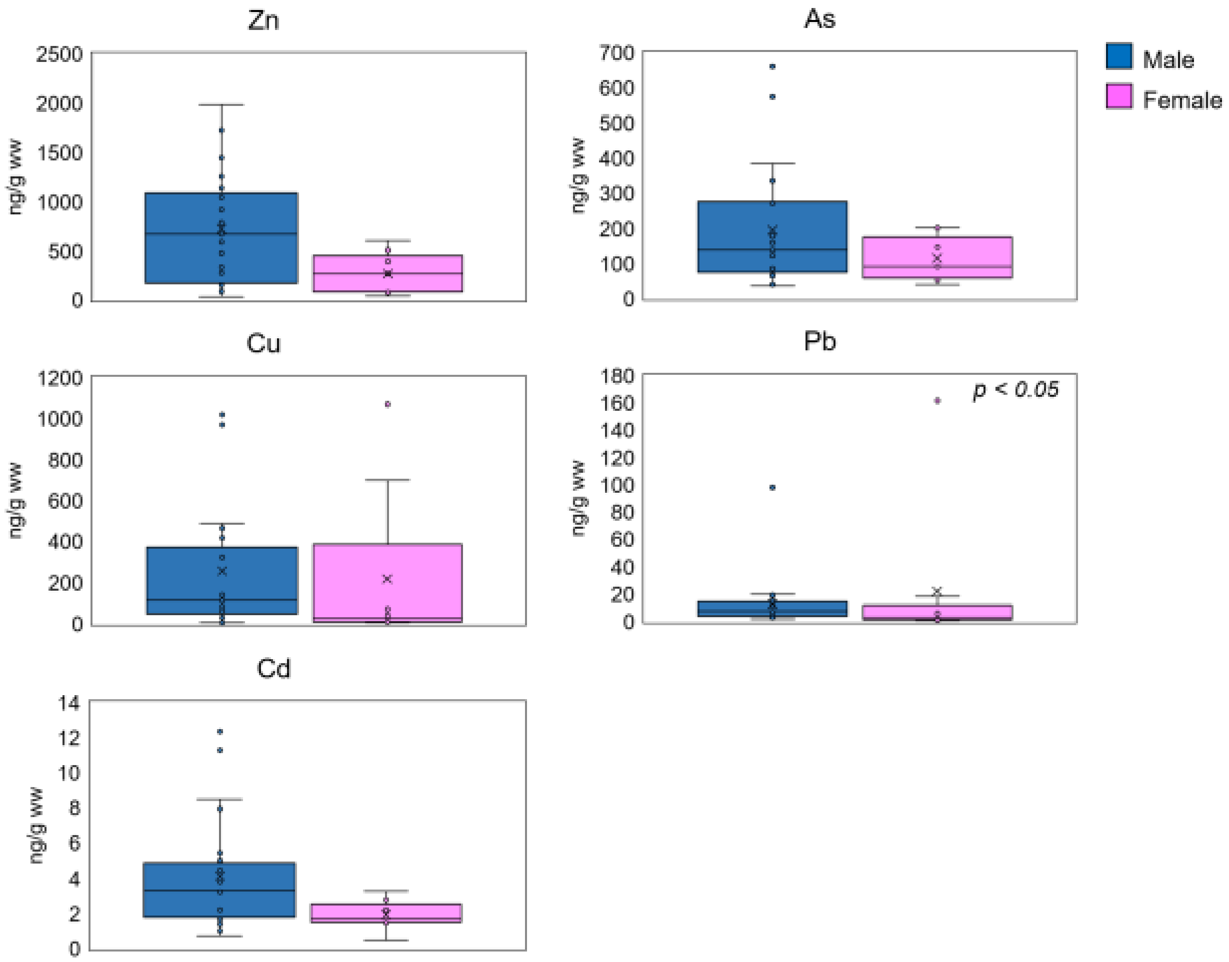

Fig. 3.3. TEs concentrations in males and females from LAP in S1. Mann-Whitney test showed significant p values ($p < 0.05$) in the concentration of Pb.

3.3.1.3 *Relation with total length (TL)*

- *Bahía de Los Ángeles*

In general, concentration of TEs seems to present a negative behaviour with the TL in males; this correlation was significative for Zn, Pb and Cd (Spearman p value < 0.05; linear equation [Zn]=−128.68 TL+ 1073.3; [Pb]=−2.16 TL+ 20.2; [Cd]=−1.09 TL+ 10.6) (Fig. 3.4) The same relationship showed a positive behaviour in females but correlation was not applied due to the low number of females.

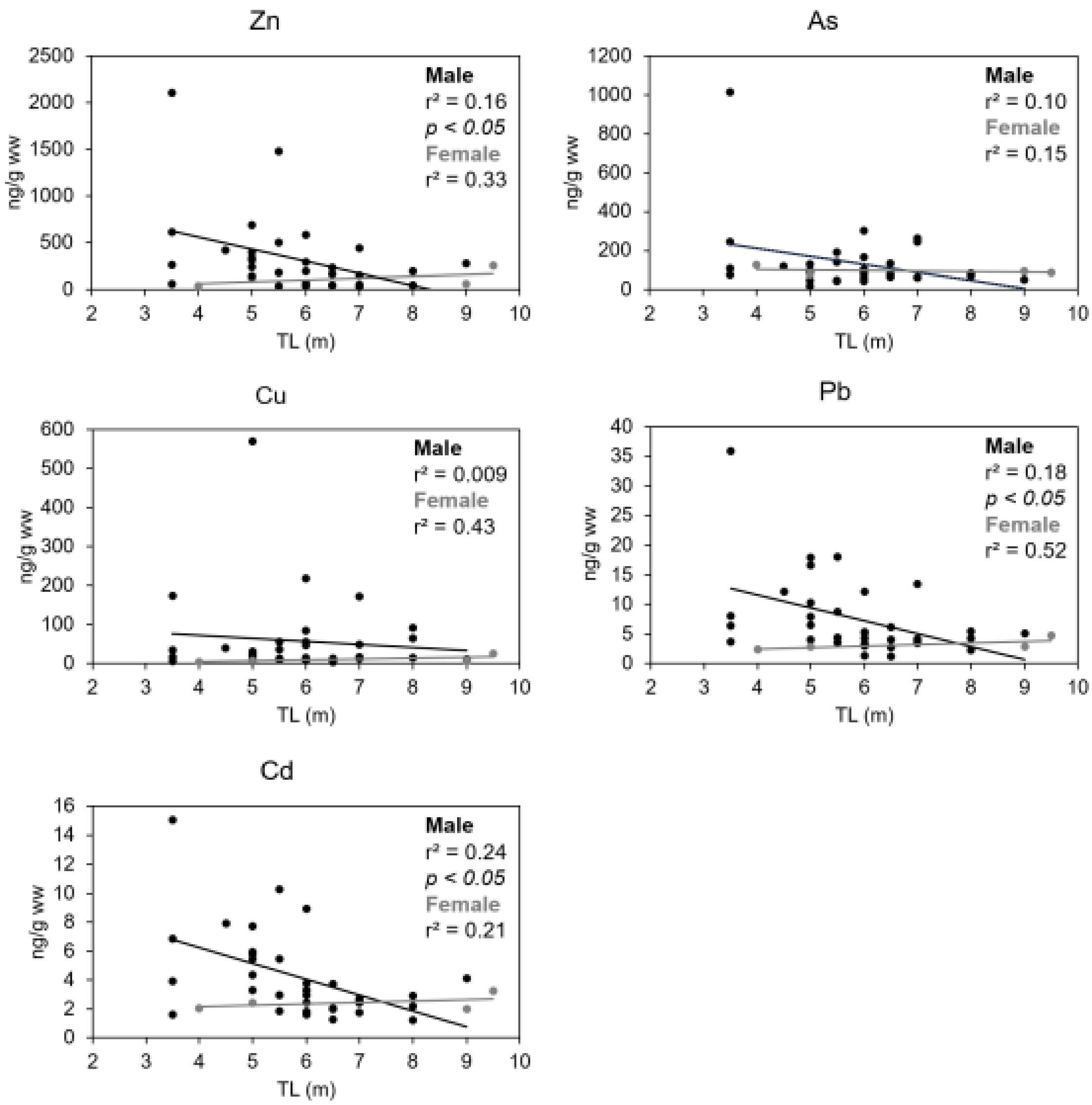

Fig. 3.4. Correlation between TEs and TL in males (black) and females (grey) from BLA in S1.

- *Bahía de La Paz*

In general, concentration of TEs (ng/g ww) seems to present a negative behaviour with the TL in males; an exception was found for Cu where the correlation in male was positive. Correlations were not significant but once we eliminate one outlier from Pb in males, we found p value < 0.05 (linear equation [Pb]=−1.75 TL+ 18) (Fig. 3.5) In females, all correlations were positive with the TL except in for Pb and Cd.

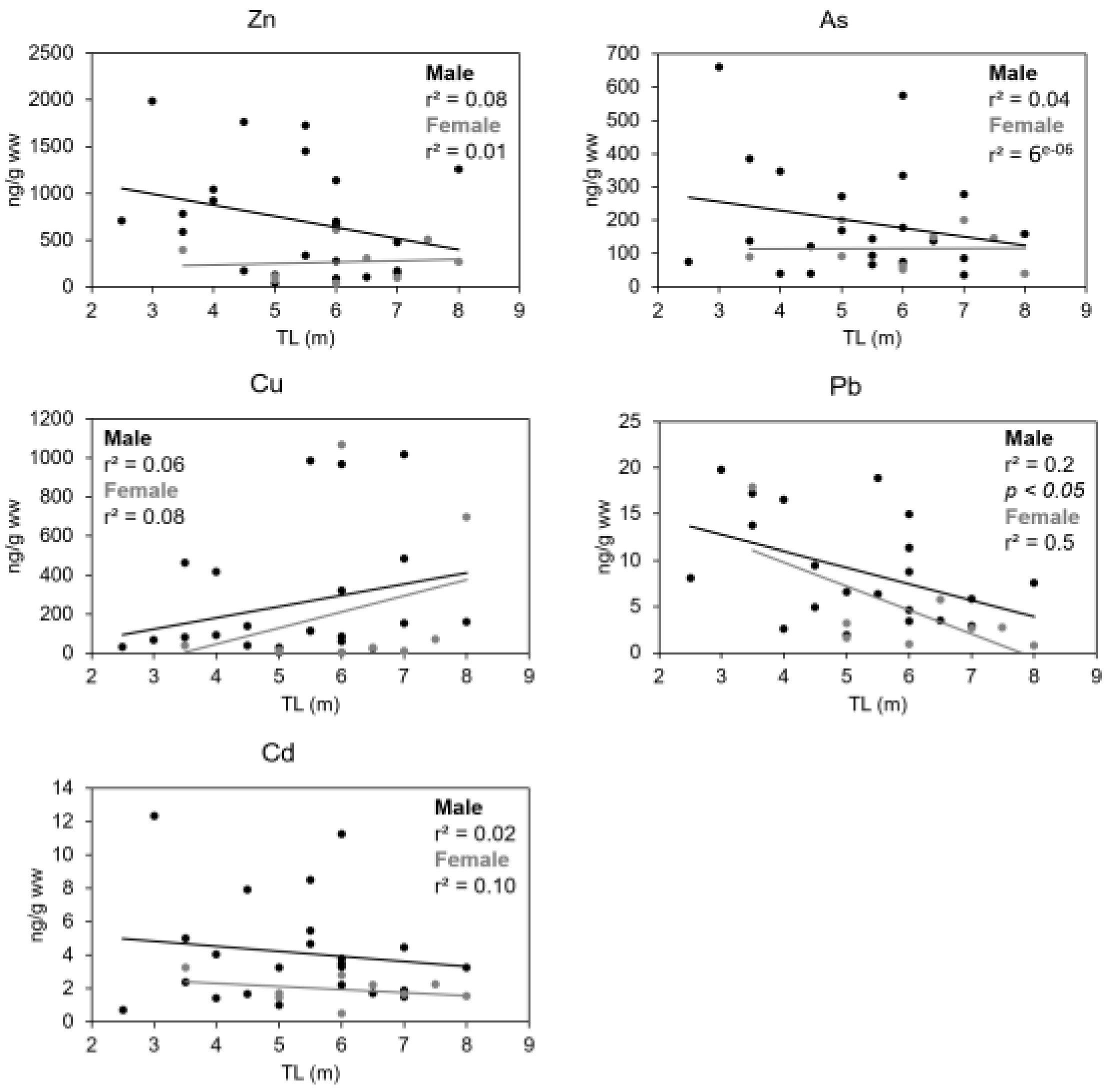

Fig. 3.5. Correlation between TE and TL in males (black) and females (grey) from LAP in S1.

3.3.2 SEASON 1: TEs concentrations in zooplankton

In the zooplankton samples, mean ± SD trace elements concentration was found in the order: Zn (1749±1171 ng/g), Cu (212±189 ng/g), As (163±183 ng/g), Pb (48±101 ng/g) and Cd (47±25 ng/g) (Fig. 3.6; Table 3.2). Chaetognatha (n=3) was the group with the highest concentration of Zn (2523±1646 ng/g) and Cu (173±148 ng/g) while copepods (n=2) was the groups with the highest concentration of Cd (71±62 ng/g), As (54.5±53.0 ng/g), and Pb (12±8 ng/g) (Fig. 3.7). Statistical tests were not applied due to low number of samples.

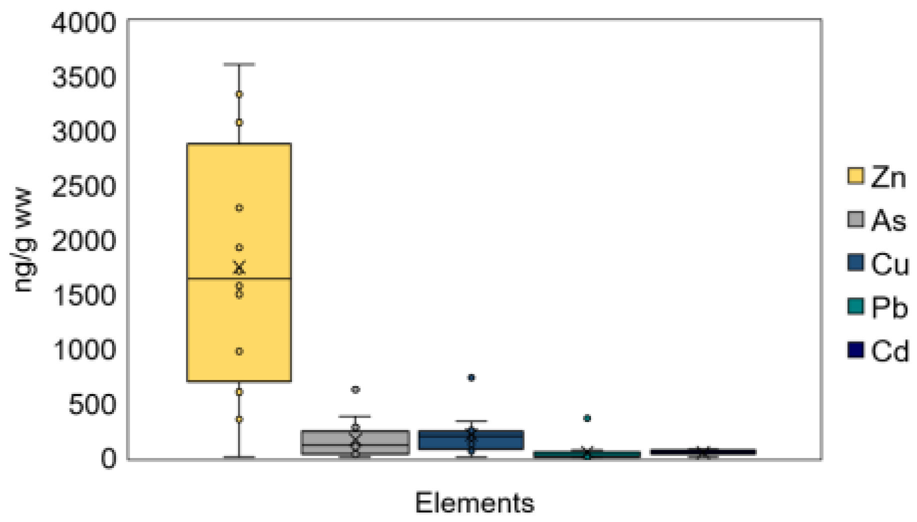

Fig. 3.6. TEs concentrations in the zooplankton collected in LAP in S1.

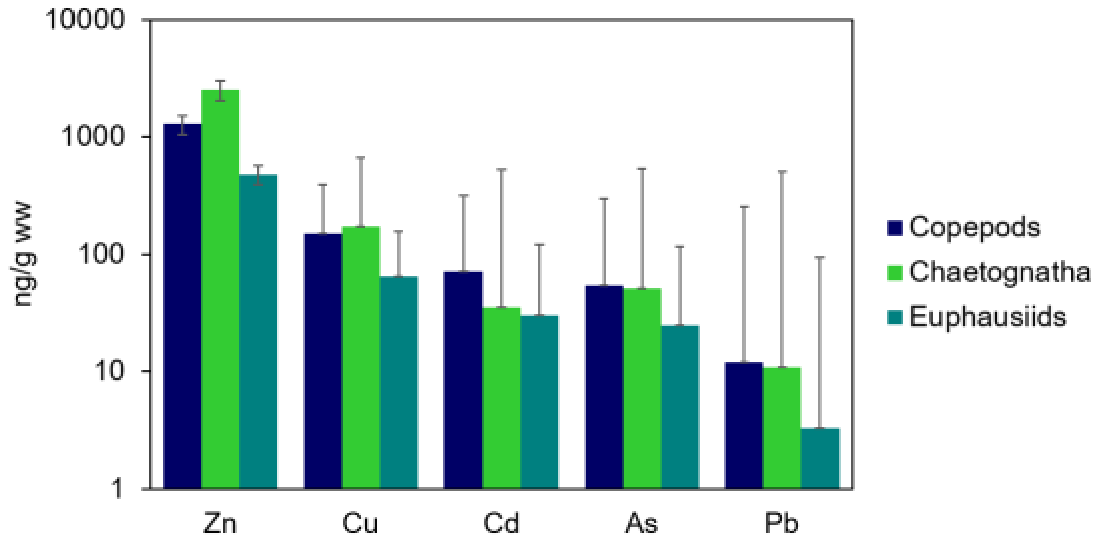

Fig. 3.7. Mean concentration of TEs and SE in the main groups of zooplankton from LAP in S1.

Table. 3.2. Concentration of Zn, Cu, As, Pb and Cd (ng/g ww) in zooplankton and main groups of zooplankton from LAP in S1.

		Month	Year	Zn	Cu	As	Pb	Cd
		Sept	2016	351	57.6	41.8	2.4	28.7
		Oct	2016	7.4	0.9	0.1	0.3	0.1
		Oct	2016	3616	739.1	133.5	363.6	70.0
		Oct	2016	3336	331.6	67.7	48.6	72.4
		Nov	2016	1716	248.3	30.6	43.0	41.5
		Nov	2016	1930	197.6	122.6	66.1	18.2
Zooplankton		Dec	2016	3076	206.8	138.0	13.0	59.4
		Dec	2016	1579	187.8	628.4	30.0	52.2
		Dec	2016	605	63.3	34.1	2.7	34.3
		Jan	2017	973	208.8	276.8	7.5	81.3
		Mar	2017	2294	121.0	101.0	1.4	30.3
		Mar	2017	1502	184.6	383.8	1.5	78.0
Average±SD				1749±1171	212±189	163±183	48±101	47±25
	Copepods	Sept	2016	2069	234.7	92.1	6.3	115.3
	Copepods	Dec	2016	512	68.2	17.0	17.7	27.1
Average±SD				1291±1101	151.4±118	54.5±53	12.0±8	71.2±6
Main groups	Chaetognatha	Oct	2016	4406	343.6	72.5	25.3	65.3
	Chaetognatha	Dec	2016	1361	83.0	17.5	3.5	21.8
	Chaetognatha	Mar	2017	1802	92.1	63.5	3.3	19.0
Average±SD				2523±1646	173±148	51±29	11±13	35±26
	Euphausiids	Jan	2017	479	92.5	41.0	5.7	43.2
	Euphausiids	Mar	2017	483	36.0	8.3	0.9	16.6
Average±SD				481±3	64.2±40	24.6±23	3.3±3	30.0±19

3.3.2.1 Biomagnification factor (BMF)

BMF for the whale shark in LAP (n = 32) was calculated for zooplankton (n = 12), copepods (n = 2), chaetognatha (n = 3) and euphuasiids (n = 2) (Table 3.3). BMF through total zooplankton was 0.34 for Zn, 0.07 for Cd, 0.32 for Pb, 1.06 for As and 1.15 for Cu. Despite As and Cu BMF were > 1, these values did not show p values statistically > 1 (t-test > 0.05). Euphausiids was the zooplankton group with the highest BMF withing all zooplankton categories.

Statistical tests were not applied to the BMF of the main groups of zooplankton due to the low number of samples.

Table 3.3. BMF (mean, min and max) for whale sharks calculated from zooplankton, copepods, chaetognatha and euphausiids and the whale shark biopsies concentrations in organisms collected in S1 in LAP.

		BMF				
	n	Zn	As	Cu	Pb	Cd
Zooplankton	12	0.34	1.06	1.15	0.32	0.07
		(0.02-1.14)	(0.21-4.05)	(0.02-5.04)	(0.02-3.34)	(0.01-0.26)
Copepods	2	0.46	3.16	1.61	1.27	0.05
		(0.03-1.54)	(0.64-12.12)	(0.02-7.07)	(0.06-13.47)	(0.01-0.17)
Chaetognatha	3	0.24	3.37	1.41	1.43	0.10
		(0.01-0.79)	(0.68-12.91)	(0.02-6.12)	(0.07-15.12)	(0.01-0.35)
Euphausiids	2	1.24	7.00	3.80	4.59	0.12
		(0.07-4.13)	(1.42-26.84)	(0.06-16.68)	(0.23-48.68)	(0.02-0.41)

3.3.3. Season 2: TEs concentrations in biopsies

3.3.3.1 Differences between sites

In biopsies of sharks sampled from BLA (n = 27), the Zn content (in ww) ranged from 520 to 12712 ng/g with a mean ± SD of 1959 ± 2545 ng/g, As ranged from 131 to 1405 ng/g with a mean ± SD of 411 ± 272 ng/g, Cu ranged from 38 to 573 with a mean ± SD of 166 ± 129, Pb ranged from 18 to 319 with a mean ± SD 69 ± 76 and Cd ranged from 6 to 71 ng/g with a mean ± SD of 17 ± 14 (Table 3.4).

In biopsies of sharks sampled from LAP (n = 33), the Zn content (in ww) ranged from 664 to 6093 ng/g with a mean ± SD of 2642 ± 1261 ng/g, As ranged from 83 to 1428 ng/g with a mean ± SD of 377 ± 273 ng/g, Cu ranged from 4 to 1032 with a mean ± SD of 244 ± 246, Pb ranged from 2 to 23 with a mean ± SD 7 ± 5 and Cd ranged from 2 to 53 ng/g with a mean ± SD of 13 ± 10 (Table 3.4).

Mann-Whitney test showed significant differences ($p < 0.001$) of Zn, As and Pb between the two areas (Fig. 3.8).

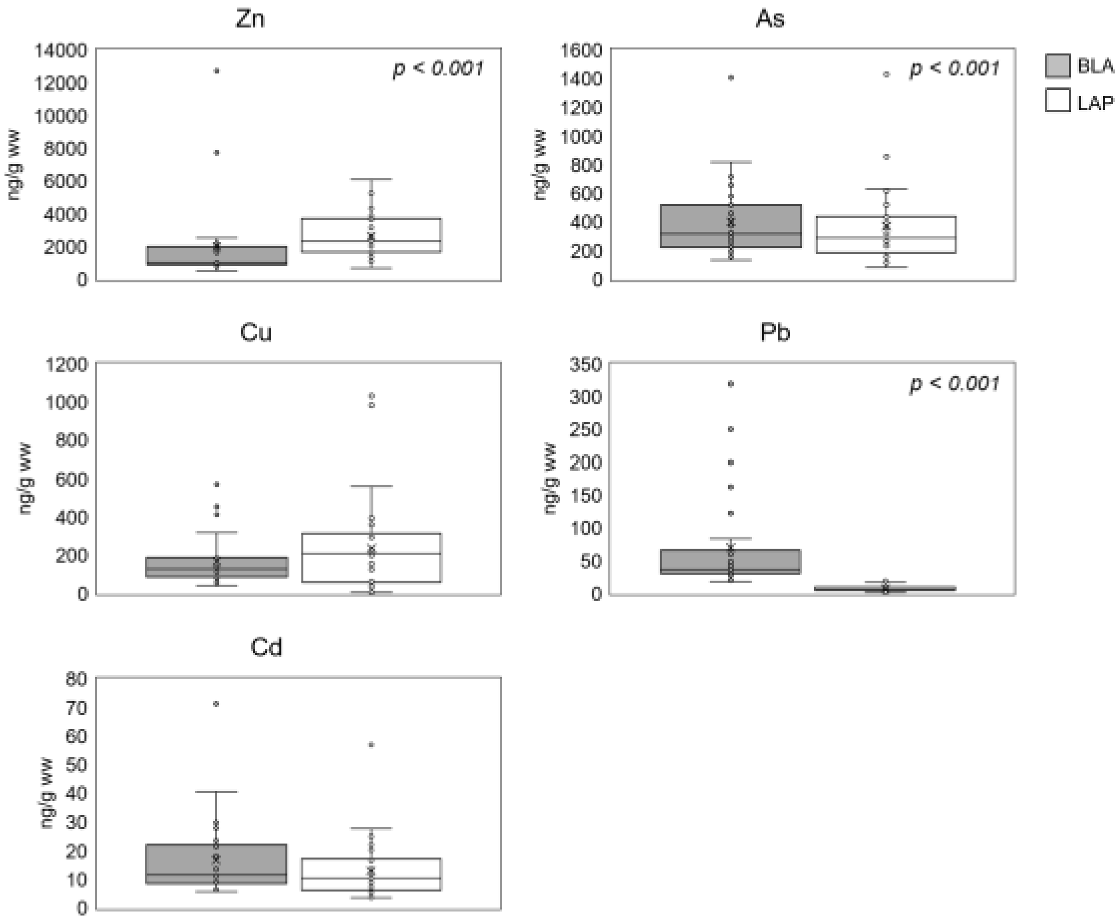

Fig. 3.8. Concentrations of Zn, As, Cu, Pb and Cd (ng/g ww) in biopsies of whale sharks collected during S2 in BLA and LAP.

Table 3.4. Concentrations (mean ± SD in ng/g ww) of Zn, As, Cu, Pb and Cd in males and females of whale shark samples in BLA and LAP during S2.

	N	Zn	As	Cu	Pb	Cd
BLA						
Male	26	1970 ± 2594	402 ± 273	169 ± 131	70 ± 78	17 ± 14
		(520-12712)	(131-1405)	(38-573)	(18-319)	(6-71)
Female	1	1472	657	108	48	21
Total	27	1959 ± 2545[a]	411 ± 272[a]	166 ± 129	69 ± 76[a]	17 ± 14
		(520-12712)	(131-1405)	(38-573)	(18-319)	(6-71)
LAP						
Male	24	2782 ± 1043	356 ± 290	289 ± 264[1]	7 ± 4	12 ± 11
		(1423-5255)	(83-1428)	(4-1032)	(2-19)	(3 -57)
Female	9	2269 ± 1737	409 ± 233	97 ± 109[2]	8 ± 6	15 ± 7
		(664-6093)	(115-855)	(5-294)	(2-23)	(5-25)
Total	33	2642 ± 1261[b]	377 ± 273[b]	244 ± 246	7 ± 5[b]	13 ± 10
		(664-6093)	(83-1428)	(4-1032)	(2-23)	(2-53)
Total of 2 bays	60	2335 ± 1958	388 ± 271	205 ± 204	35 ± 60	15 ± 12
		(520-12712)	(83-1428)	(4-1032)	(2-319)	(3-71)

Different superscript letter indicates significantly different ($p < 0.001$) mean concentrations between sites for the same element; different superscript number indicate significantly different ($p < 0.05$) mean concentrations between sexes for the same element. Statistical were performed by a Mann-Whitney test.

3.3.3.2 Differences between sex

- *Bahía de Los Ángeles*

During S2 (2017-2018), only one female was observed in BLA so statistical tests were not applied to our data.

- *Bahía de La Paz*

In LAP, mean concentration of Zn and Cu were higher in males (n = 23) compared to females (n = 9) with significant differences found in the concentration of Cu ($p < 0.05$) between sex. Arsenic, Pb and Cd mean concentrations were higher in females compared to males but with no statistical differences found (Fig. 3.9).

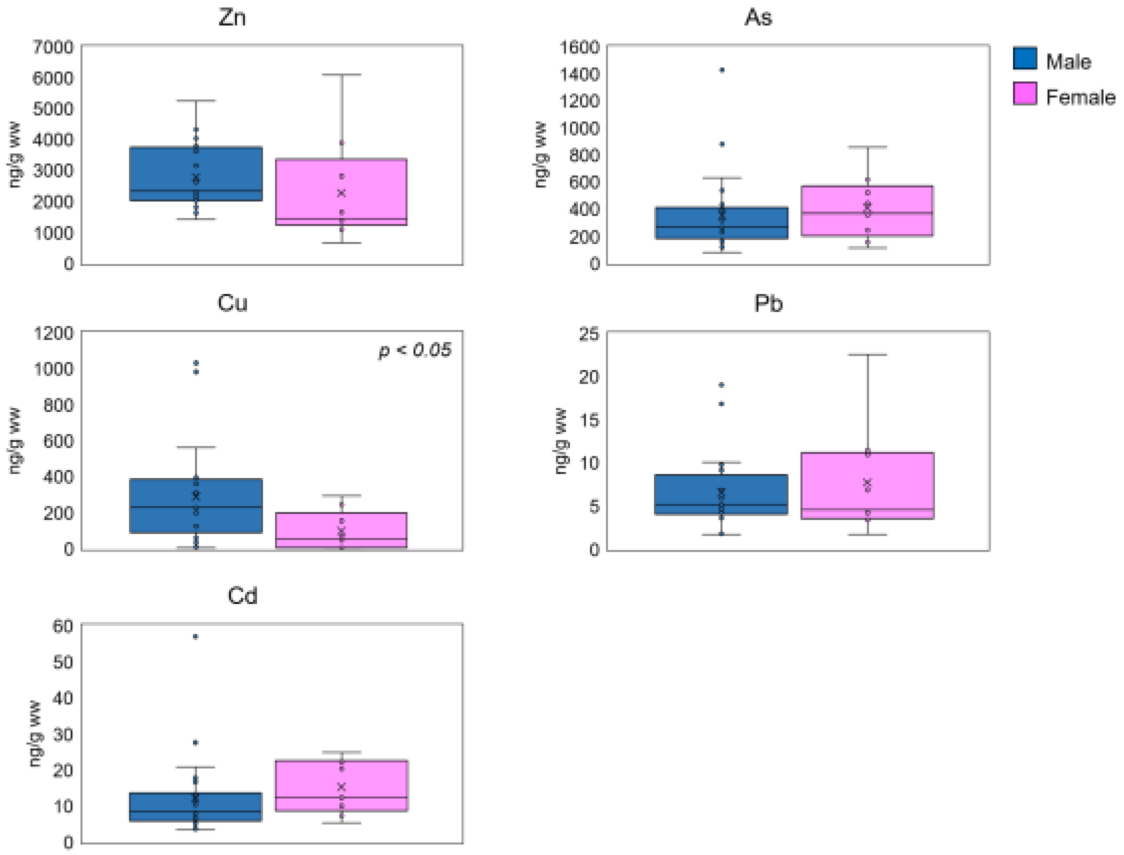

Fig. 3.9. TEs concentrations in whale shark biopsies of males and females from LAP during S2.

3.3.3.3 Relation with total length

- *Bahía de Los Ángeles*

Correlation between TL of the animals and TE concentration of the biopsies was performed only in males (n = 26) in this area. In general, concentration of TEs (ng/g ww) present a negative behaviour with the TL in males; this correlation was significative for all elements; Spearman p value < 0.05; linear equation [Zn]=−249.18 TL+ 3559.3; rho = -0.60; [As]=−90.497 TL+ 975.87; rho = -0.51; [Cu]=−27.069 TL+ 340.33; rho = -0.50; [Pb]=−14.463 TL+ 161.93; rho = -0.56 [Cd]=−2.7247 TL+ 34.057; rho = -0.50) (Fig. 3.10).

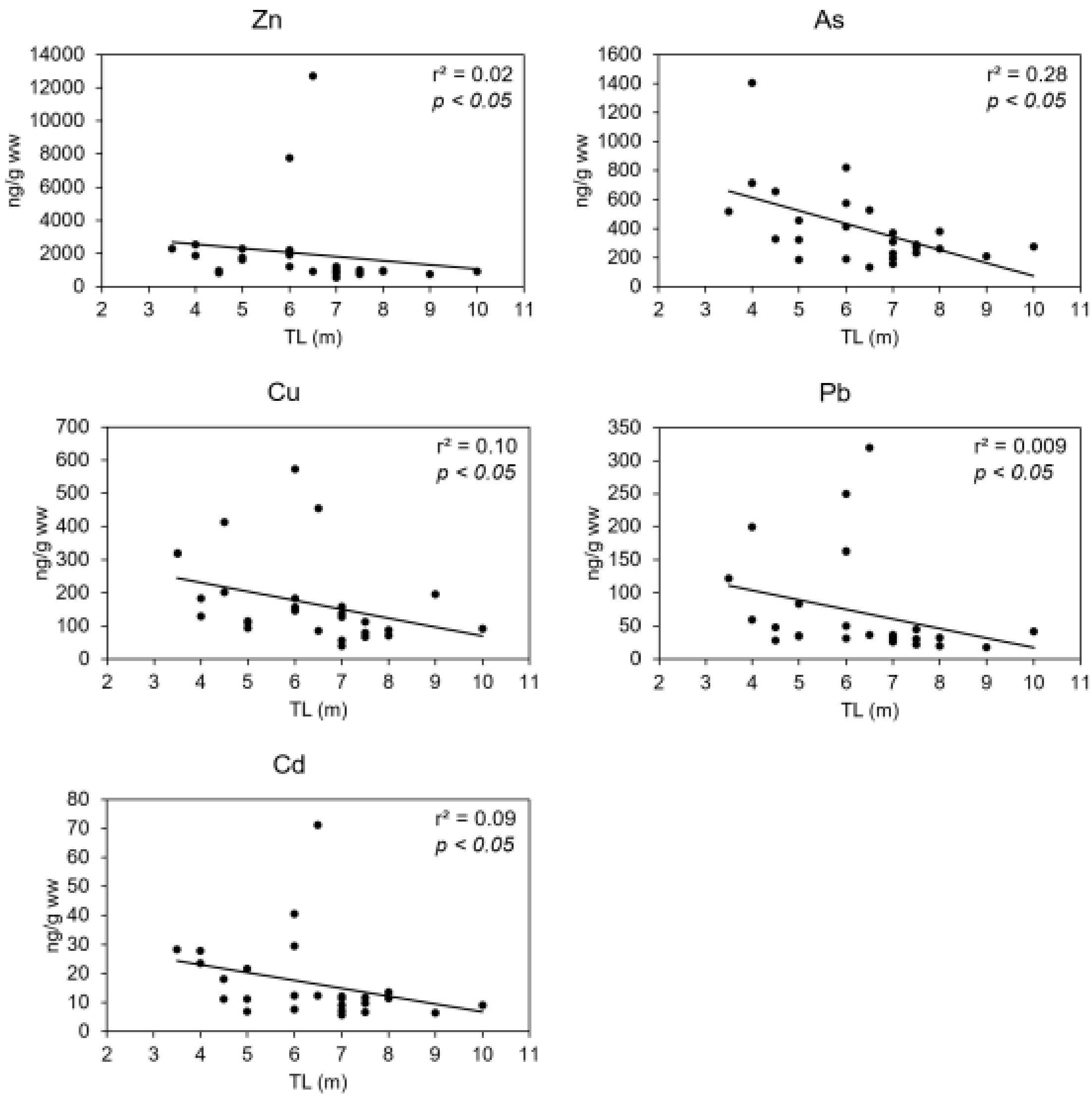

Fig. 3.10. Spearman correlation applied to TL and TEs concentration in biopsies of males of whale shark from BLA during S2.

- *Bahía de La Paz*

In general, we did not find any significant correlation between TEs concentration and TL in LAP; correlations seems to present a more positive behaviour in females (n = 9) respect to males (n = 24) but the coefficients of correlations were low for all elements (Fig. 3.11).

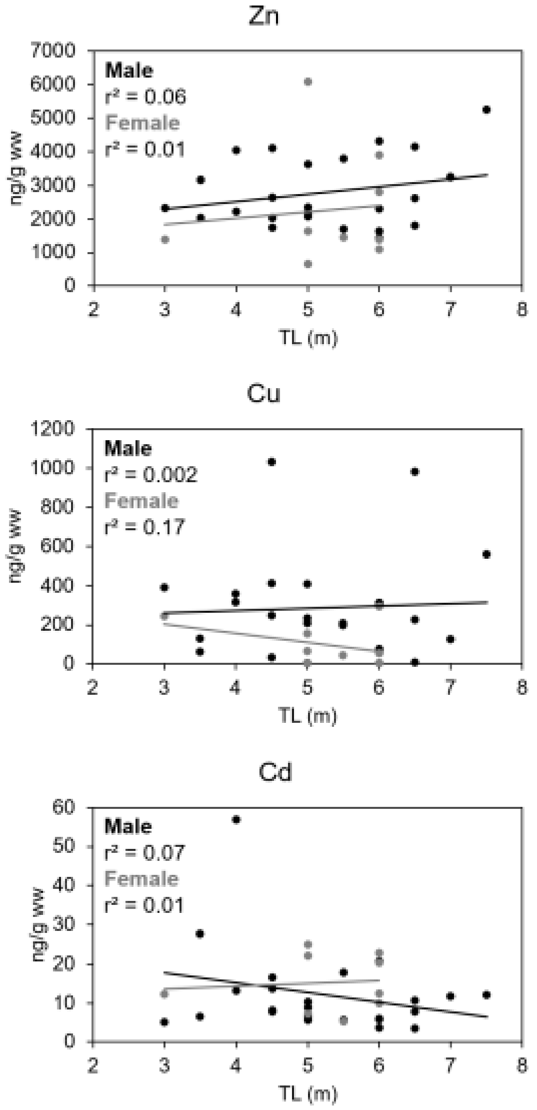

Fig. 3.11. Spearman correlation applied to TL and TEs concentration in biopsies of males and females of whale shark from LAP during S2.

3.3.4 SEASON 2: TEs concentrations in zooplankton

In the zooplankton samples collected from BLA (n =6), mean ± SD trace elements concentration (ng/g ww) was found in the order: Zn (6735±2988), Cu (1032±568), As (562±320), Cd (519±219) and Pb (20±20) (Fig. 3.12; Table 3.5). While in the zooplankton samples collected from LAP (n = 7), mean ± SD trace elements concentration was found in the order: Zn (5884±3577), As (639±491), Cd (627±229), Cu (548±431) and Pb (2±2) (Table 3.5). Copepods collected in LAP (n = 5) showed higher concentration of all elements compared with copepods from BLA (n = 5), but no statistical tests were applied due to the low number of samples (Fig. 3.13). In LAP, copepods showed higher concentrations of Zn, As and Pb, while chaetognatha (n = 2) showed higher concentrations of Cu and Cd (Fig. 3.13).

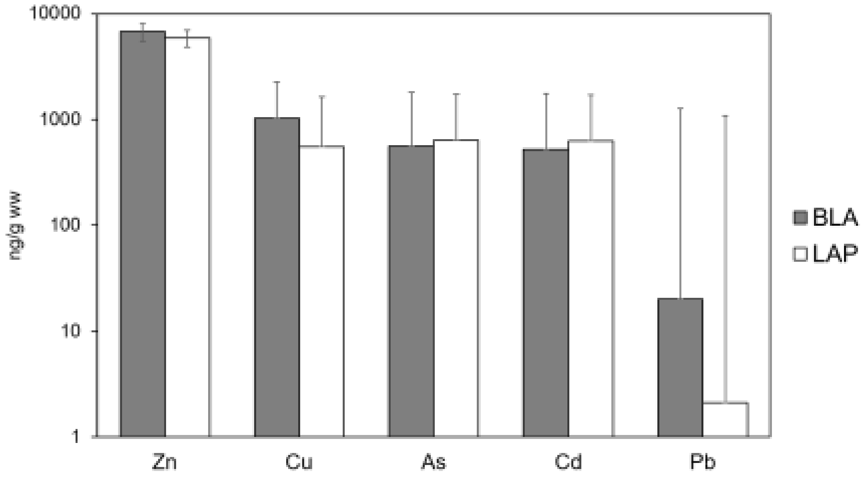

Fig. 3.12. Mean TEs concentration and SD in zooplankton samples collected from BLA and LAP during S2.

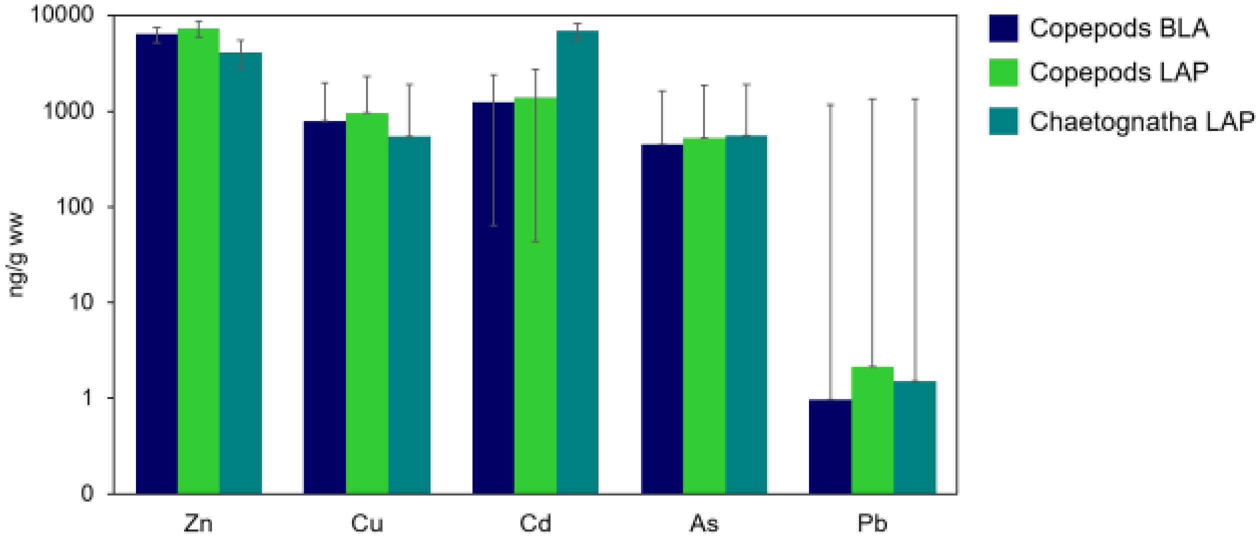

Fig. 3.13. Mean TEs concentrations in the main groups of zooplankton samples collected in BLA and LAP during S2.

Table 3.5. TEs concentrations (mean ± SD (ng/g ww)) in zooplankton and in the main zooplankton groups collected in BLA and LAP during S2.

	Site		Month	Year	Zn	Cu	As	Cd	Pb
Zooplankton	BLA	Mixt	Sept	2017	7556	1218	821	529	34
	BLA	Mixt	Sept	2017	5245	458	386	342	6
	BLA	Mixt	Sept	2017	5439	997	409	748	5
	BLA	Mixt	Sept	2017	12454	1802	1010	819	53
	BLA	Mixt	Oct	2017	5256	1405	617	364	20
	BLA	Mixt	Oct	2017	4459	313	129	313	1
		Average±SD			6735±2988	1032±568	562±320	519±219	20±20
	LAP	Mixt	Oct	2017	44	3	101	1068	0.9
	LAP	Mixt	Nov	2017	11488	1408	518	479	4.5
	LAP	Mixt	Nov	2017	4766	419	295	527	5.3
	LAP	Mixt	Dec	2017	7597	550	1037	801	2.3
	LAP	Mixt	Jan	2018	4323	348	413	445	0.7
	LAP	Mixt	Jan	2018	7885	451	1540	473	0.5
	LAP	Mixt	Feb	2018	5082	659	568	594	0.6
		Average±SD			5884±3577	548±431	639±491	627±229	2±2
Main groups	BLA	Copepods	Sept	2017	4605	811	821	439	0.8
	BLA	Copepods	Sept	2017	9420	1313	1276	872	1.3
	BLA	Copepods	Sept	2017	5129	906	884	381	0.8

BLA	Copepods	Sept	2017	68	8	1657	148	1.6
BLA	Copepods	Oct	2017	12387	875	1475	419	0.3
	Average±SD			6322±4740	783±476	1223±365	452±262	1.0±0.5
LAP	Copepods	Oct	2017	12715	2837	1101	634	6.0
LAP	Copepods	Nov	2017	5675	610	690	354	1.8
LAP	Copepods	Dec	2017	7028	571	1097	1009	1.6
LAP	Copepods	Jan	2018	8822	675	974	390	0.5
LAP	Copepods	Feb	2018	2135	130	3012	226	0.8
	Average±SD			7275±3906	965±1069	1373±930	523±309	2.1±2.2
LAP	Chaetognats	Oct	2017	8075	1079	10929	868	0.3
LAP	Chaetognats	Nov	2017	109	14	2831	253	2.7
	Average±SD			4092±5633	547±753	6880±5726	561±434	1.5±1.7

3.3.4.1 Biomagnification factor (BMF)

BMF for whale sharks of BLA (n = 27) was calculated for zooplankton samples (n = 6) and copepods (n = 5) (Table 3.6). BMF through total zooplankton was 0.30 for Zn, 0.73 for As, 0.16 for Cu, 3.53 for Pb and 0.03 for Cd. Pb BMF through total zooplankton and copepods was consistent and statistically >1.0 (Table 3.6).

In whale sharks from LAP (n = 33), BMF was statistically > 1 ($p < 0.001$) only for Pb through zooplankton (n = 7) and copepods (n = 5). Chaetognatha BMF was also > 1 but t-test was not applied (n = 2).

Table 3.6. BMF (mean, min and max in ng/g ww) of whale sharks calculated from samples of zooplankton, copepods and chaetognatha collected in BLA and LAP during S2. Asterisks indicate p values < 0.05 (*) and < 0.001 (**). Statistical test applied was t-test.

				BMF BLA		
	n	Zn	As	Cu	Pb	Cd
Zooplankton	6	0.30 (0.08-1.89)	0.73 (0.23-2.50)	0.16 (0.04-0.56)	3.53* (0.89-16.0)	0.03 (0.01-0.14)
Copepods	5	0.32 (0.08-2.01)	0.91 (0.29-3.11)	0.21 (0.05-0.73)	73.1** (18.5-330)	0.01 (0.005-0.060)
				BMF LAP		
Zooplankton	7	0.44 (0.11-1.04)	0.58 (0.13-2.24)	0.43 (0.01-1.88)	3.69** (0.80-17.5)	0.02 (0.01-0.09)
Copepods	5	0.36 (0.09-0.84)	0.71 (0.16-2.73)	0.24 (0.004-1.07)	3.63** (0.79-17.2)	0.01 (0.003-0.04)
Chaetognatha	2	0.63 (0.16-1.49)	0.66 (0.15-2.55)	0.43 (0.01-1.89)	5.21 (1.1-24.6)	0.002 (0.001-0.008)

3.3.5 Comparison of TEs concentration between seasons

- *In whale shark biopsies*

In general, TEs concentration increased in both areas from S1 to S2; Mann-Whitney test showed significant differences ($p < 0.001$) in the concentration of Zn, As and Cd between seasons in both areas (Fig. 3.14). Cu and Pb concentrations were statistically different ($p < 0.001$) during season S1 and during season S2 only in BLA.

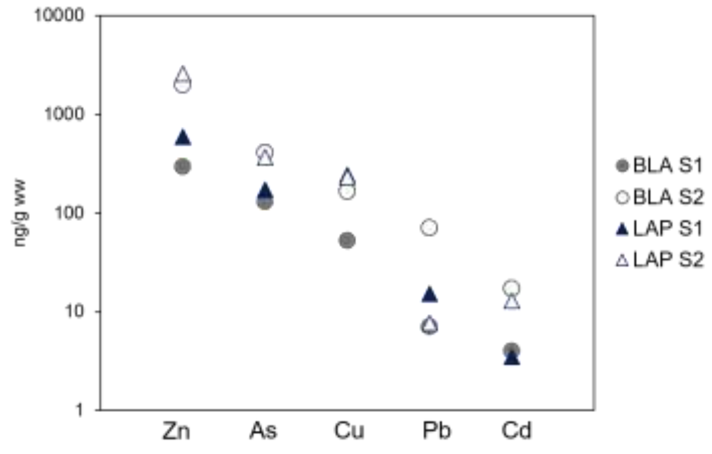

Fig. 3.14. Mean TEs concentrations in biopsies of whale sharks collected in BLA and LAP during S1 and during S2.

➢ *In zooplankton from LAP*

The TEs concentrations in the mixt zooplankton were most high during S2 (n = 12) than during S2 (n = 7). Mann-Whitney p values < 0.05 was found in the comparison of the mean concentration of Zn, As, Cu and Pb between S1 and S2. Cd showed a p value < 0.001 (Fig. 3.15). Statistical tests were not applied to the concentrations on TEs in the copepods (S1n = 2; S2n = 5) and chaetognatha (S1n = 3; S2n = 2) due the number of samples was too low.

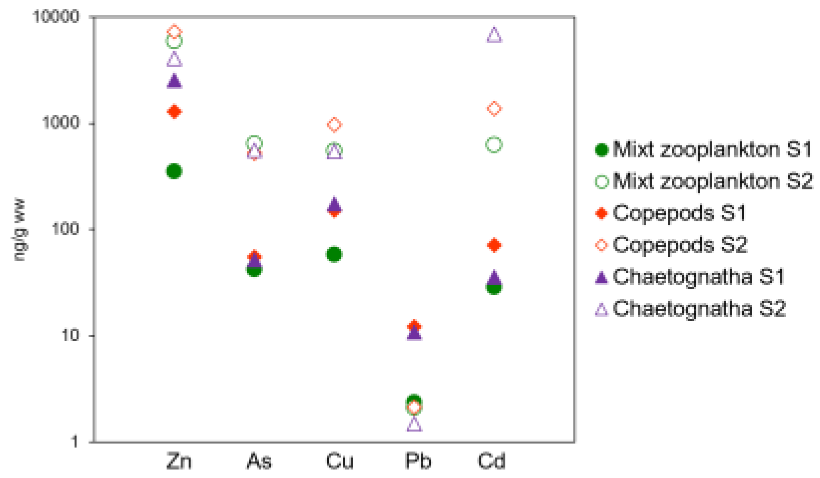

Fig. 3.15. Mean TEs concentrations in samples of zooplankton, copepods and chaetognatha collected in LAP during S1 and S2.

➢ *Changes in TEs concentration in the same individuals*

Generally, in BLA, TEs concentration (ng/g ww) in biopsies was higher in all six animals (Fig. 3.16); mean ± SD increase for Zn was 3401 ± 1154 ng/g, 1105 ± 582 ng/g for As, 444 ± 492 ng/g for Cu, 103 ± 32 ng/g for Pb and 39 ± 11 ng/g for Cd.

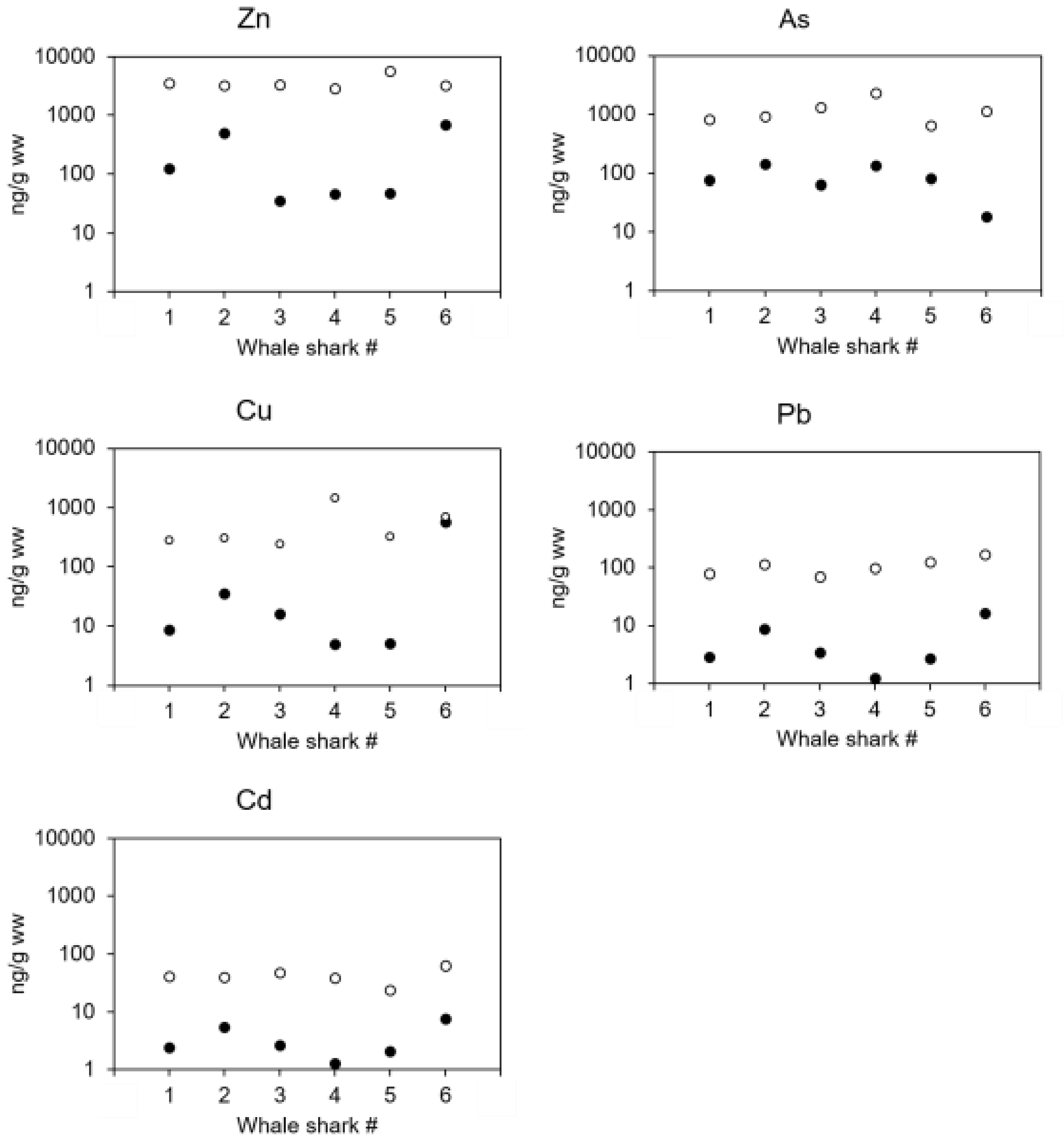

Fig. 3.16. Variability in TEs concentrations during S1 (black circle) and during S2 (white circle) in six whale sharks spotted in BLA.

In LAP, Zn presented an increase (mean±sd) of 7865 ± 8331 ng/g in the animals (Fig. 3.17). Arsenic decreased in whale shark #1 (345 ng/g), #2 (668 ng/g) and #3 (212 ng/g) and increased in the other six sharks. Cu increased in

WS #1 (494 ng/g), #3 (465 ng/g), #4 (243 ng/g), #5 (929 ng/g), #7 (991 ng/g), and decreased in WS #2 (71 ng/g), #6 (243 ng/g), #8 (5826 ng/g) and #9 (5690 ng/g) (Fig. 3.17). Pb generally decreased but presented an increase in concentration in three animals. Finally, Cd only decreased in one animal (WS #6; 26 ng/g) and it was the element that presented the least variation within animals (Fig. 3.17).

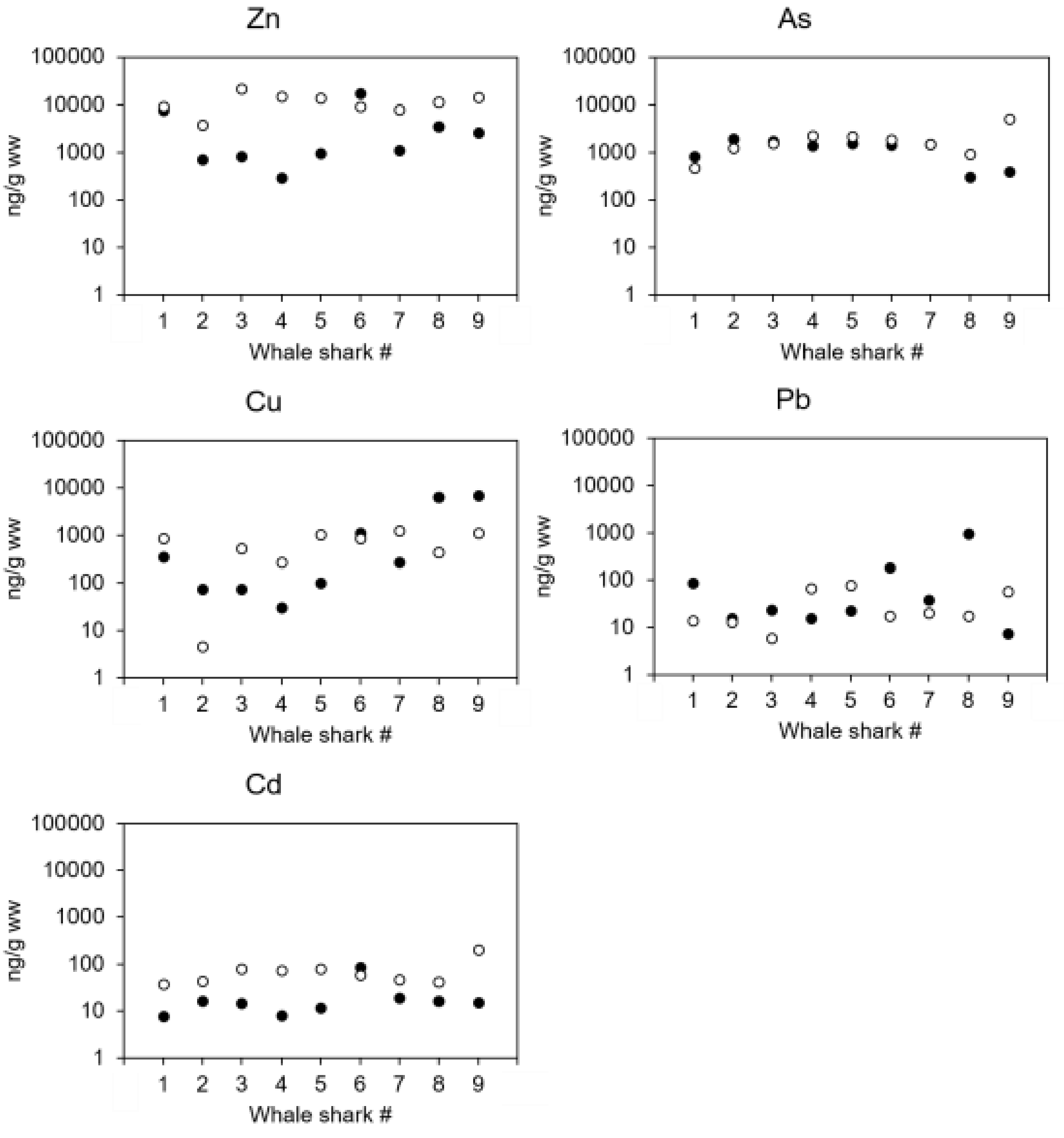

Fig. 3.17. Changes in TEs concentrations of biopsies during S1 (black circle) and during S2 (white circle) in nine whale sharks spotted in LAP.

3.3.6 Molar ratios

Molar ratios Zn:As, Zn:Cd, Cd:Pb, Cd:As, Se:Cd, Se:As, Cu:Cd, Zn:Pb and Cu:Pb were calculated in the biopsies of whale shark collected in both study areas and both seasons. Data of Se are showed in Chapter 2. Molar ratios were compared with another study on trace elements found in epidermis of two dead whale sharks from China (Table 3.7). Statistical differences (t-test applied) of the molar ratios in BLA and LAP in both seasons are shown in Table 3.8.

Antagonist behaviour was explored between these elements and results are shown in Fig. 3.18, 3.19, 3.20, 3.21, 3.22, 3.23, 3.24, 3.25 and 3.26.

Table 3.7. TEs (mean ± SD, min - max) found in whale shark biopsies collected during S1 and during S2 in BLA and LAP.

	n	Zn nmol/g	As nmol/g	Pb nmol/g	Cd nmol/g	Se nmol/g	Cu nmol/g
S1							
BLA	38	4.5 ± 6.2 (0.5 – 32.2)	1.7 ± 2.1 (0.2 – 13.5)	0.03 ± 0.03 (0.01 – 0.20)	0.04 ±0.03 (0.01 – 0.13)	89.3 ± 82.7 (15.4 – 454.0)	0.83 ± 1.57 (0.07 – 8.96)
LAP	32	9.0 ± 8.5 (0.5 – 30.3)	2.3 ± 2.0 (0.5 – 8.8)	0.07 ± 0.15 (0.004 – 0.80)	0.03 ± 0.03 (0.004 – 0.10)	3.6 ± 2.5 (0.2 – 10.7)	3.84 ± 5.33 (0.06 – 16.84)
S2							
BLA	27	30.0 ± 39.0 (8.0 – 194.4)	5.5 ± 3.6 (1.7 – 18.7)	0.3 ± 0.3 (0.1 – 1.5)	0.2 ± 0.1 (0.05 – 0.60)	3.3 ± 5.2 (0.3 – 23.2)	2.62 ± 2.02 (0.60 – 9.02)
LAP	33	40.4 ± 19.3 (10.0 – 93.2)	5.0 ± 3.6 (1.0 – 19.0)	0.03 ± 0.02 (0.01 – 0.10)	0.1 ± 0.1 (0.03 – 0.50)	4.2 ± 2.4 (1.0 – 15.0)	3.72 ± 3.88 (0.06 – 16.24)

Table 3.8. Molar ratio (mean ± SD, min - max) calculated in whale shark biopsies collected during S1 and during S2 in BLA and LAP. Results from this study are compared with the ones found in Wang et al. (2014) on two whale sharks from China.

	n	Zn:As	Zn:Cd	Cd:Pb	Cd:As	Se:Cd	Se:As	Cu:Cd	Zn:Pb	Cu:Pb
S1										
BLA	38	4.7 ± 9.0*	102 ± 61**	1.2 ± 0.4*	0.04 ± 0.05	2520 ±1537**	89.3 ± 147.3**	25.3 ± 40.3**	120.7 ± 72.8**	25.1 ± 33.2**
		(0.3 – 43.8)	(23.2 – 280)	(0.3 – 2.6)	(0.004 – 0.3)	(1011 – 10822)	(8.7 – 880.4)	(2.1 – 173.1)	(13.5 – 356.8)	(1.3 – 134.5)
LAP	32	7.0 ± 10.2*	327 ± 352**	1.0 ± 0.7	0.02 ± 0.03	193 ± 200*	2.4 ± 2.0*	156.4 ± 225.8**	261.4 ± 271.0**	187.4 ± 532.0**
		(0.3 – 50.3)	(61.2 – 1735)	(0.03 – 3.7)	(0.003 – 0.4)	(2.5 – 801)	(0.03 – 8.6)	(3.6 – 801.3)	(12.0 – 1107.5)	(3.8 – 2969.3)
S2										
BLA	27	5.8 ± 5.4**	191.2 ± 92.5**	0.6 ± 0.3	0.03 ± 0.02	18.2 ± 12.5*	0.6 ± 0.6	20.6 ± 13.7**	98.2 ± 38.7**	11.3. ± 9.8**
		(1.4 – 27.5)	(88.6 – 435)	(0.1 – 1.3)	(0.01 – 0.09)	(3.0 – 64.5)	(0.1 – 3.0)	(8.2 – 65.2)	(37.3 – 207.7)	(3.0 – 48.4)
LAP	33	11.0 ± 6.8**	460 ± 245**	4.8 ± 5.8**	0.03 ± 0.01	55.0 ± 52.0*	1.2 ± 1.0*	57.0 ± 94.5**	1756.1 ± 1912.8**	163.5 ± 191.2**
		(0.9 – 30.0)	(45.7 – 951)	(1.0 – 29.0)	(0.01 – 0.06)	(10.0 - 254.5)	(0.3 – 4.5)	(0.4 – 499.1)	(184.0 – 11407.2)	(1.2 – 763.0)
Wang et al., 2014	2		247.1	0.9				24.7	217.0	21.7
			235.0	0.7				30.4	169.0	22.0

Asterisks indicate p values < 0.05 (*) and < 0.001 (**). Statistical test applied was t-test.

> *Zn versus As*

Spearman correlation showed significative values ($p < 0.05$) between concentrations of Zn and As in BLA during S2 and LAP during S2 (Fig. 3.18 b and d); this indicate the such elements follow a same metabolic route in this tissue.

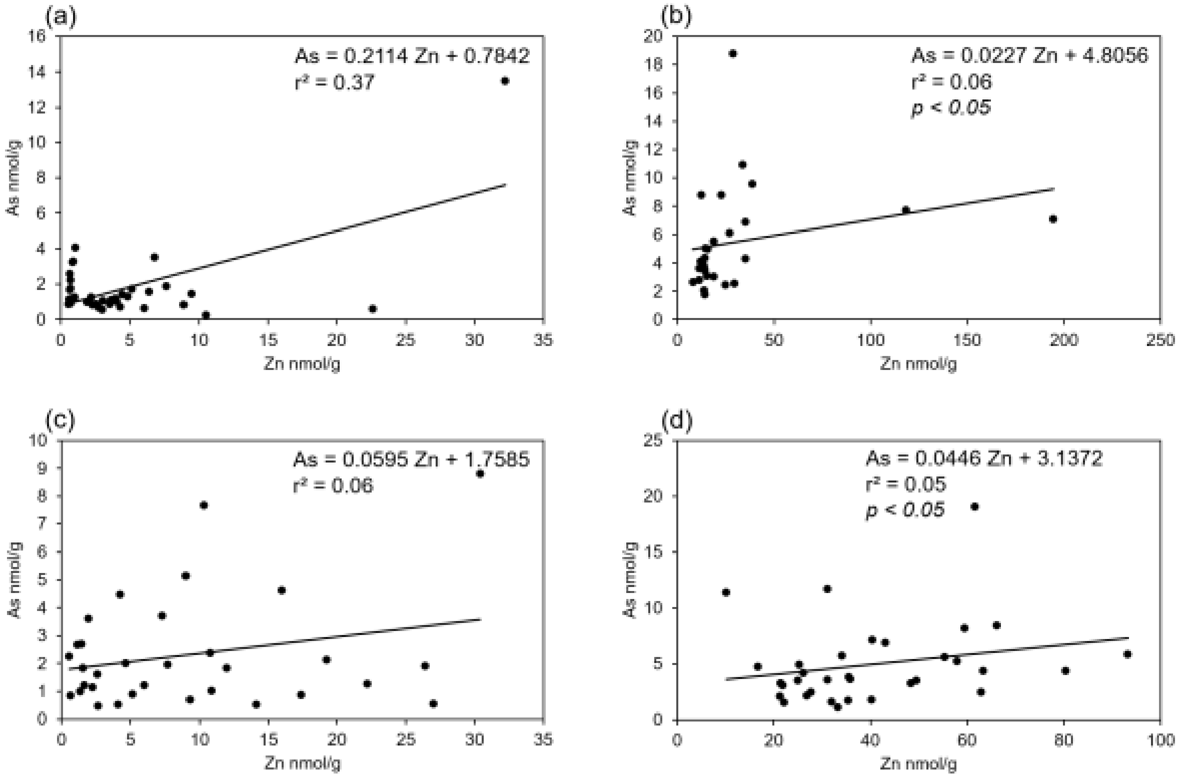

Fig. 3.18. Variation of Zn with As in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

- *Zn versus Cd*

Spearman correlation showed significative values ($p < 0.001$ and $p < 0.05$) between Zn and As concentrations in both study areas and both seasons (Fig. 3.19); this indicate the such elements follow a same metabolic route in this tissue.

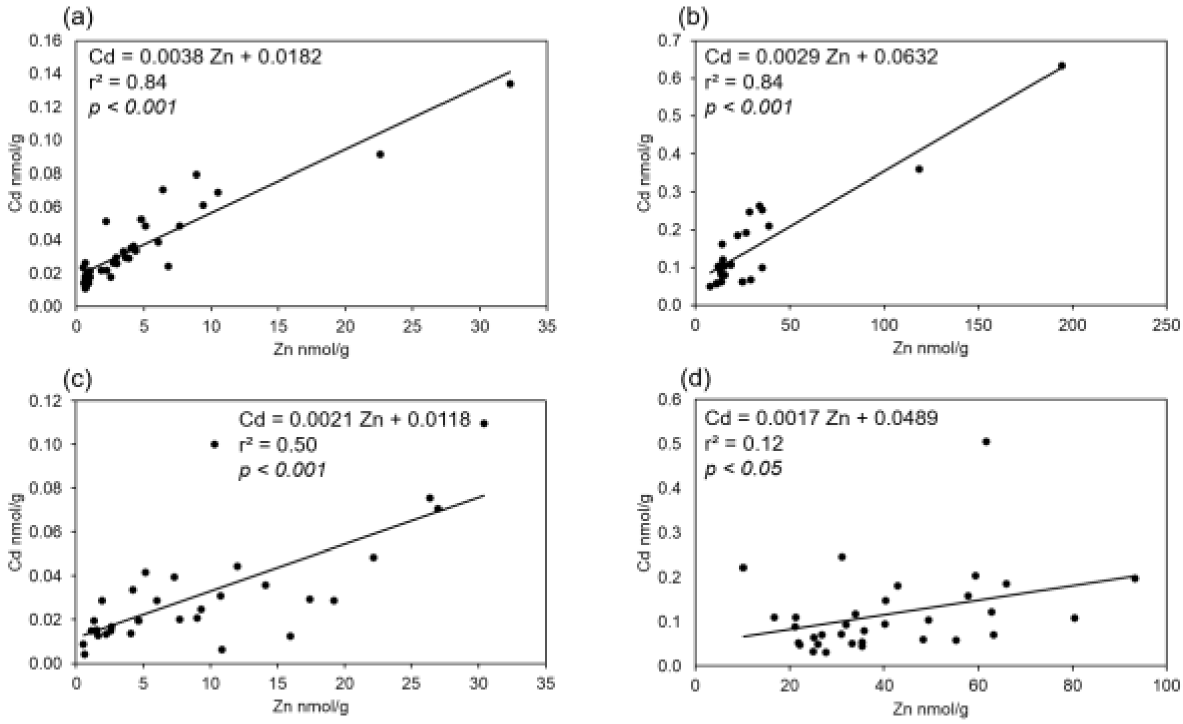

Fig. 3.19. Variation of Zn with Cd in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

➢ *Cd versus Pb*

The variation of Cd with Pb was significantly correlated ($p < 0.001$) in BLA A during S1 and during S2 (Fig. 3.20 a and b). In LAP, this correlation were not significant; nevertheless, we found significant correlation ($p < 0.05$) in S1 by not considering two outliers (white circles Fig. 3.20 c). The original equation of this correlation was Pb= 0.7547 Cd + 0.05 and r^2=0.02.

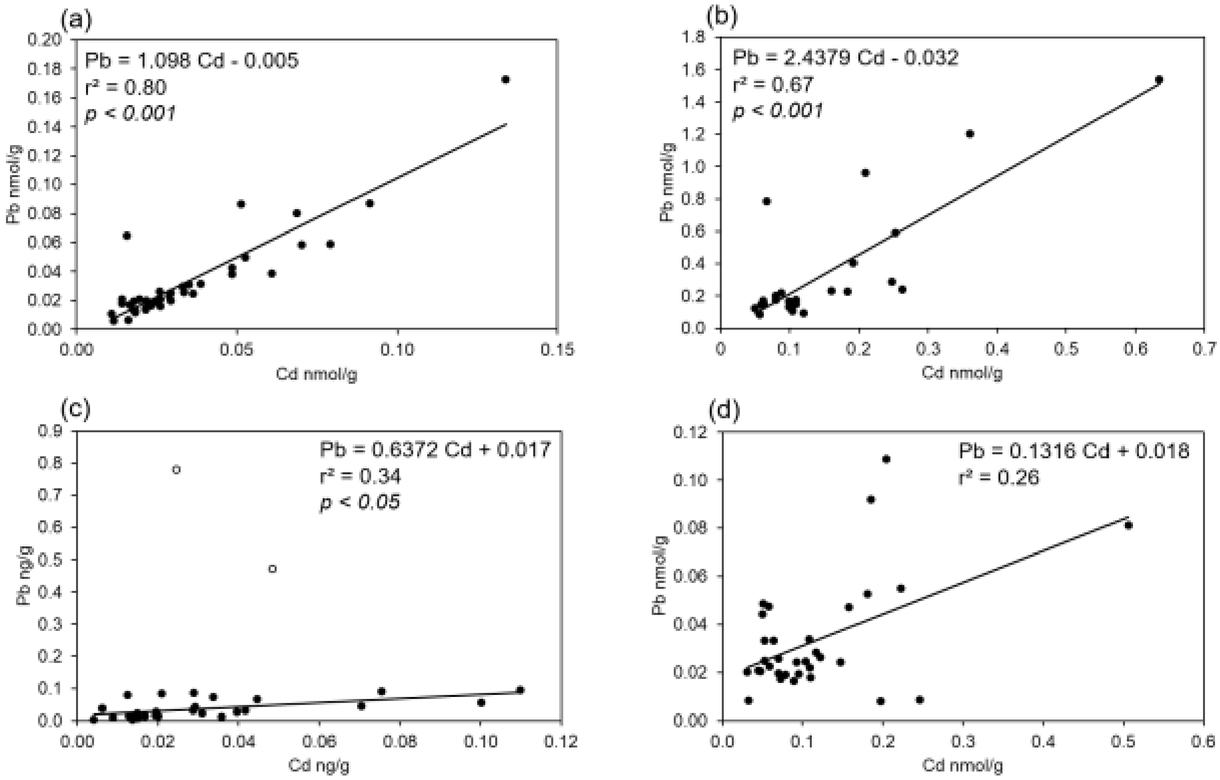

Fig. 3.20. Variation of Cd with Pb in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

➢ *Cd versus As*

The variation of Cd with As concentrations showed a significant correlation ($p < 0.001$) in both study areas but only during S2 (Fig. 3.21 b and d).

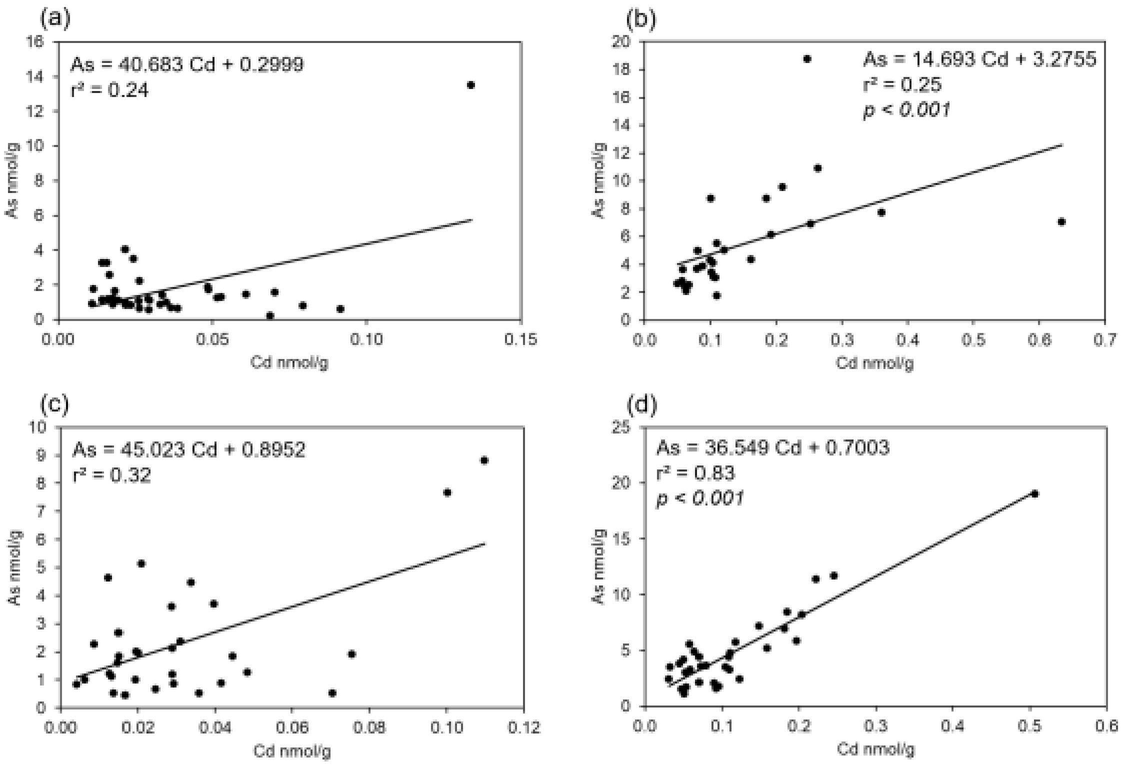

Fig. 3.21. Variation of Cd with As in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

➢ *Se versus Cd*

The variation of Se with Cd showed a correlation significative in both seasons only in BLA ($p < 0.001$) (Fig. 3.22 a and b); which indicate that during such season the two elements follow a same metabolic route.

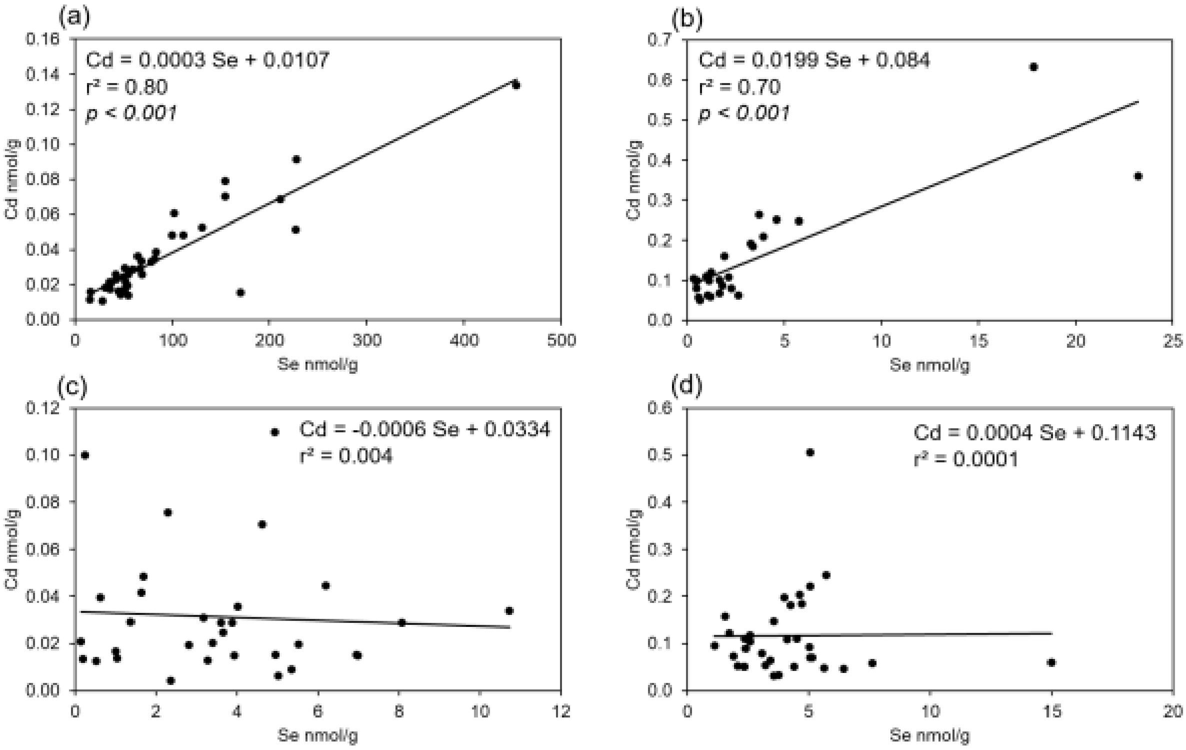

Fig. 3.22. Variation of Se with Cd in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

➢ *Se versus As*

In general, this variation did not showed a significant correlation, except in BLA during S2 ($p< 0.001$; Fig. 3.23 b); which indicate that these two elements follwed a different metabolic route.

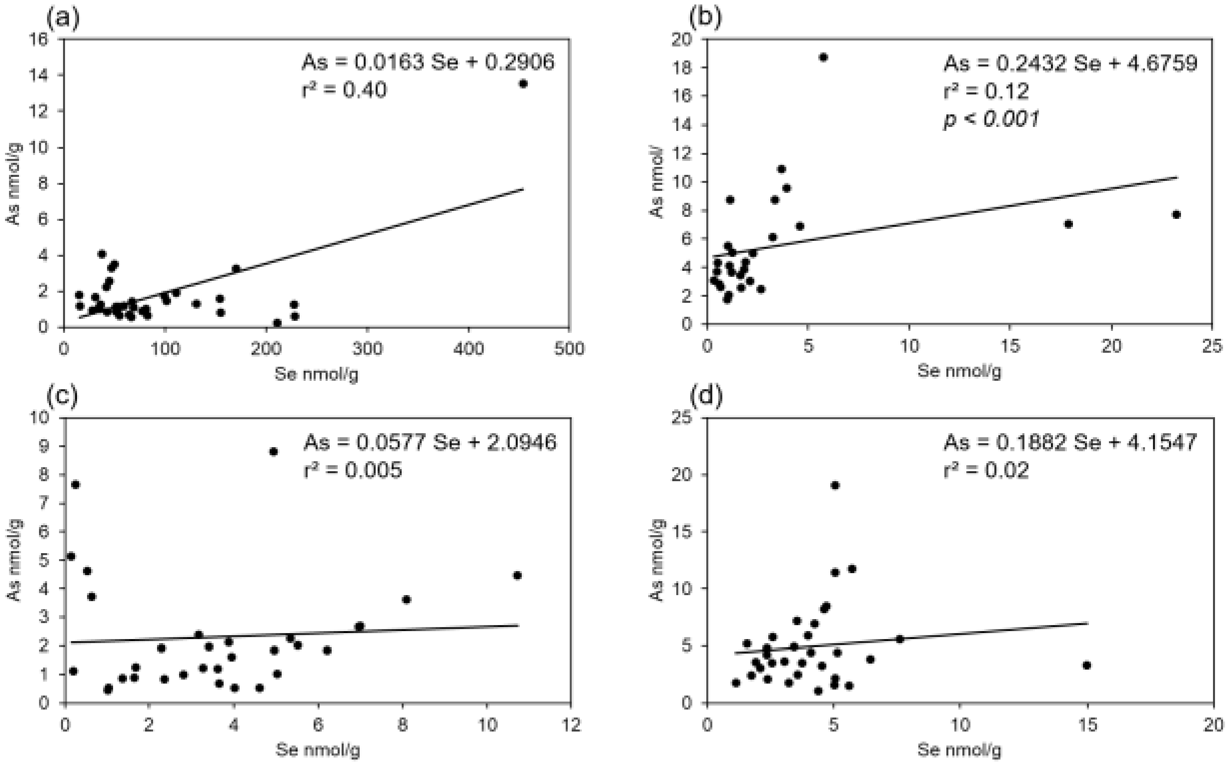

Fig. 3.23. Variation of Se with As in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

➢ *Cu versus Cd*

This correlation was significant in BLA in both seasons ($p < 0.05$; Fig. 3.24 a,b).

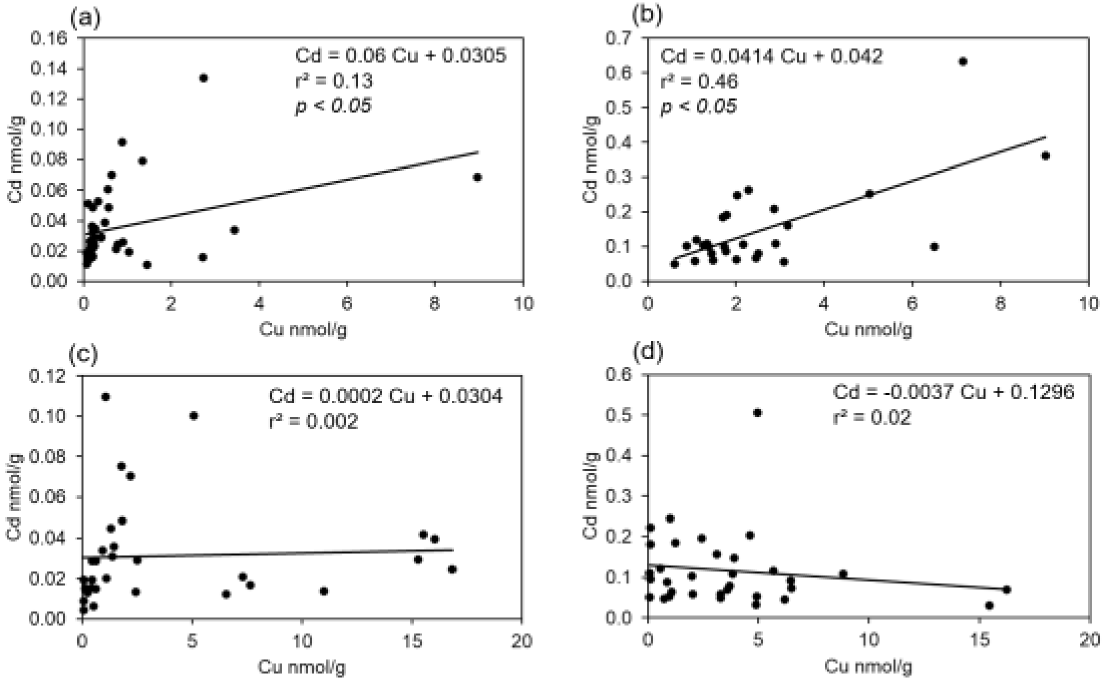

Fig. 3.24. Variation of Cu with Cd in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

➢ *Zn versus Pb*

In BLA, this correlation showed Spearman p values < 0.001 in both seasons (Fig. 3.25 a,b) with strong r^2 values. On the contrary, in LAP Spearman p value was signifcant only in S1 ($p < 0.05$; Fig. 3.25 c) even if the r^2 was low.

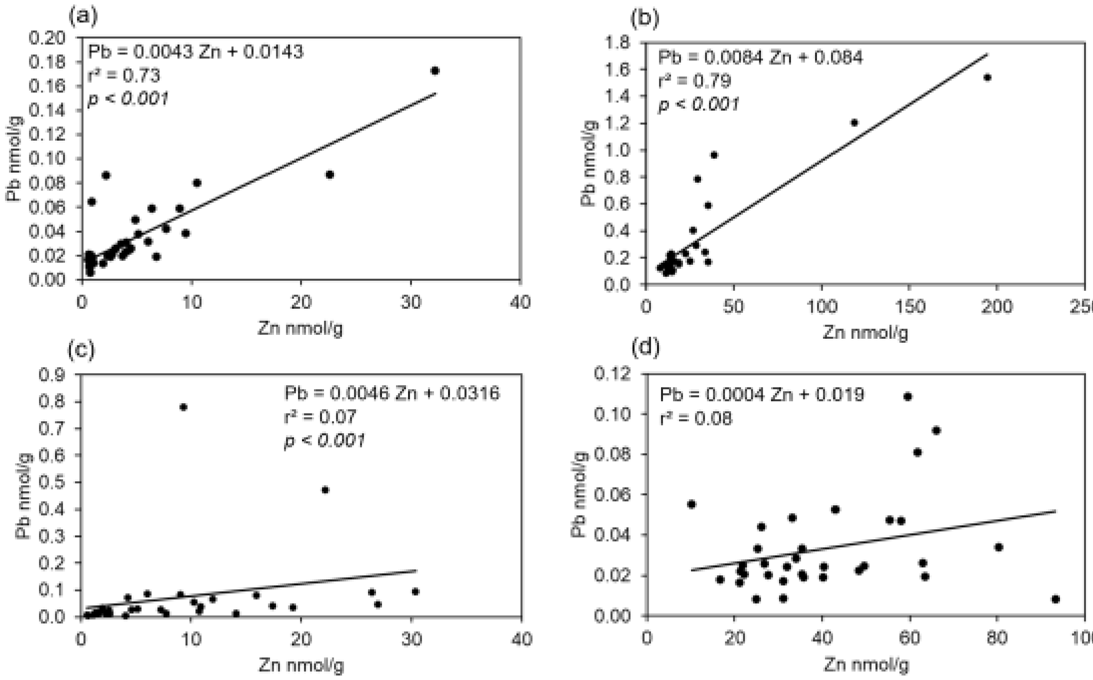

Fig. 3.25. Variation of Zn with Pb in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

➢ *Cu versus Pb*

In BLA, this correlation showed Spearman p values < 0.05 in both seasons (Fig. 3.26 a,b). On the contrary, in LAP Spearman p value was signifcant only in S1 (p < 0.05; Fig. 3.26 c).

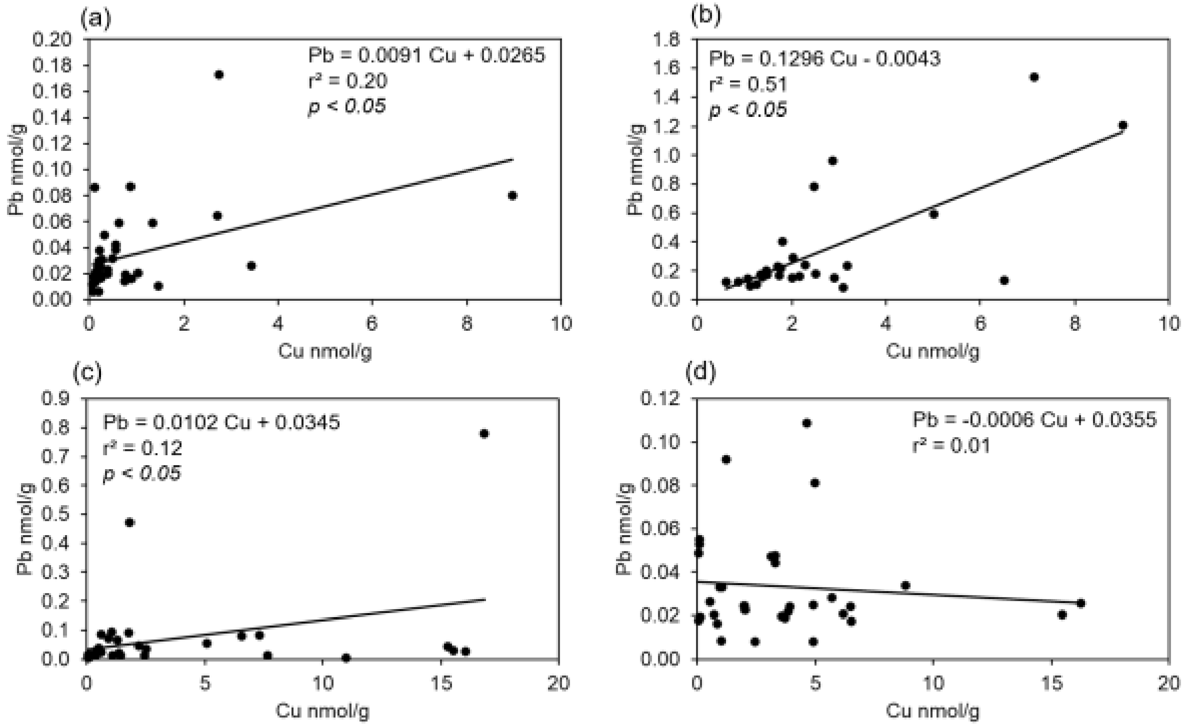

Fig.3.26. Variation of Cu with Pb in biopsies of whale shark from BLA during S1 (a), BLA during S2 (b), LAP during S1 (c) and LAP during S2 (d).

3.4. DISCUSSION

Concentrations of As, Zn, Cd, Cu and Pb found in the epidermis of whale sharks from BLA and from LAP in two seasons of sampling were lower than those reported in epidermis of whale sharks from China (Wang *et al.,* 2014) and Dijibouti (Boldrocchi *et al.,* 2020). Heavily developed urbanization and mayor port traffic, such as China and Dijibouti, could increase the concentrations of heavy metals in the ocean leading to higher concentrations in shark tissues compared to areas that are less populated such as the Baja California Peninsula.

These differences are shown in Table 3.9 in order to facilitate the comparison expressed in µg/g (ww).

Zinc was the most abundant elements in the whale sharks sampled from the Gulf of California. As an essential element, Zn is involved in the formation of over 300 proteins, nucleic acids, carbohydrates and fatty acids (Hogstrand & Wood, 1996). It is highly present in lysosomes that are responsible of its deposition in scales in teleostean fish (Hogstrand & Wood, 1996). Zinc maintains a fundamental role in the replication and transcription of the DNA (Vallee & Falchuk, 1993) and in the induction of metallothioneins. In fish, Zn is mostly found in skin, muscle and bone where it constitutes the 50-60% of the total body Zn (Glynn, 1991). Mean concentrations of Zn in fish can vary from 10 to 40 µg/g ww in most tissues, even if some tissues such as the retina and the liver can accumulate higher concentrations (Hogstrand & Wood, 1996).

Heavy metals like Pb and Cd could be higher in tissues of whale shark found in heavily urbanized areas like China, respect to area that are less populated such as the Baja California Peninsula. In addition, information on the size of the dead whale shark animals found in China could be useful to understand the differences in concentration found with the whale sharks from the Gulf of California; nevertheless, this kind of information was not available in the paper published by Wang *et al.,* (2014).

Table 3.9. Trace element concentrations found in whale shark biopsies from this study and those found in two dead whale sharks from the coast of China (n = 2; Wang *et al.,* 2014) and Dijibouti (n = 12; Boldrocchi *et al.,* 2020). Concentrations are expressed in µg/g (ww).

TE	BLA S1	LAP S1	BLA S2	LAP S2	*Wang et al., 2015*	*Boldrocchi et al., 2020*
Zn	0.30 ± 0.41	0.60 ± 0.55	1.96 ± 2.55	2.64 ± 1.26	28.74 ± 0.05 21.87 ± 0.09	37.8 ± 42.6
Cd	0.004 ± 0.003	0.004 ± 0.003	0.02 ± 0.01	0.01 ± 0.01	0.20 ± 0.0008 0.16 ± 0.0008	0.06 ± 0.03
Cu	0.05 ± 0.10	0.24 ± 0.34	0.17 ± 0.13	0.24 ± 0.25	2.79 ± 0.20 2.75 ± 0.06	8.1 ± 11
Pb	0.01 ± 0.01	0.02 ± 0.03	0.07 ± 0.08	0.007 ± 0.005	0.42 ± 0.006 0.41 ± 0.006	1.24 ± 1.12
As	0.13 ± 0.16	0.17 ± 0.15	0.41 ± 0.27	0.37 ± 0.27		0.96 ± 0.7

3.4.1 TEs concentrations in sites

We found significant differences in the concentration of As, Zn and Cu, more abundant in the whale sharks from LAP respect to BLA, and in the concentration of Pb, which was more abundant in the sharks from BLA. La Paz bay is enriched in As and Zn probably due to liberation of materials from the phosphorite rock present in San Juan de La costa. This area has been an active mining during over 80 years and according with Castañón & Bañuelos (2016), the mine is responsible to provide 96% of the phosphorite used in the whole Mexico. Phosphorite is used in pesticides and as preservative in the food industry.

Trace element concentration in the marine environment depends on environmental factors such as wind patterns, raining and sea temperature (Renteria-Cano, 2011); in addition to the magnitude of the sources, there are intrinsic factors as pH, salinity, organic matter composition, redox, alkalinity, and dissolved oxygen that influence directly on the bioavailability of trace metals. In the Gulf of California, precipitation (Brito-Castillo *et al.,* 2010) and storm from the Baja Peninsula and Sonora desert, and the atmospheric precipitation are the main sources of such elements (Ortiz-Figueroa, 2009).

3.4.2 Relation with sex and length

Sex, apparently, was not a significant influential factor in TEs concentration; the exceptions were Pb and Cu which were found in higher concentration in males of LAP, during S1 and S2, respectively. Generally, TEs concentrations showed a negative correlation with the total length of males, and a more positive correlation with females. These results could be related with several factors: essential elements like Zn could be involved in the development of the claspers or in the male sexual development. Zinc and Cu are essential elements both are regulated by homeostasis processes; and their concentrations in the body depend on many factors including starvation (Jakimska *et al.,* 2011). In the muscle of fish, the concentrations of Zn and Cu tend to decrease during the growth period (Zhang & Wang, 2006; Endo *et al.,* 2008; Storelli *et al.,* 2011). Another explanation could be related with feeding preferences between sex; females could be feeding on more proteinic preys respect to males in order to compensate the energy expenditure related to eggs development. Whale sharks are aplacental viviparous and females produce over 300 eggs (Joung *et al.,* 1996). On the other hand, males could be developing better elimination mechanisms according with growing and this would explain the lower concentrations of elements in larger animals.

Finally, the different use of feeding grounds already observed between juveniles and adult whale sharks could explain –in part or totally- our results; juvenile sharks, in fact, feed in coastal areas that are usually richer in trace elements (Ramírez-Macías *et al.,* 2017). Adults sharks instead have been observed in oceanic, meso and bathypelagic waters (Ramírez-Macías *et al.,* 2017). Even though we still do not know what the shark behaviour is at these depths, feeding activity cannot be discarded.

In general, individuals sampled in both seasons showed a higher variation in the concentration of essential elements such as Cu, Se and Zn. These differences could be related, firstly, by differences in the bioavailability of such elements; and second, by the metabolic use involving essential elements in the organism. Zn for example is rapidly eliminated though gills, liver and kindney and it is been observed that in *Cyprinidae* spp. this elimination take between 2 days to one week (Hogstrand & Wood, 1996).

The fact that As concentrations in the sharks from La Paz presented the same variation respect to essential elements could be a sign and a confirmation that the Bay is enriched of this element and that whale sharks in La Paz are capturing As through diet and breathing following the same metabolic route that essential elements.

3.4.3 TEs in zooplankton

The results of this study confirm that whale sharks mainly feed on patched of copepods and chaetognatha in the Gulf of California. Eventually, eufasiids are also a main group of zooplankton found in the feeding grounds of these sharks. Zn was the element with the highest concentrations found in the zooplankton collected in both study areas, LAP and BLA.

These results are in accordance with previous studies performed in the southeast Gulf of California (Jara-Marini *et al.,* 2009) and in other areas such as Greenland (Ritterhoff & Zauke, 1997) and the North Sea (Zauke *et al.,* 2006). Zn is an essential element involved in metabolic and photosynthetic processes (Hogstrand & Wood, 1996). The decapods *Palaemon elegans*, for example, is able to regulate its body concentration of zinc (to about 90 µg/g) when exposed to a wide range of dissolved zinc bioavailabilities. Zn uptake rate in decapods is balanced by the excretion rate so that the body concentration remains constant (Rainbow, 2007) with little stored in detoxified form. In zooplankton samples collected in Dijibouti, concentration of TEs (mean ±sd) were: 242 ± 155 of Zn in µg/g ww, 231 ± 246 of Pb in µg/g ww and 1.14 ± 1.65 of Hg in µg/g ww (Boldrocchi *et al.,* 2020).

Concentrations of elements showed a peak of Cd in LAP in the second season of sampling (S2); it has been observed that in the area of Baja California, concentrations of Cd on the surface have been associated with upwelling events and algal blooms, having observed levels up to three times more in upwelling events in relation to situations where these events do not occur (Renteria-Cano, 2011). In non-upwelling zone, Cd concentrations are usually low, but they rise starting from 200 m of depth. According with the distribution that Cd presents in the Gulf of California, it is considered a nutrient type; its biogeochemical cycle is controlled by phytoplanktonic organisms and is involved in photosynthesis processes and as a key component of enzymes (Renteria-Cano, 2011). Crustaceans instead cannot regulate Cd concentration in the body (Rainbow, 2007). All cadmium taken up from solution is accumulated without excretion over at least a 28-day period (Rainbow & White, 1989). Finally, the accumulated Cd is necessarily detoxified, typically as metallothionein (Rainbow, 2007).

Chaetognats presented the highest concentrations of trace elements; in particular, higher concentrations of Cd were observed in this zooplankton group. This could be related to environmental factors such us upwelling associated to trade winds. In addition, chaetognatha is a carnivorous group which mainly feed on copepods so Cd biomagnification might be occurring.

3.4.4 Biomagnification

Lead biomagnification through zooplankton and copepods was statistically > 1 in whale shark biopsies from both areas. According with Rubio-Franchini *et al.,* (2016) Pb bioaccumulation was reported in nine zooplanktonic species from the Tula River (Mexico) and, as a result of biomagnification also in the muscle of tilapias (*Oreochromis nilotica*). These and our results stand in contrast to reports that suggest that Pb biomagnification does not occur or is restricted to low trophic levels (Tulasi *et al.,* 1992).

Lead has been detected in practically all environments and it is considered one of the most toxic heavy metals for aquatic organisms (Hodgson & Levi, 1997; Klaassen & Watkins, 2001). It can substitute for calcium and probably Zn in ion dependent phenomena in the synapses, causing damages in different neurotransmission systems (Rubio-Franchini *et al.,* 2008). According with Slaveykova & Wilkinson (2002) Pb uptake is mostly influenced by the concentration of Pb^{2+} in the marine environment.

3.4.5 Molar ratios

Molar ratio Zn:As, Zn:Cd, Cu:Cd, Zn:Pb and Cu:Pb were statistically > 1 ($p < 0.001$) in all sharks from both areas and both seasons.

Moreover, interaction between Zn and Cd was explored and it showed strong p values in animals from both study areas and season. These results indicate that such elements follow a same metabolic route in the whale shark, which is reflected in the epidermis and that Zn might be involved in the elimination of Cd. This is in accordance with studies performed in stomach (Kamunde & MacPhail, 2011) and liver of bony fish (Eroglu *et al.,* 2005; Siscar *et al.,* 2014), elasmobranch (Barrera-García *et al.,* 2013) and mammals (Messaoudi *et al.,* 2009) where interaction between these elements indicate that Zn is involved in Cd detoxification. Cadmium-zinc interaction reviewed in humans and animals showed that Zn intake strongly increases during Cd intoxication, and that Cd toxicity increases during Zn deficiency (Brzóska & Moniuszko-Jakoniuk, 2001). Even if epidermis could not be the tissue where these elements are stored, our results could give a hind of the mechanisms happening in other tissues of the organisms.

Our results show significant correlation between Se and Cd which could indicate that Se is involved in the elimination of Cd as it is for Hg; and also that both elements follow the same metabolic route. A non-toxic compound Se-Cd, resulted as a metabolite of selenium, has been observed in the liver and kidney of marine and terrestrial mammals (Arai *et al.,* 2004). Once again, a comparison between epidermis and liver or kidney is not correct but so far, no studies have been performed on metals molar ratio in epidermis of fish. Despite this, our results might suggest that in the epidermis of this shark species Se and Cd present synergic mechanisms.

Correlation between Cd and Pb was significant in the sharks from BLA from both seasons, which could be related with the fact that these two elements compete for absorption site in the whale sharks as have been found in other marine organisms (Komjarova & Blust 2009). The inhibition of Cd uptake by Pb and Zn found in Zebrafish (Komjarova & Blust 2009) is in agreement with other studies that indicate competitive interactions between these metals for entry into the cell via Ca^{2+} channels (Birceanu *et al.,* 2008; Verbost *et al.,* 1987).

Correlation between Cu and Cd was positive and significant in the sharks sampled from BLA during both seasons. Antagonist interactions between these elements has been observed in stomach and pyloric caeca of rainbow trout (*Oncorhynchus mykiss*) where reduced whole-body Cd concentration were induced by enhancement of Cu accumulation (Kamunde & MacPhail, 2011). The results from these authors suggest that high dietary Cd intake could induced synthesis of metallothionein with subsequent increased sequestration and retention of Cu (Kamunde & MacPhail, 2011). According with Eroglu et al., 2005, Cd levels decrease sharply when concentration of Cu increases in Nile fish (*Oreochromis niloticus*). Pelgrom et al., 1994 showed that exposure to Cu, Cd and mixture of these metals resulted different accumulation levels of Cu and Cd in fish *Oreochromis mossambicus* which could be a result of interaction between these two elements.

Molar ratio Cu:Pb indicates interaction between these elements. Lead intake increases Cu uptake in Zebrafish (*Danio rerio*; Komjarova & Blust, 2009) and in Neon tetra (*Paracheirodon innesi*; Tao *et al.,* 1999).

According with our results, it seems that the explored correlations indicated different behaviours depending on the study area; positive and significant in BLA and not significant in LAP (except in few correlation); this result might confirm that: (i) connectivity between BLA and LAP is low as previously observed (between 16 and 21% according to Ramírez-Macías et al., 2012) and (ii) whale sharks from BLA use different feeding grounds compared to whale sharks from LAP. As already documented, feeding aggregation in BLA include a higher number of larger animals compared to LAP where the animals are usually very juvenile (Ramírez-Macías et al., 2012). After reaching maturity, whale sharks larger than 9 mt often move offshore from the Gulf of California into the eastern Pacific Ocean, possibly performing vertical migrations that reach meso and bathypelagic depths (Ramírez-Macías et al., 2017) linked to upwelling events. The result of a different use of feeding ground and movement between inshore and offshore waters would be eventually reflected in the chemical interaction of the elements observed in this work.

3.5. CONCLUSIONS

The conclusions derivated from this chapter are:

Considering all the elements analyzed (As, Cd, Cu, Pb, Zn), Zn was the most concentrated trace element presents in the zooplankton and whale shark biopsies from both areas BLA and LAP, during the seasons 2016-2017 and 2017-2018, while Cd was the less concentrated. Significant differences between site and season were found in the concentration of trace elements in the zooplankton and the biopsies, which could be related to environmental factors that affect the presence and the bioavailability of the elements.

Sex was not a significant influential factor in the concentration changes of most of the analized elements.

Correlations with the total length of the whale sharks suggest that trace elements concentration decreases in males and increases in females, even the number of females sampled was too low to apply statistical tests. These differences could be due to the different use of feeding habitat that exists between juvenile and adult whale sharks and between sex, and/or it could be related to the different metabolic use made by males and females of some elements as Zn.

Essential elements (Cu and Zn) presented a great variability in the individuals sampled during S1 and S2 which could be related to the metabolic uses involving these elements, and biological availability of such metals.

Lead was biomagnificated by whale sharks (biopsies) from both sites through zooplankton and copepods. The remain elements As, Cd, Cu, and Zn exhibited trophic dilution or bioreduction.

The concentrations of trace elements evaluated in the epidermis of the whale sharks sampled in the lower and center part of the Gulf of California were lower compared with those found in two dead sharks studied from the coast of China and from whale sharks of Dijibouti.

Correlation and molar ratios indicate interaction between selected elements which show differences depending on the study area.

CHAPTER 4

TRACE ELEMENTS IN TISSUES OF WHALE SHARKS STRANDED IN THE GULF OF CALIFORNIA, MEXICO

Chapter partially based on the published paper: Pancaldi F., Páez-Osuna, F., Soto-Jimenez, M.F., González-Armas, R., O'Hara, T., Marmolejo-Rodríguez, A.J., Vázquez-Haikin, A., Galván-Magaña, F. (2019). Trace elements in tissues of whale shark stranded in the Gulf of California, Mexico. Bulletin of Environmental Contamination and Toxicology, 103(4), 515-520.

4.1. ABSTRACT

Concentration of essential (Se, Zn and Cu) and non-essential (As, Cd, Hg and Pb) trace elements were measured in selected tissues of two dead whale sharks (*Rhincodon typus*) stranded in the Gulf of California (GC) in 2017 and 2018. Concentrations of Cd and Pb in the skeletal muscle of the whale shark from La Paz Bay, GC were higher compared to a previous study on whale shark from the coast of China. The shark from La Paz Bay also presented higher concentration of Pb in the epidermis, compared to the same tissue of the other whale shark stranded in Punta Bufeo, GC. The Hg in all analysed tissues was lower than those documented in carnivorous sharks. Molar ratio Se:Hg showed an excess of Se over Hg in all the tissues sampled in both sharks.

4.2. INTRODUCTION

The whale shark (*Rhincodon typus*) is a pelagic filter feeder with a circumglobal distribution found in tropical and temperate waters and in mesopelagic, oceanic and coastal areas (Stevens, 2007). In the Gulf of California (México), whale shark aggregations are found in Bahía de Los Ángeles (Ramírez-Macías *et al.,* 2012), Bahía de la Paz (Ketchum *et al.,* 2013), and Nayarit (Ramírez-Macías *et al.,* 2015). The species is enlisted by the IUCN Red list as endangered since 2016, in the Appendix II of the CMS (1999) of Wild Animals, and the CITES (2002) as endangered species.

In Mexico, *R. typus* is protected by two legal regulations (DOF 2006, 2010), therefore capture and/or trading is forbidden. Natural mortality in this species is rarely investigated but appears to be higher in very juvenile animals and pups due to natural predation (Rowat & Brooks, 2012) by the blue marlin *Makaira nigricans* (Colman, 1997) and the blue shark *Prionace glauca* (Kukuyev, 1995).

Stranding has been observed in very few areas such as the Indian Ocean coast of South Africa (Beckley *et al.,* 1997) and coasts of Australia (Speed *et al.,* 2009). In the Gulf of California, 14 whale shark strandings have been recorded from 2000 to 2018 (Whitehead *et al.,* 2018). Causes of the strandings were not apparent; nevertheless, local topography, marine pollution, fisheries pressure, and disease have been speculated. Due to the high persistency in the environment and their bioaccumulation in the trophic chain, the study of essential and non-essential trace elements in marine biota is a fundamental tool to assess the health of the organisms. Chronic or intermittent exposure to metals and metalloids can produce harmful effects in marine organisms at different levels including physiological, cellular and behavioural (Ralston *et al.,* 2016).

The protective action of Se over Hg has been described in several organisms. This mechanism involves the binding of Hg to Se which give rise to a non-toxic compound (SeHg). Due Se is involved in the correct function of the brain through the action of selenoproteins, a sufficient quantity of free Se in the tissue is fundamental to maintain the correct activity of the organism. The Se:Hg molar ratio was calculated to assess the risks of Hg exposure, ratio > 1 and < 1 reflect a healthy (protective) or non-healthy, respectively, intake of Se regarding Hg (Ralston *et al.,* 2016). Only two studies are available on *R. typus* and toxic trace element concentrations (Wang *et al.,* 2014; McKinney *et al.,* 2016). Also, lack of knowledge about whale shark physiology represents an additional limitation to understand the effect of toxic substances in this species. The primary goal of this research was to examine the concentrations of As, Cd, Cu, Hg, Pb, Se and Zn as well as to calculate de molar ratio Se:Hg in whale shark tissues collected from two stranded animals along the coast of the Gulf of California.

4.3. MATERIAL AND METHODS

On 24 May 2017, a whale shark stranded on the coast of Punta Bufeo (PB) (29°54′28N; 114°26′19W) Baja California, Mexico (Fig. 4.1). The shark was fresh and still in the water at the time of the sighting. It had no signs of recent injuries and cause of death could not be determined. Samples of epidermis, liver, skeletal muscle, and filtering pads were taken from the shark, sent to the Interdisciplinary Center for Marine Sciences (CICIMAR) which were preserved frozen at − 40°C. The stranding location is near two feeding sites for *R. typus*: San Luis Gonzaga Bay, located about 20 km south, and Bahía de Los Ángeles, located about 180 km south.

On 16 February 2018, a second whale shark was found stranded inside the bight of La Paz Bay (LAP) (24.0832°N, 110.1839°W), Baja California Sur, Mexico (Fig. 4.1). The dead shark was immediately transported to CICIMAR, it had no signs of recent injuries and visual assessment of the stomach content revealed no plastic, therefore, by their appearance, the cause of death could not be determined. Samples of epidermis, liver, skeletal muscle, filtering pads, kidney, testicles, stomach, brain, and gill were taken from the shark and preserved frozen at − 40°C. About 0.25 g of each sampled tissue was freeze-dried for 72 h (−49°C and 133×10^{-3} mbar, 48 h), pulverized and homogenized, blanks and reference material were digested in Teflon vials with caps (Savillex) with concentrated HNO_3 (70%, for trace metal analysis, J.T. Baker) and H_2O_2 on hotplates (120°C) for 4 h, and digested samples were gauged with a solution of In115. Water percentages of tissues are shown in Table 4.1.

Trace elements analyses and calculation of molar ratios are described in Chapter 1. Quality control of the analysis included blanks (one each 15 samples) and certified reference materials (DORM-4 fish muscle, n = 6 and DOLT-5 dogfish liver, n = 6). Recovery percentage are shown in Tables 1.3 and Table 1.4 of Chapter 1.

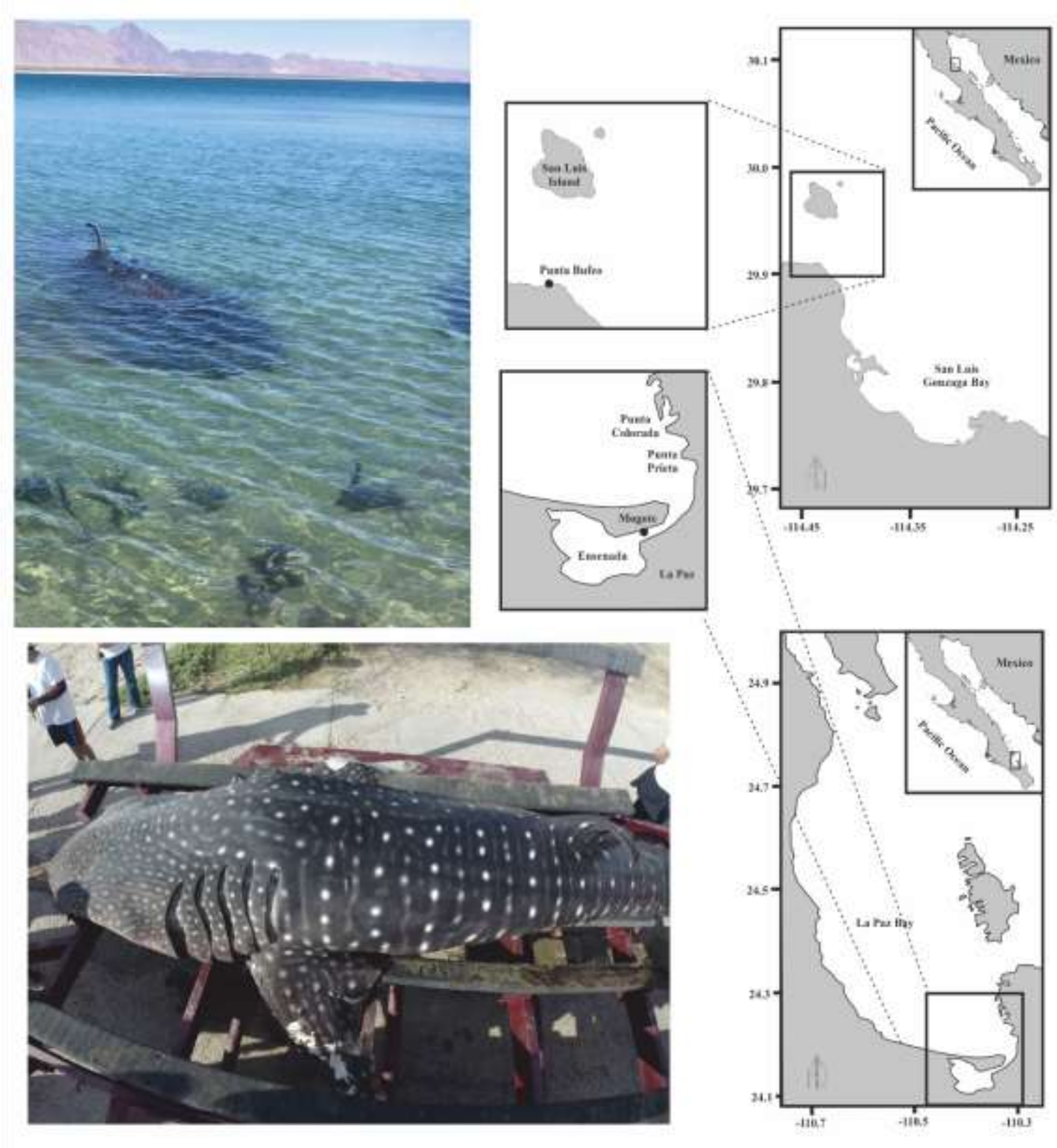

Fig. 4.1. Location of stranding site of two whale sharks at Punta Bufeo (top) and La Paz Bay (bottom), Gulf of California.

4.4. RESULTS AND DISCUSSION

The whale shark from PB was a 9.44 m total length (TL) mature male seasonally spotted in Bahía de Los Angeles. Maturity was confirmed by the calcification and the length of the same organs, longer than the pelvic fins (Wintner, 2000). The whale shark found in LAP was a 5.48 m TL immature male. Concentrations of trace elements in the tissues of the sharks are shown in Table 4.1.

Table 4.1. Concentrations (ng/g ww) and water % of trace elements in tissues of stranded whale sharks sampled in Punta Bufeo (PB) and La Paz Bay (LAP), Baja California Sur, Mexico.

TL (m)	Site	Tissue	Water %	Cu	Se	Zn	As	Cd	Hg	Pb
9.4	PB	Dermal denticles	66	200	100	3800	530	10	31	60
		Skeletal muscle	76	920	20	8500	1050	30	29	20
		Liver	21	1590	400	9500	24200	5130	73	40
		Filtering pads	79	1830	1580	8700	260	1480	16	20
		Epidermis	79	1560	60	200	70	510	26	680
5.5	LAP	Liver	45	3060	400	38100	33700	17500	56	50
		Kidney	93	930	890	9400	210	680	7	10
		Heart	82	2600	1890	13300	300	1870	31	10
		Testicle	89	1230	400	5200	140	580	22	20
		Stomach	89	160	400	1800	100	890	13	10
		Gill	85	1240	450	3500	180	1240	22	50
		Filtering pads	79	730	1160	10800	300	2240	15	40
		Skeletal muscle	72	3030	1130	26200	710	2790	45	13700
		Brain	97	350	180	3100	320	340	6	30
		Epidermis	75	5790	70	10	30	310	86	6770

Table 4.2. Molar ratio of trace elements in tissues of stranded whale sharks sampled in Punta Bufeo (PB) and La Paz Bay (LAP), Baja California Sur, Mexico.

TL (m)	Site	Tissue	Se:Hg	Zn:As	Zn:Cd	Cd:Pb	Cd:As	Se:Cd	Se:As
9.4	PB	Dermal denticles	8.3	8.2	653.4	0.3	0.01	14.24	0.18
		Skeletal muscle	1.4	9.3	487.1	2.8	0.02	0.95	0.02
		Liver	13.8	0.4	3.2	236.4	0.14	0.11	0.02
		Filtering pads	259.3	38.3	10.1	136.4	3.79	1.52	5.77
		Epidermis	5.9	3.3	0.7	1.4	4.86	0.17	0.81
5.5	LAP	Liver	18.4	1.3	3.7	645.1	0.35	0.03	0.01
		Kidney	336.3	51.3	23.8	125.3	2.16	1.86	4.02
		Heart	157.2	50.8	12.2	344.7	4.15	1.44	5.98
		Testicle	44.9	42.6	15.4	53.5	2.76	0.98	2.71
		Stomach	78.6	20.6	3.5	164.0	5.93	0.64	3.80
		Gill	51.9	22.3	4.9	45.7	4.59	0.52	2.37
		Filtering pads	190.9	41.3	8.3	103.2	4.98	0.74	3.67
		Skeletal muscle	64.0	42.3	16.1	0.4	2.62	0.58	1.51
		Brain	77.8	11.1	15.7	20.9	0.71	0.75	0.53
		Epidermis	2.2	0.4	0.1	0.1	6.89	0.32	2.21

In the specimen sampled in PB, can be observed that Zn in comparison to other elements exhibited the higher values in the liver (9500 ng g^{-1}), filtering pads (8700 ng g^{-1}) and skeletal muscle (8500 ng g^{-1}); whereas As was relatively elevated or moderate in dermal denticles (530 ng g^{-1}), skeletal muscle (1050 ng g^{-1}) and liver (24200 ng g^{-1}). Contrarily, Hg and Pb were the lowest levels (16–73 ng g^{-1}) in dermal denticles, red muscle, liver and filtering pads. Selenium showed intermediate and lower levels (16–1580 ng g^{-1}) in the five tissues examined. The molar ratio Se:Hg followed the order: filtering pads > liver > dermal denticles > epidermis > skeletal muscle.

Concentrations of trace elements found in the whale shark from LAP showed a different sequence in the order of their concentrations. Except for the epidermis, Zn was the element with the highest concentrations (up to 3800 ng/g) in the tissues. Copper was found in high concentration in muscle (300 ng/g), liver (3060 ng/g) and epidermis (5790 ng/g). Contrarily, Hg showed the lowest levels (6–86 ng/g) in most of the tissues. Lead was also found in low concentrations in most of the tissues except in epidermis and muscle where it reaches 13700 ng/g. Se:Hg ratio followed the order: kidney > filtering pads > heart > stomach > brain > muscle > gill > testicle > liver > epidermis (Table 4.2). Molar ratio Zn:Cd was 653.4 in dermal denticles and 487.1 in skeletal muscle, on the other hand, Se:Cd was 14.2 for dermal denticles and 0.95 for skeletal muscle (Table 4.2). As observed in previous chapters, Zn could be more efficient in the detoxication of Cd.

Table 4.3 shows the results of this work compared with other published studies on other species of sharks; in order to facilitate the comparison, our concentrations of elements have been converted to µg/g ww. Concentrations from other publications were converted from dw to ww for a better comparison.

Table 4.3 Trace elements concentrations (µg/g ww) in tissues of whale shark and other species of sharks. Ref. 1 (Wang et al., 2014), 2 (Fulgencio de Moura et al., 2015), 3 (Alves et al., 2016), 4 (Branco et al., 2007), 5 (Barrera-García et al., 2012), 6 (Storelli and Marcotrignano, 2004), 7 (Velez Alavez et al., 2013), 8 (Storelli et al., 2003), 9 (Bergés-Tiznado et al., 2015), 10 (Nam et al., 2010), 11 (Vas, 1991), 12 (Escobar-Sánchez et al., 2010), 13 (Storelli et al., 2011), 14 (this work).

Tissue	Species	Sampling site	Cu	Se	Zn	As	Cd	Hg	Pb	Ref.
Liver	*R.typus*	China	22.04±0.08		141.29±0.31		31.75±0.43	0.0033±0.00001	2.27±0.006	1
	M. pelagios	SO Atlantic	2.6	1.61	19.57	13.93	7.46	0.0264	0.07	2
	P. glauca	NE Atlantic	6.81±3.89	0.0	43.99±39.65	39.98±27.76	4.52±3.60	0.28±0.35	1.30±4.35	3
	P. glauca	NE Atlantic		0.47–3.00				0.032–0.96		4
	P. glauca	Pacific	9.28±8.39	1.67±0.58	49.94±27.1	10.62±4.76	34.66±29.61	0.22±0.35	0.37±0.37	5
	P. glauca	Mediterranean				5.95±2.67				6
	I. oxyrinchus	Pacific				0.0001-216.38	0.0001-1.05	0.0001-74.28	0.0001-1.17	7
	S. zygaena	Mediterranean	4.96-7.34	6.54-9.45	24.98-28.65	42.00-46.43	18.43-21.00	32.31-39.48	0.14-0.19	8
	S. zygaena	Gulf of California		7.7±0.5				0.15±0.01		9
	N. brevirostris	Florida		0.25±0.16				0.15±0.08		10
	R. typus	**Gulf of California PB**	**1.59**	**0.397**	**9.52**	**24.2**	**5.13**	**0.073**	**0.040**	**14**
	R.typus	**Gulf of California LAP**	**3.06**	**0.403**	**38.09**	**33.7**	**17.51**	**0.056**	**0.055**	**14**
Muscle	*R.typus*	China	3.58±0.07		11.12±0.04		0.12±0.0008	0.0004±0.00001	0.33±0.006	1
	R.typus	China	1.69±0.07		7.43±0.04		0.11±0.0008	0.0023±0.00001	0.30±0.007	1
	M. pelagios	SO Atlantic	0.23	0.76	18.37	5.57	0.05	0.09958	0.01	2
	P. glauca	NE Atlantic	1.15±0.55	0.29±0.93	24.61±15.51	78.19±21.98	0.01±0.03	1.36±0.83	0.12±0.11	3
	P. glauca	NE Atlantic		0.084–0.30				0.22–1.3		4
	P. glauca	Pacific	1.64±0.13	0.22±0.02	6.1±0.37	6.66±0.55	0.2±0.12	1.03±0.08	<0.07±0.01	5
	P. glauca	Mediterranean				7.20±3.05				6
	P. glauca	N Atlantic					0.45			11
	I. oxyrinchus	Pacific				0.0001-155.70	0.0001-0.55	86.32-429.51	0.0001-1.11	7
	S. zygaena	Mediterranean	1.01–1.82	2.86–3.58	6.76-7.13	15.65–20.21	0.02-0.03	8.55-21.07	0.02–0.04	8

	S. zygaena	Gulf of California		1.2 ± 0.1				0.63±0.04		9
	S. zygaena	Pacific		0.11-1.63				0.005-1.93		12
	N. brevirostris	Florida		0.044±0.026				0.311±0.152		19
	M. mustelus	Mediterranean	0.33-2.23		2.64-5.06		0.01-0.06	1.03-2.58	0.02-0.16	13
	R. typus	**Gulf of California PB**	**0.92**	**0.016**	**8.49**	**1.054**	**0.03**	**0.029**	**0.016**	**14**
	R.typus	**Gulf of California LAP**	**3.03**	**1.131**	**26.24**	**0.707**	**2.79**	**0.045**	**13.749**	**14**
Kidney	*P. glauca*	Pacific	8.17±2.59	1.84±0.89	61.27±12.43	5.14±16.03	6.68±7.28	0.38±0.36	1.39±0.35	5
	I. oxyrinchus	Pacific				0.0001-56.48	0.0001-1.63	0.0001-113.60	0.0001-1.01	7
	S. zygaena	Gulf of California		10.0 ± 0.8				0.23±0.01		9
	N. brevirostris	Florida		1.16±0.81				0.13±0.04		10
	R. typus	**Gulf of California LAP**	**0.93**	**0.89**	**9.41**	**0.207**	**0.68**	**0.007**	**0.012**	**14**
Brain	*S. zygaena*	Gulf of California		1.3±0.1				0.11±0.01		9
	N. brevirostris	Florida		0.151±0.085				0.043±0.023		10
	M. mustelus	Mediterranean	0.90-4.02		5.90-7.35		0.01-0.05	0.04-0.34	0.03-0.59	13
	R. typus	**Gulf of California LAP**	**0.352**	**0.182**	**3.088**	**0.322**	**0.339**	**0.006**	**0.026**	**14**

Concentrations of As found in the liver of the whale shark stranded in PB and LAP were higher compared to the concentrations found in the liver of *Megachasma pelagios* (13.9 µg/g) stranded in the south-eastern coast of Brazil (Fulgencio de Moura *et al.,* 2015) and from *Prionace glauca* collected in Mexico (10.7 ± 4.8 µg/g; Barrera-García *et al.,* 2012) and in England (5.95 ± 2.97 µg/g; Vas, 1991). In the aquatic environment, As is principally absorbed through the gills, gastrointestinal tract and skin (Páez-Osuna *et al.,* 2017). Liver and kidney are the principal storing organs, nevertheless, As also is accumulated in the skin, gills and muscle where inorganic As is transformed to the organic form which is liposoluble and hydrosoluble (Ruelas-Inzunza *et al.,* 2018). Toxic effects of As in fish include neuromotor damages (McGeachy & Dixon, 1992), diminish of red cell production in the bone marrow (Oladimeji *et al.,* 1984), teratogenic effects and lesions to liver and kidney (Kotsanis & Iliopoulou-Georgudaki, 1999).

Concentration of Cd found in the liver of the two whale sharks stranded in the Gulf of California were lower (PB, 5.12 µg/g; LAP, 17.5 µg/g) compared to the whale shark from China (31.7 ± 0.4 µg/g) and to the liver of *P. glauca* (34.7 ± 29.6 µg/g; Barrera-García *et al.,* 2012) and *Sphyrna zygaena* (18.4–21.0 µg/g; Storelli *et al.,* 2003). Particularly, high concentrations of Cd and Pb were found in the skeletal muscle of the dead whale shark from LAP; Cd and Pb concentrations were higher compared to the level found in the whale shark from PB and in the dead whale sharks found in China (Wang *et al.,* 2014). Even if *R. typus* is not consumed in Mexico due to its conservation status, the levels of Cd and Pb in the muscle of the stranded whale shark from LAP could be considered potentially toxic for humans, as they exceed the maximum level permissible (0.5 µg/g ww) from the official Mexican regulation (NOM 242-SSA1, 2009).

Despite the lack of knowledge on Cd and Pb toxicity in *R. typus*, numerous studies have proved that these heavy metals produce toxic effects in fish including the induction of reactive oxygen species, changes in antioxidant enzyme activity and oxidative damages (Barrera-García *et al.,* 2012), changes in reproduction and behaviour (Weber & Dingel, 1997). It is well known that metallothioneins (MTs) binds to essential elements such as Zn and Cu. If Cd is present in the organism it mimics this mechanism by binding to MTs and release Zn and Cu which induce the novo synthesis of MTs (Auclair & Gagné, 2014).

However, specific information on the Cd and Pb toxicity in *R. typus* is not available.

The Gulf of California waters are characterized by high productivity caused by upwelling (Páez-Osuna *et al.,* 2017); these events bring nutrients and trace metals (included Fe and Cd) to the surface waters where phytoplankton growth, so the Cd of the phytoplankton can be transferred causing the enrichment in tissues of *R. typus*.

The highest concentrations of Hg were found in the epidermis of the whale shark from LAP followed by the liver of the whale shark from PB (Table 4.1); these values exceed the levels found in both epidermis (0.00022 ± 0.00001 and 0.0012 ± 0.00001 µg/g) and the liver (0.0033 ± 0.00001 µg/g) sampled in the whale shark specimen of China (Wang *et al.,* 2014). Hg concentrations from the muscle of our sharks also exceeded the levels found in the muscle (0.0004 ± 0.00001 and 0.0023 ± 0.00001 µg/g) of the two dead whale sharks from China. McKinney *et al.,* (2016) found a mean of 0.26 ± 0.08 µg/g Hg on dry weight basis (~ 0.068 µg/g of Hg ww) in the muscle of whale sharks stranded in South Africa. Due to its feeding habits, whale shark is not expected to exhibit Hg and other trace elements at the same concentration than carnivorous sharks such as *P. glauca* from the Atlantic (1.36 ± 0.83 µg/g; Alves *et al.,* 2016) and Pacific (1.03 ± 0.08 µg/g; Barrera-García *et al.,* 2012), *I. oxyrinchus* from the Pacific Ocean (86.32–429.51 µg/g; Vélez-Alavez *et al.,* 2013) and *S. zygaena* from the Mediterranean sea (8.55–21.07 µg/g; Storelli *et al.,* 2003). Nevertheless, the wide home range of *R. typus* and the bioavailability of trace elements could finally affect the uptake mechanism and bioaccumulation in the animals.

Filtering pads from both dead whale sharks from the Gulf of California presented the highest concentration of Se in comparison to other tissues (Table 4.1); the function of these structures is to filter the water and improve feeding activity. However, the molecular composition of these structures is unknown. The molar ratio Se:Hg was > 1.0 in all the tissues sampled from the sharks of this study, which indicate a protective action of Se over Hg toxicity (Ralston *et al.,* 2008, 2016). Similar ratios have been reported in other species of elasmobranchs (Bergés-Tiznado *et al.,* 2015) and teleostean fish (Burger *et al.,* 2013). However, other studies show Se:Hg molar ratio < 1.0 (Correa *et al.,* 2014).

The difference between the muscle Se:Hg molar ratio between the two individuals sampled in PB (1.4) and LAP (64.0) could be associated with dissimilarities in their size and exposure history to such elements. In both whale sharks of this study, the highest concentration of Zn was found in the liver; nevertheless, the whale shark from LAP presented higher concentration of this element (38.1 µg/g ww) compared to the shark from PB (9.52 µg/g). These levels of Zn were lower compared to those found in the liver of the dead whale shark from China (141.3 ± 0.3 µg/g) (Wang *et al.,* 2014). The whale shark from LAP also presented higher Zn concentration in the muscle (26.2 µg/g) and filtering pads (10.8 µg/g) compared to the whale shark from PB (8.48 and 8.73 µg/g, respectively).

Concentrations of Zn in the muscle of the two sharks of this study do not differ from the study of China for the whale shark (Wang *et al.,* 2014) and other species of carnivorous sharks such as *P. glauca* (24.61 ± 15.51 µg/g; Alves *et al.,* 2016) and *M. pelagios* (18.37 µg/g; Fulgencio de Moura *et al.,* 2015). Zinc, like other trace elements, is absorbed through the water and the diet, and its function in fish is related with a healthy growth and development due to the role in several enzymatic components (Hogstrand & Wood, 1996).

Copper in the whale shark from LAP was higher in epidermis, liver, and muscle compared to the whale shark from PB (Table 4.1). Cu concentrations in muscle were in the same range of concentrations to the whale sharks from China (Wang *et al.,* 2014). Contents of Cu in the liver of the Chinese whale shark was higher (22.4 µg/g) compared to our results (Table 4.1). Metal affinity with a specific tissue and the role of each organ in the metabolism system could explain the differences of trace element concentration in different tissues (Barrera-García *et al.,* 2013). Besides the lack of knowledge on the movements of the two dead whale sharks from the Gulf of California is a limitation to determinate the possible exposure sites to trace elements.

4.5. CONCLUSIONS

In conclusion for this chapter, essential and non-essential elements were detected (Table 4.1) in the two dead whale sharks stranded in the Gulf of California. Arsenic concentrations in the liver were found to be higher or in the same range of concentration of other species of carnivorous sharks, typically top predators of the trophic chain. Particular attention is due to the levels of Cd found in the liver and filtering pads of the two specimens stranded in the Gulf of California, which could indicate: (i) high biological availability of Cd in the areas inhabited by the whale shark, and/or (ii) *R. typus* ability to accumulate Cd in specific tissues rather than others. Further research is needed to assess the movements of this species in the Gulf of California and continuous monitoring on metals and metalloids levels in the feeding areas of *R. typus*. Additionally, it is necessary to develop a protocol including proper investigation (necropsy and biochemical analyses) to examine the cause of such stranding.

CHAPTER 5

TRACE ELEMENTS IN THE WHALE SHARK LIVER: INNER ORGANOTROPISM?

5.1. ABSTRACT

Essential (Cu, Se and Zn Cu) and non-essential (As, Cd, Hg and Pb) element concentrations were determined in three areas (proximal, median and distal) of the right (RL) and left (LL) lobe of liver obtained from a stranded whale shark in the Gulf of California, Mexico. Results showed high levels of Zn (mean ± SE in µg/g ww; RL: 22.5 ± 2.1; LL: 26.5 ± 7.1), As (RL: 33.0 ± 1.6; LL: 20.0 ± 9.9) and Cd (RL: 15.5 ± 0.9; LL: 11.3 ± 3.7); and low levels of Cu (RL: 3.2 ± 0.3; LL: 2.2 ± 0.9), Se (RL: 0.5 ± 0.1; LL: 1.2 ± 0.6), Hg (RL: 0.06 ± 0.02; LL: 0.05 ± 0.004) and Pb (RL: 0.05 ± 0.02; LL: 0.05 ± 0.01). Trace element concentrations showed significant ($p<0.05$) differences within the same lobe but not between lobes, which suggests an inner organotropism of Zn and Se in the liver. The proximal area of the left lobe showed the highest concentration of Se that corresponded to the lowest of Cd and As, which may be related to detoxification action of Se. Molar Se:Hg ratios indicate sufficient concentration of Se to detoxicate Hg in all areas of the two lobes.

5.2. INTRODUCTION

The liver is a highly metabolic organ where up taking, storing, as well as excretion of nutrients and other molecules occur (Hinton *et al.,* 2001). Several studies describe this organ as a target for toxic substances (Pethybridge *et al.,* 2010), as bioaccumulation of contaminants often occurs due to the large blood supply, along with its interaction with anthropogenic compounds, such as heavy metals and methylmercury (Bosch *et al.,* 2016) and organochlorines (Bezerra *et al.,* 2019). One of the main functions of the liver is the detoxification through the transformation of poorly excretable chemicals in more excretable ones, as well as the synthetize of metallothionines proteins which bind to different metals such as Cd, As and Zn (Hinton *et al.,* 2001). Therefore, the liver plays a key role in an organism´s health. Top predators with large lipid-rich livers, such as sharks, are particularly susceptible to the uptake and bioaccumulation of contaminants (Barrera-García *et al.,* 2013) which can increase throughout their lifetime. Bioaccumulation occurs differentially between organs and tissues in a process known as organotropism (Correa *et al.,* 2014), where specific organs are the main storage and detoxification sites of trace elements (Régine *et al.,* 2006; Mieiro *et al.,* 2011). The interaction between different elements is an additional tool for body detoxification; selenium, for example, is an essential element that maintains cellular oxidative homeostasis and mitigate the toxicity of heavy metals such as Hg (Endo *et al.,* 2005) and Cd (Jamwal *et al.,* 2018) through the action of selenoproteins (Belzile *et al.,* 2006; Palmisano *et al.,* 1995).

By being the largest fish of the planet, the whale shark *Rhincodon typus* (Smith, 1828) is a circumglobal species (Norman *et al.,* 2019) that is found in several areas of the Gulf of California, such as San Luis Gonzaga (Eckert & Stewart, 2001), Bahía de los Ángeles (Ramírez-Macías *et al.,* 2012) and La Paz Bay (Ketchum *et al.,* 2013; Whitehead *et al.,* 2019). It is a long-lived animal with a life span of more than 100 years; additionally, it is an opportunistic filter feeding shark (Colman, 1997).

Feeding aggregations in the Gulf of California show that whale shark prey mostly on zooplankton patches that are rich in copepods (Nelson & Eckert, 2007), euphasiids (Ketchum *et al.,* 2013) and chaetognats (Pancaldi *et al.,* 2019b). Studies on trace elements bioaccumulation in this species are scarce and show that whale sharks accumulate trace elements such as As, Hg, Cd, Cu, Pb, Se and Zn in the liver (Pancaldi *et al.,* 2019a; Wang *et al.,* 2014). This study complements a previous report on the element's concentrations in the tissues of this animal (Pancaldi *et al.,* 2019a). The main objectives of this study were to examine the concentrations of essential (Cu, Se, Zn) and non-essential elements (As, Cd, Hg and Pb) as well as to determinate the potential antagonism between Se, Zn, Hg, Cd and As throughout the molar ratio in three areas (proximal, median and distal) of the right and left lobes of the liver of a stranded whale shark from La Paz, Mexico.

5.3. MATERIAL AND METHODS

On 16 February 2018 a whale shark was found stranded inside the lagoon of La Paz Bay (24.0832°N, 110.1839°W; Fig.5.1), Baja California Sur, Mexico (Whitehead *et al.,* 2019). The dead shark was immediately transported to CICIMAR where liver was extracted. Samples from the proximal, median and distal part of both lobes were taken and preserved frozen at − 20°C. About 0.25 g of each sampled tissue was freeze-dried for 72 h (− 49°C and 133 × 10−3 mbar, 48 h), pulverized and homogenized. Blanks and reference material were digested in Teflon vials with caps (Savillex) using concentrated HNO_3 (70%, for trace metal analysis, J.T. Baker) and H_2O_2 on hotplates (120°C) for 4 h, and digested samples were gauged with a solution of In115. Water percentages of tissues are shown in Table 5.1.

Quality control of the analysis included blanks and certified reference materials (DOLT-5 dogfish liver, n = 6). Blanks were performed with no anomalies detected. Recoveries percentages are shown in Table 1.2, Chapter 1. Trace elements concentrations are expressed in µg/g per wet weight (ww).

5.4. RESULTS

The stranded whale shark was a 548 cm total length (TL) immature male (Whitehead *et al.,* 2019). Sex was confirmed by the presence of claspers and sexual maturity by the length of the same organs that were shorter than the pelvic fins (Wintner, 2000). Right lobe of the liver (Fig. 5.1) was 152 cm TL and 38 cm in its wider area. Gallbladder (Fig. 5.1) was attached to the right lobe (near proximal area).

Left lobe of the liver was 151 cm TL and 39 cm in its wider area. In the right lobe, water percentage was 32.8% (proximal area), 15.2% (median area) and 42.3% (distal area; Table 5.1). In the left lobe water percentage was 21.4% in the proximal area, 19.3% in the median area and 44.8% in the distal area (Table 5.1).

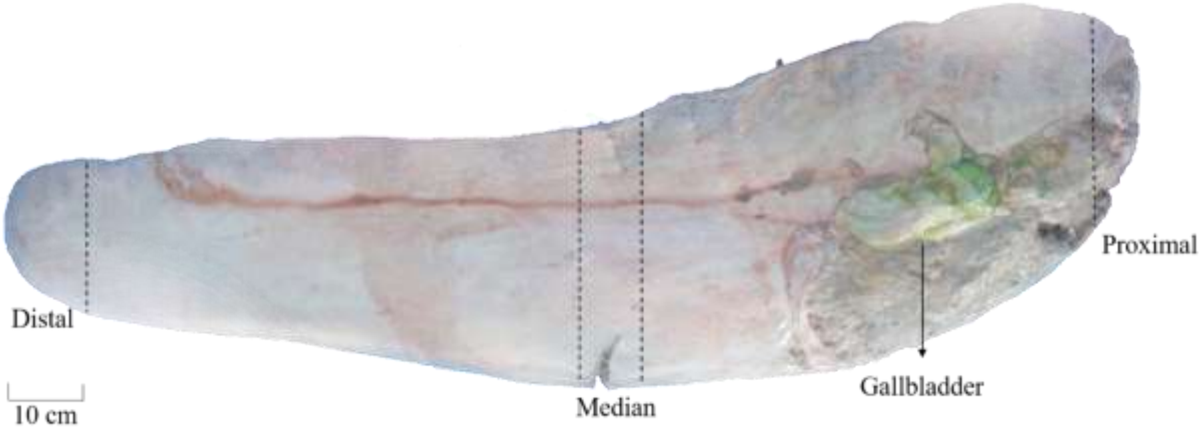

Fig. 5.1. *Rhincodon typus* liver right lobe with the proximal, median and distal area and the gallbladder.

In general, As, Zn and Cd showed the highest concentrations within both lobes, while Cu, Hg and Pb were the less concentred elements (Table 5.1). In the right lobe the mean±SE in µg/g (ww) of As, Zn and Cd was 33.0±1.6, 22.5±2.1 and 15.5±0.9, respectively. Mean±SE was 3.2±0.3 for Cu, 0.5±0.1 for Se, 0.06±0.02 for Hg and 0.05±0.02 for Pb (Table 5.1). In the left lobe the mean concentration of As and Cd was lower compared to the right lobe (20.0±9.9 and 11.3±3.7 respectively) while Zn was higher 26.5±7.1 (Table 5.1). Mean±SE was 2.2±0.8 for Cu, 1.2±0.6 for Se, 0.05±0.004 for Hg and 0.05±0.01 for Pb (Table 5.1). Despite these differences, concentrations of all trace elements were not statistically different between lobes ($p>0.05$).

Table 5.1. Trace elements concentrations, mean and standard error (SE) and molar ratio Se:Hg, Se:Cd, Se:As, Zn:Cd, Zn:As in the right and left lobe of the whale shark liver. Concentrations are expressed in µg/g (ww).

Lobe	Sample	Water %	Cu	Se	Zn	As	Cd	Hg	Pb	Se:Hg	Se:Cd	Se:As	Zn:Cd	Zn:As
Right	Proximal	32.8	3.1	0.6	26.3	30.0	16.5	0.05	0.08	30.0	0.05	0.02	2.7	1.0
	Median	15.2	3.8	0.5	19.1	33.5	13.7	0.10	0.04	13.8	0.06	0.02	2.4	0.7
	Distal	42.3	2.7	0.4	22.1	35.3	16.4	0.04	0.02	20.8	0.03	0.01	2.3	0.7
	mean		3.2	0.5	22.5	33.0	15.5	0.06	0.05	21.4*	0.05**	0.01**	2.5*	0.8
	SE		0.3	0.1	2.1	1.6	0.9	0.02	0.02	4.6	0.01	0.003	0.1	0.1
Left	Proximal	21.4	0.7	2.3	13.5	0.9	4.6	0.05	0.03	108.4	0.70	2.50	5.0	17.6
	Median	19.3	2.8	0.8	27.7	25.8	11.8	0.04	0.05	44.6	0.09	0.03	4.0	1.2
	Distal	44.8	3.1	0.4	38.1	33.7	17.5	0.06	0.06	17.6	0.03	0.01	3.7	1.3
	mean		2.2	1.2	26.5	20.0	11.3	0.05	0.05	57.0	0.30	0.84	4.3*	6.7
	SE		0.8	0.6	7.1	9.9	3.7	0.004	0.01	26.9	0.20	0.82	0.4	5.4

Asterisks indicate molar ratio that are significantly different (*$p < 0.05$; ** $p < 0.001$) than 1. Statistical were performed by a *t*-test.

5.4.1 TEs concentrations in the right lobe (RL)

In the RL, As showed the highest concentrations (35.3 µg/g in the distal, 33.5 µg/g in the median and 30.0 µg/g in the proximal area) followed by Zn (26.3 µg/g in the proximal, 22.1 µg/g in the distal and 19.1 µg/g in the median area). Cd concentrations were 16.5 µg/g in the proximal area, 16.4 µg/g in the distal area and 13.7 µg/g in the median area. Cu, Se, Hg and Pb were found in lower concentrations ranging from 3.8 µg/g of Cu in the median area to 0.02 µg/g of Pb in the distal area (Table 5.1). In this lobe, significant statistical differences were found between As with Cu, Se, Hg ($p<0.05$) and Pb ($p<0.001$), Zn with Se, Hg ($p<0.05$) and Pb ($p<0.001$), Cd with Pb and Hg ($p<0.05$).

5.4.2 TEs concentrations in the left lobe (LL)

In the LL, Zn showed the highest concentrations (38.1 µg/g in the distal and 27.7 µg/g in the median), which were also higher compared to the observed levels of the same element in the right lobe. Arsenic concentrations were 33.7 µg/g in the distal and 25.8 µg/g in the median area. Cd concentrations were 17.5 µg/g in the distal and 11.8 µg/g in the median area. Unexpectedly, these three elements (Zn, As, and Cd) were found in lower concentrations in the proximal area of the left lobe (13.5 µg/g for Zn, 0.9 µg/g for As and 4.6 µg/g for Cd) compared to the proximal area of the right one.

The Hg was found in the same concentrations in the proximal area of the right (0.05 µg/g) and left lobe (0.05 µg/g). In this lobe, significant statistical differences were found between As with Pb and Hg ($p<0.05$), Zn with Se, Hg and Pb ($p <0.05$), Cd with Pb and Hg ($p<0.05$).

No statistical differences (Mann-Whitney test $p > 0.05$) of the same elements were found between lobes.

5.4.3 Molar ratios

In the right lobe, Se:Hg molar ratio was 30.0:1.0 in the proximal area, 20.8:1.0 in the distal area, and 13.8:1.0 in the median area (Table 5.1). In the left lobe, Se showed the highest level in the proximal area (2.3 µg/g) and molar ratio Se:Hg was 108.4:1.0 (Table 5.1). In the median and distal area, Se:Hg molar ratio was 17.6:1.0 and 44.6:1.0, respectively.

Mean Se:Cd molar ratio was 0.05 in the right lobe and 0.30 in the left lobe instead, molar ratio Zn:Cd was > 1 in all areas of both lobes (RL: 2.7, 2.4 and 2.3; LL: 5.0, 4.0 and 3.7 in the proximal, median and distal area respectively).

In the right lobe, molar ratio Se:As was 0.02 in the proximal and median and 0.01 in the distal area (Table 5.1). In the left lobe, molar ratio Se:As was 2.50 in the proximal, 0.03 in the median and 0.01 in the distal area. Statistical test applied to the mean of the molar ratio Se:Cd and Se:As were significantly < 1 ($p < 0.001$), while Zn:Cd in both lobes showed a p value < 0.01 (Table 5.1).

5.4.4 Antagonist effects between TEs

Correlation between elements pairs is showed in Fig. 5.2 Spearman correlation applied to trace elements shows strong relations ($r^2>0.8$) (Fig.5.2 b,d,e). The antagonism between Se and As in whale shark liver (right and left lobe) is shown in Figure 5.2(e) where Spearman correlation shows a negative and significative ($r^2=0.99$; $p=0.002$; $n=6$) relation between these elements. A strong ($r^2=0.90$) negative and not significative ($p=0.13$; $n=6$) Spearman correlation is also found between Se and Cd (Fig. 5.2 d) however, p value could be due to the low sample size. The highest concentration of Se (2.3 µg/g; Table 5.1) found in the whale shark liver, corresponds to the lowest concentrations of Cd (4.6 µg/g) and As (0.9 µg/g) which could indicate antagonism.

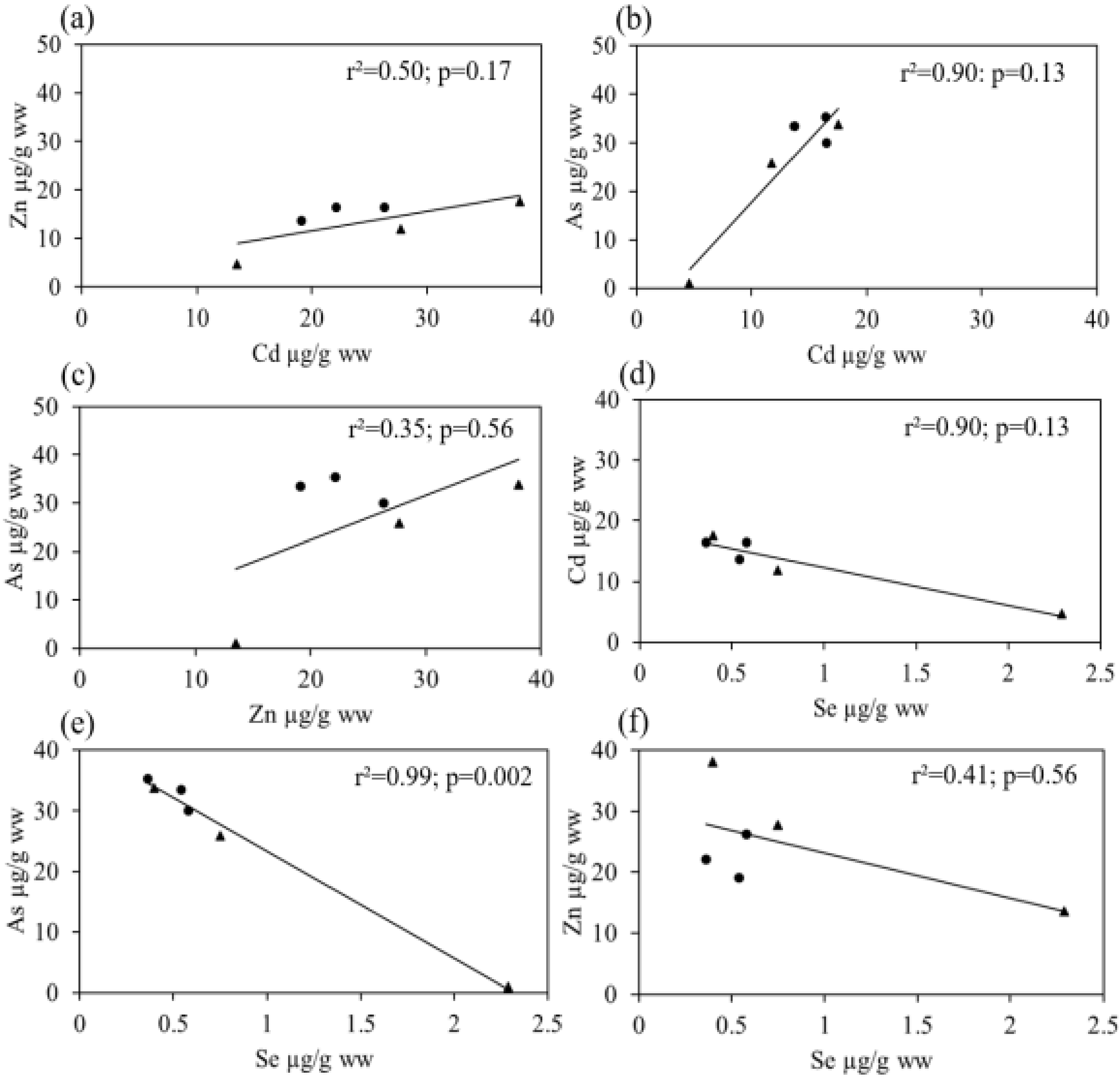

Fig. 5.2. Variation of concentrations Zn vs Cd (a), As vs Cd (b), As vs Zn (c), Cd vs Se (d), As vs Se (e) and Zn vs Se (f) in the right (●) and left (▲) lobe of whale shark liver.

5.5. DISCUSSION

Our results indicate enriched levels of As and Cd in whale shark liver; according to Pérez-Tribouillier *et al.,* (2015), the surface sediments around the Mogote area, where feeding aggregations have been observed, are naturally enriched in As and Cd as these elements are bound within phosphorites rocks (Piper, 1991). Results from Romero-Bañuelos (2003) showed that Cd concentrations in the surface water of La Paz Lagoon are higher compared with the mean concentrations of the earth crust reported by Taylor (1964; 0.2 µg/g^{-1}) because of the strong presence of phosphorite rock. Phosphorite mining activity in San Juan de la Costa during the past 80 years (Castañón & Bañuelos, 2016; 2019) could have affected the levels of As and Cd in whale shark feeding grounds leading to bioaccumulation in the liver.

Marcovecchio (1991) explains the high concentrations of Cd and Zn in the liver of three species of sharks through the action of metallothionein which are concentrated in hepatic tissues and retain these elements.

Significant differences found between the concentrations of TEs in the different parts of liver suggest organotropism of As, Zn and Cd in whale shark liver. The proximal area of the right lobe of whale shark liver is located near the gallbladder which contains the bile, excrete substance of the organisms. The proximity between the proximal area and the gallbladder in the right lobe could be a factor that increases the concentrations of As and Cd.

The levels of Hg in both lobes were higher (0.10 µg/g in the median, 0.05 µg/g in the proximal, and 0.04 µg/g in the distal area of the right lobe and 0.06 µg/g in the distal, 0.05 µg/g in the proximal and 0.04 µg/g in the median area of the left lobe) compared to the level found in the liver of a dead whale shark found in China (0.0033±0.00001 µg/g; Wang *et al.,* 2014); nevertheless, Hg levels in the whale shark from La Paz were lower compared to the levels found in the liver of the blue shark *Prionace glauca* from the Mexican Pacific (0.22±0.35 µg/g; Barrera-García *et al.,* 2013). The uptake of Hg principally occurs thought the diet therefore Hg concentrations are expected to be higher in top predator sharks rather than filter feeder's species (Storelli *et al.,* 2003).

The antagonist effect of Se with some heavy metals such as Hg and Cd is been observed in several organisms including elasmobranch (Escobar-Sánchez *et al.,* 2010; Siscar *et al.,* 2014). Even though mechanism of these interactions is not well understood, molar ratio between the elements is proposed to be 1:1 or close in order for Se to be efficient against heavy metal toxicity (Ralston *et al.,* 2016; Raymond & Ralston, 2004).

In the left lobe, Se showed the highest level in the proximal area (2.3 µg/g; Table 5.1); consequently, molar ratio Se:Hg in this area presented the highest value (108.4:1; Table 5.1) which indicate a more evident detoxification process in this area. The other areas of the left and right lobe indicated molar ratio Se:Hg >1 which indicate sufficient concentrations of Se to detoxify the action of Hg.

Molar ratio Se:Cd was < 1 (Table 5.1) in all sampled area of the liver which could indicate that Se concentrations are not high enough to protect the organ from the damage of Cd. Cadmium is a non-essential metal and it is known to affect the uptake of calcium through fish gills (Niyogi & Wood, 2004). In addition, the exposure to this element may leads to accumulation of reactive oxygen species and oxidative damage (Bertin & Averbeck, 2006; Waisberg *et al.,* 2003).

Our results show that molar ratio Zn:Cd was >1 (Table 5.1), which could indicate that in whale shark live Zn might exert a more efficient detoxification action against Cd respect to Se. This same result has been found in the liver of some deep fish species (Siscar *et al.,* 2014) and mammals (Jihen *et al.,* 2009) where Zn is more efficient than Se in protecting the liver from Cd damages.

Molar ratio Se:As was < 1 in all sampled areas of the liver except in the proximal area of the left lobe while molar ratio Zn:As was close or greater than 1 (Table 5.1). According with these results, Zn could also act more efficiently against As respect to Se. An antagonistic effect of Zn over As and a synergetic effect of As over Zn has been observed in the cladoceran *Daphnia obtusa* (Gaete & Chavez, 2008); nevertheless, information on this interaction in elasmobranch is absent.

5.6. CONCLUSIONS

In conclusion, trace elements were not uniformly distributed in whale shark liver: Cd, As and Zn were found in high concentrations in both right and left lobe probably due to natural enrichment of the area, while Hg and Pb were found in lower concentrations. The proximal area of the left lobe presented the lowest concentrations of Zn, As and Cd and the highest concentrations of Se which could be due to the distance from the gallbladder (which is connected to the right lobe) or to a detoxification action from Se throughout the action of selenoproteins. Molar ratio Se:Hg indicates sufficient levels of Se to detoxify Hg in all sampled areas of the lobes. On the other hand, molar ratio Se:Cd was <1 and molar ratio Zn:Cd was >1 which could indicate that Zn acts more efficiently in Cd detoxification in this species.

CHAPTER 6

GENERAL REMARKS

The results of trace element concentrations analysed from whale shark biopsies and in two stranded individuals are shown in Chapter 2,3,4 and 5. To estimate quantitatively the biomagnification, the TEs concentrations were determined in zooplankton.

The main remarks are:

a) Whale sharks, *Rhincodon typus*, from the Gulf of California accumulate TEs, where Zn is the most concentrated element that could be related with the metabolic role of this metal in the organism.
b) Some elements such as As, Cu, and Zn are significantly higher in the whale sharks from LAP and this might be due to the occurrence of naturally phosphorite enriched rocks located in San Juan de la Costa.
c) Differences found in TEs concentrations between sharks sampled in BLA and LAP confirm low connectivity between these two feeding grounds; sharks from one area do not necessarily move to the other one, and as results were observed different concentrations of elements in sharks.
d) Sex is not a significant influential factor to determine changes in TEs concentrations.
e) Concentration of TEs related with body size of the sharks was different between sexes; males show negative correlation between TEs concentrations and size, while females show positive correlation. These differences might confirm a different use of feeding grounds and different prey preferences between males and females. Also, males could develop better elimination mechanisms according with growing.
f) Copepods and chaetognatha identified as the main preys of zooplankton in both study areas. According with our results Hg and Se varied greatly in the zooplankton. Zinc was at the highest concentration compared to other TEs, and this was reflected in the sharks. Cd concentration in the zooplankton vary significantly different between season according with upwelling events and environmental conditions.

g) Hg and Pb were biomagnified through zooplankton, on the contrary, Se and other elements (As, Cd, Cu, Pb, Zn) were not biomagnified by the sharks. Hg biomagnification showed differences between sharks below 4 m (juveniles) and shark above 4 m (adults). This could indicate a shift in the shark diet or metabolic changes once the animal reaches this size.

h) Molar ratio Se:Hg, Zn:As, Zn:Cd, Cd:Pb, Cd:As, Se:Cd and Se:As were calculated in the epidermis from alive sharks and different tissues of two stranded sharks. Se:Hg in the epidermis and other tissues of whale shark indicate sufficient quantity of Se in order to detoxify Hg. In addition, molar ratio Zn:Cd in the epidermis and the liver showed a strong correlation which indicate that both metals follow a same metabolic route and that Zn might be more efficient in the elimination of Cd respect to Se in these tissues. Other tissues such as dermal denticles, heart, kidney, gills, filtering parched and skeletal muscle also exhibited molar ratio Zn:Cd greater than 1.0 which could also indicate a more efficient role of Zn over Cd respect to Se. A synergic mechanism between Cd and Pb could be involved in epidermis, filtering pads, kidney, heart, testicle, stomach, gills and brain.

i) Continuous monitoring is necessary to determinate the levels of metals and metalloids in the animals of the GC. Further studies are necessary to establish the physiology of *R. typus*, to determine the uptake mechanism, distribution, and elimination of TEs and establish if a true toxic risk exists for this species. Additionally, it is necessary to develop a protocol including proper investigation (necropsy and biochemical analyses) to examine the cause of stranding.

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