INSTITUTO POLITECNICO NACIONAL

CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

PLASTIC POLLUTION AS A POTENTIAL THREAT FOR OCEANIC MANTA RAYS IN THE MEXICAN PACIFIC OCEAN

TESIS

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PRESENTA

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ACTA DE REVISIÓN DE TESIS

En la Ciudad de La Paz, B.C.S., siendo las 12:00 horas del día 12 del mes de Noviembre del 2019 se reunieron los miembros de la Comisión Revisora de Tesis designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de CICIMAR para examinar la tesis titulada:

"PLASTIC POLLUTION AS A POTENTIAL THREAT FOR OCEANIC MANTA RAYS IN THE MEXICAN PACIFIC OCEAN"

Presentada por el alumno:

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Aspirante de:

DOCTORADO EN CIENCIAS MARINAS

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA DEFENSA DE LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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Dedication

“There is a time when one must decide either to risk everything to fulfill one’s dreams or sit for the rest of one’s life in the backyard.”

Robert M. Manry

A Robbè,
sei stato il vento che ha spinto e guidato questa piccola deriva attraverso mari ed oceani.

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Abstract

The oceanic manta ray, *Mobula birostris*, filters large volumes of water while feeding on zooplankton. Thus, they are potentially exposed to the growing threat of plastic pollution. Ingested plastics can leach adsorbed toxic pollutants and plastic additives (e.g. phthalates, used as indicators of plastic contamination in animal tissues) that are recognized as endocrine disruptors and are toxic for many species of marine animals. The oceanic manta ray populations in the Gulf of California have been drastically reduced in recent decades, making the Revillagigedo Archipelago (AR) and Banderas Bay (BB) their last refuges and aggregation areas in the Mexican Pacific Ocean. Samples (n=94 in BB, n=47 in AR) were collected with a manta net (333µm mesh): floating plastics were found in both areas and we determined the abundance, size and polymer composition of the plastic debris through Fourier transform infrared spectroscopy (µFT-IR). Tissue samples (skin and muscle biopsies, n=38) of manta rays were collected while scuba diving and freediving using a spear pole with a modified stainless steel tip and underwent chemical extraction and subsequent analysis to measure the concentration of phthalates, organochlorine compounds (OCs) and polycyclic aromatic hydrocarbons (PAHs). The same compounds were also analyzed in plastics found in both areas (n=15) to quantify the pollutants adsorbed on the surface of plastic debris. A clear seasonality was found in the abundance of floating plastics in BB, probably driven by the offload caused by the rains during the hurricane season. In AR, the abundance of floating plastics is homogeneous throughout the archipelago and the lack of information available from the hurricane season makes it difficult to determine a seasonal pattern. In both areas, 3 of every 4 plastics were smaller than 5mm in length, making them bioavailable to be ingested by lifeforms from the base of the food web. PAHs, PCBs and pesticides were found adsorbed on the plastic samples. PAHs and PCBs were also detected in biopsies from both areas, while pesticides and phthalates were not found in any manta ray. The absence of phtalates in the biopsies suggests that the ingestion of plastics in manta rays does not occur or occurs seldom, and the plasticizers from the plastics are not detectable in skin and muscle. This research is a baseline study for plastic debris contamination in both areas and for possible ingestion of plastics by oceanic manta rays.

Resumen

Las mantas gigantes oceánicas, *Mobula birostris*, filtran grandes volúmenes de agua al alimentarse de zooplancton. Por esta razón, están potencialmente expuestas a la basura marina. Los plásticos ingeridos pueden liberar compuestos tóxicos adsorbidos y aditivos de la producción del plástico (e.g. ftalatos, que son usados como indicadores de ingestión de plástico en los tejidos animales), los cuales son reconocidos disruptores endocrinos y tóxicos para muchas especies marinas. La población de mantas gigantes del Golfo de California se ha reducido drasticamente en las últimas decadas, haciendo del Archipiélago de Revillagigedo (AR) y Bahía de Banderas (BB) sus últimos refugios y áreas de agregación en el Pacífico Mexicano. Se colectaron muestras de plancton (n=94 in BB, n=47 in AR) con una red manta (malla de 333µm): se encontraron plásticos flotantes en ambas areas y se determinaron: abundancia, estacionalidad, clases de talla y composición a través de espectometría de infrarrojo (µFT-IR). Muestras de piel y músculo (n=38) de mantas gigantes fueron colectadas durante buceo libre y scuba usando una vara hawaiiana con una punta especial, se realizó una extracción y análisis químico para determinar la concentración de ftalatos, compuestos organoclorados (OCs) e hidrocarburos aromaticos policiclicos (PAHs). Los mismos compuestos fueron analizados también en los plásticos (n=15) encontrados en ambas áreas, para determinar la concentración de contaminantes adsorbidos en la superficie de los desechos plásticos. Se encontró una marcada estacionalidad en la abundancia de plasticos flotantes en BB, probablemente derivado de la descarga causada por las lluvias en la estación de huracanes. En el AR la abundancia de plásticos fue homogénea en las cuatro islas. La falta de información en la temporada de huracanes hace difícl la determinación de un patrón de estacionalidad. En ambas áreas, 3 de cada 4 plásticos fueron menores a 5mm de largo, lo que sugiere que son biodisponibles para ser ingeridos desde los eslabones mas bajos de la red trófica. Se encontraron PAHs, PCBs y DDTs adsorbidos en los plásticos. En las muestras de manta gigante se encontraron PAHs y PCBs, mientras que no se detectaron pesticidas ni ftalatos, lo cual sugiere que la ingestión de plástico en las mantas no está pasando, o que es un evento raro y no llegan a detectarse plastificantes en músculo y piel. Estos resultados constituyen una linea base sobre la contaminación

de plásticos en las dos áreas, y son el primer estudio de contaminantes orgánicos en mantas gigantes a nivel mundial.

1 General introduction

1.1 Plastics

1.1.1 Plastic production and use

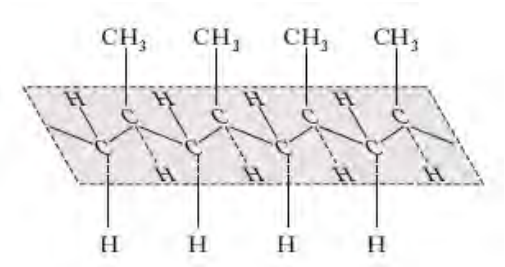


Figure 1: Chemical structure of polypropylene (www.treccani.it/enciclopedia/polipropilene/)

Plastics are a family of synthetic materials that are obtained from organic raw materials such as cellulose, oil, natural gas, etc. They are constituted by long chains of monomers with a carbon skeleton (Figure 1), and can be shaped through heat and pressure (Thompson *et al.*, 2009).

Plastics are incredibly versatile materials They are cheap, lightweight, hydrophobic, strong, durable, corrosion- resistant and bio-inert, with high thermal and electrical insulation properties that allow them to be used in many fields. The rising demand of plastic items has dramatically boosted annual plastic production from 1.5 million tonnes in the 1950s to more than 300 million tons in 2015 (PlasticsEurope, 2016). A wide variety of polymers are currently on the market, the most produced and used being: polypropylene (PP), polyethylene (PE), polyvinylchloride (PVC), polyurethane (PU), polystyrene (PS) and polyamides (PA) (PlasticsEurope, 2016).

These polymers are usually mixed with organic compounds (phthalates, bisphenol A, etc.) that are used as additives during manufacture to improve the flexibility, durability and to lower the processing temperature of plastics (Teuten *et al.*, 2009). Thanks to the additives, and the properties that they give to the resins, plastics are able to substitute many of the natural materials that were used in the past, with a much lower production cost and longer durability.

Plastics are extremely versatile materials and that facilitates their use for the production of a vast array of plastic products (Thompson *et al.*, 2009). Currently, more than a third of plastic is used for packaging and single-use products (Figure 2), raising a growing environmental concern for their disposal after use. These types of materials have a very short life, ending up in landfills or in the environment in less than one year from manufacture (PlasticsEurope, 2016). The major source of plastics that find their way into the environment is the result of inappropriate waste management and irresponsible human behavior (Barnes *et al.*, 2009).

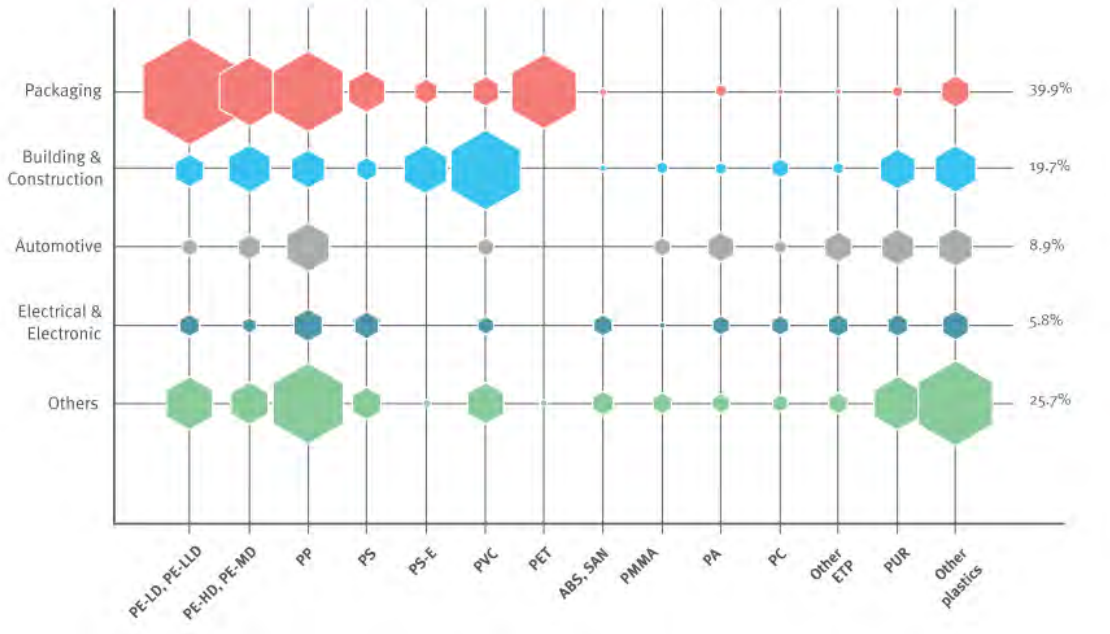


Figure 2: Plastics demand by polymer and market segment (PlasticsEurope, 2016).

1.1.2 Plastic Debris

In 1960, it was estimated that only 1% of urban solid waste consisted of plastics (Envirionmental Protection Agency, 2010). Currently, it has been estimated that 10% of municipal waste is made up of plastics (Barnes *et al.*, 2009).

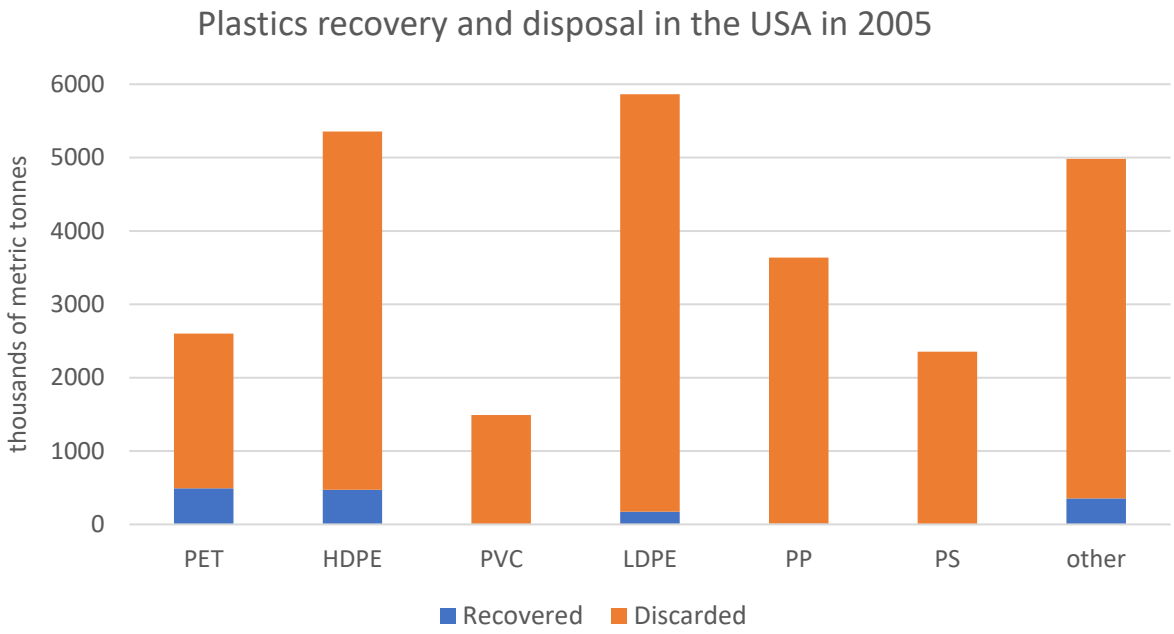


Figure 3: Municipal waste plastics recovery and disposal in the USA in 2005 (modified from Barnes et al., 2009)

Of these, only a small fraction is recycled (Figure 3), while most of it goes to landfill where it accumulates and can undergo the process of photo-oxidation that leads to its fragmentation into smaller pieces and eventual dispersal into the environment. Since half of the world population lives less than 50 miles away from the coastline, lighter debris is the most likely to be transported by winds and rains, ending up in the sea (Moore, 2008). Packaging products are almost always discarded with their functional characteristics virtually intact, permitting both easy re-use and recycling. However, only about 9.4% of plastics are presently recycled in the US, mainly due to a number of problems: high collection costs, lack of infrastructure and low demand by the industry for recycled plastic granulate (Lebreton & Andrady, 2019). Packaging-related plastics have a particularly short in-use phase and therefore dominate municipal plastic waste and subsequently the mismanaged waste as well.

In Mexico, the organization and planning of the public waste collection service is very rudimentary. Therefore, data on urban solid waste composition, quantity and recycling is scarce (Buenrostro & Bocco, 2003). Research has shown a general similarity in the composition of solid waste between developing countries and developed ones. In Mexico, it has been estimated that plastics represent 7-12% of the urban solid waste (Buenrosto *et al.*, 2001; Castrejón-Godínez *et al.*, 2015)

1.1.3 Plastics in the marine environment

In 1972, the presence of polystyrene spherules in surface waters in the Sargassum Sea was reported for the first time. Since then, the plastics problem has become a significant concern for the scientific community worldwide (Carpenter & Smith, 1972). It was estimated that between 4.8 and 12.7 million metric tons of plastic debris enters the oceans every year (Figure 4) (Jambeck *et al.*, 2015).

Different estimates exist about the amount of debris entering the oceans. Some authors suggest that the majority of marine debris is dumped directly through maritime activities such as commercial fishing, and transport shipping, *etc*. (Gregory, 2009). Other studies have estimated that up to 80% of the plastics that reach the oceans have a terrestrial origin (mismanaged trash, beach tourism, river pollution, natural disasters *etc.*) (Jambeck *et al.*, 2015). This discordance is due to the difficulties in estimating the amount of plastics that is lost during each human activity. Furthermore, there is a significant quantity of plastics that is lost during natural disasters (tsunamis, hurricanes, monsoons etc.) and that is impossible to estimate.

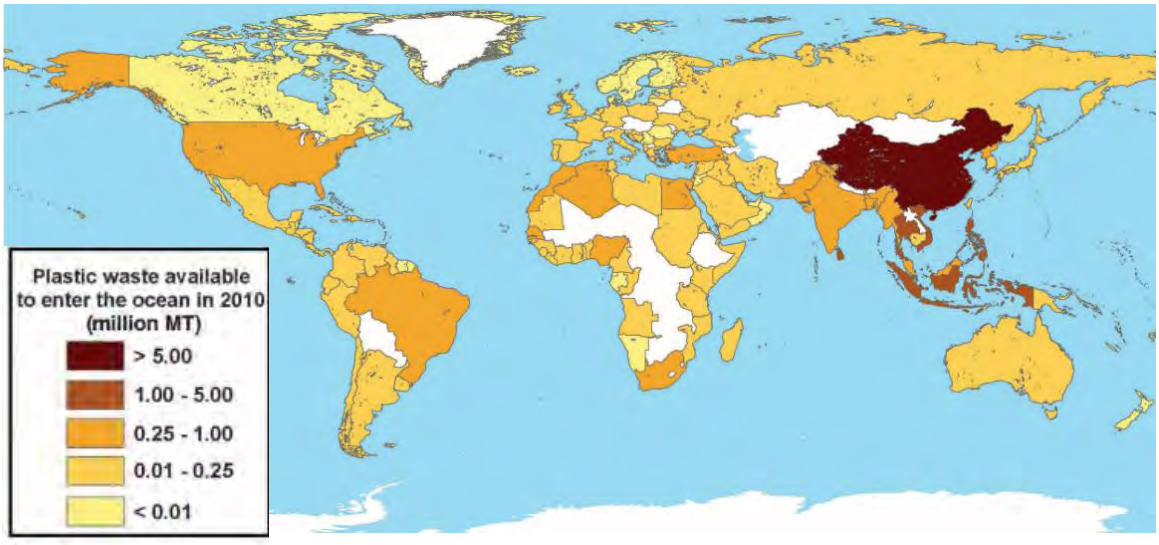


Figure 4: Plastic debris estimated to enter in the ocean for each country in 2010, in million tonnes. Only the countries that have access to sea are being considered (Jambeck et al., 2015).

Plastic debris found in the ocean includes food packaging, cigarette filters, fishing lines, rope and fishing gear, baby diapers, six-pack rings, beverage bottles, disposable syringes, and pre-production resin pellets (Sheavly & Register, 2007).

Once the plastics reach the ocean, disperse throughout the water column, according to their density: polymers that are heavier that seawater tend to sink to the bottom, the lighter ones float on the surface (Ivar do Sul & Costa, 2013; Anderson *et al.*, 2015) (Figure 5). Floating microplastics can also disperse deeper into the water column due to hydrodynamism, the ingestion by organisms and the fouling that can cause plastic debris to sink.

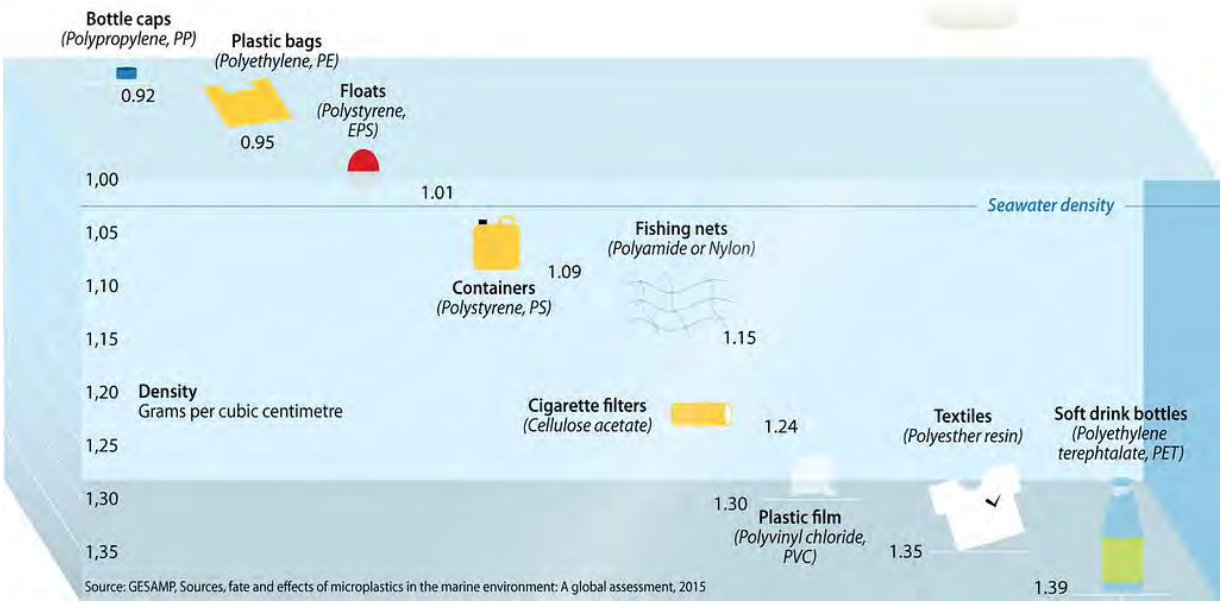


Figure 5: Densities of the most common polymers and objects. Modified from Anderson et al.,*2015.*

Heavier plastics tend to accumulate close to the source of contamination, sinking to the ocean floor, where they can be buried in the sediment and be ingested by benthic organisms. In this way, plastic can enter the food web and eventually be resuspended and transported back to the water column (Engler, 2012) (Figure 6).

Plastics that reach the ocean, disperse horizontally according to the location of the pollution source, the characteristics of the debris and currents and winds (Lusher, 2015). Heavier objects usually accumulate in the sediments on the ocean floor, near the source of entry of the plastics, and can eventually be transported by deep currents, finally contaminating canyons and trenches (Woodall *et al.*, 2014). Wind stress is the main driving force for upper ocean circulation, so the distribution of floating plastics

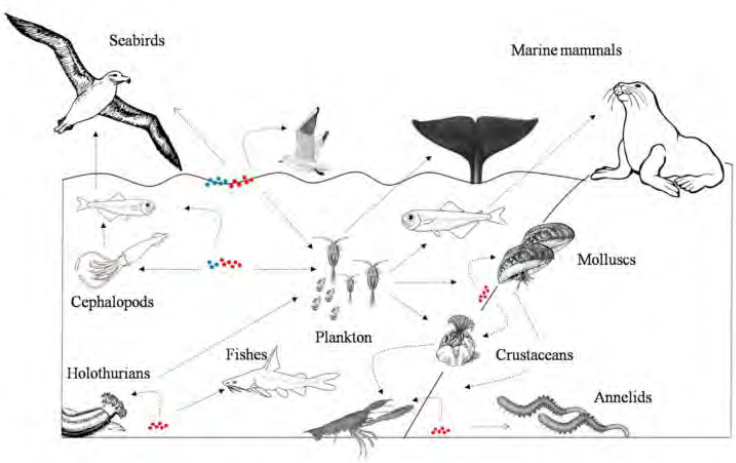


Figure 6: Distribution and possible trophic transfer of microplastics at sea. In blue, microplastics lighter than seawater, in red heavier (Ivar do Sul & Costa, 2013)

depends mainly on the wind and currents. In the convergence zones, also referred to as gyres, plastics have been accumulating for decades, creating areas of high debris concentrations, commonly known as “garbage patches” (Moore *et al.,* 2001). In these areas, the concentration of macro and microplastics can be several orders of magnitude higher than in the surrounding areas.

At least, five major patches have been identified around the world: in the Pacific (north and south), Atlantic (north and south), and Indian Ocean. Recently, with the aid of mathematic models integrating sources of contamination with oceanic surface currents, a sixth possible patch was identified in the Barents Sea (Figure 7). The garbage patches are not persistent entities: depending on the variations in the plastic ingress into the oceans, and on the atmospheric events, they can change their position, shape and concentration of floating objects (Sebille *et al.,* 2012). The Antarctic Circumpolar Current has a great potential for dispersion of plastics into the waters of the southern hemisphere, since it’s a fast moving current and is close to three garbage patches.

All the oceans are connected to each other, and not even virgin remote islands are safe from plastic pollution. In Hawaii, for example, more than 2500 pieces of plastic were found on a single square foot of beach (Moore, 2008).

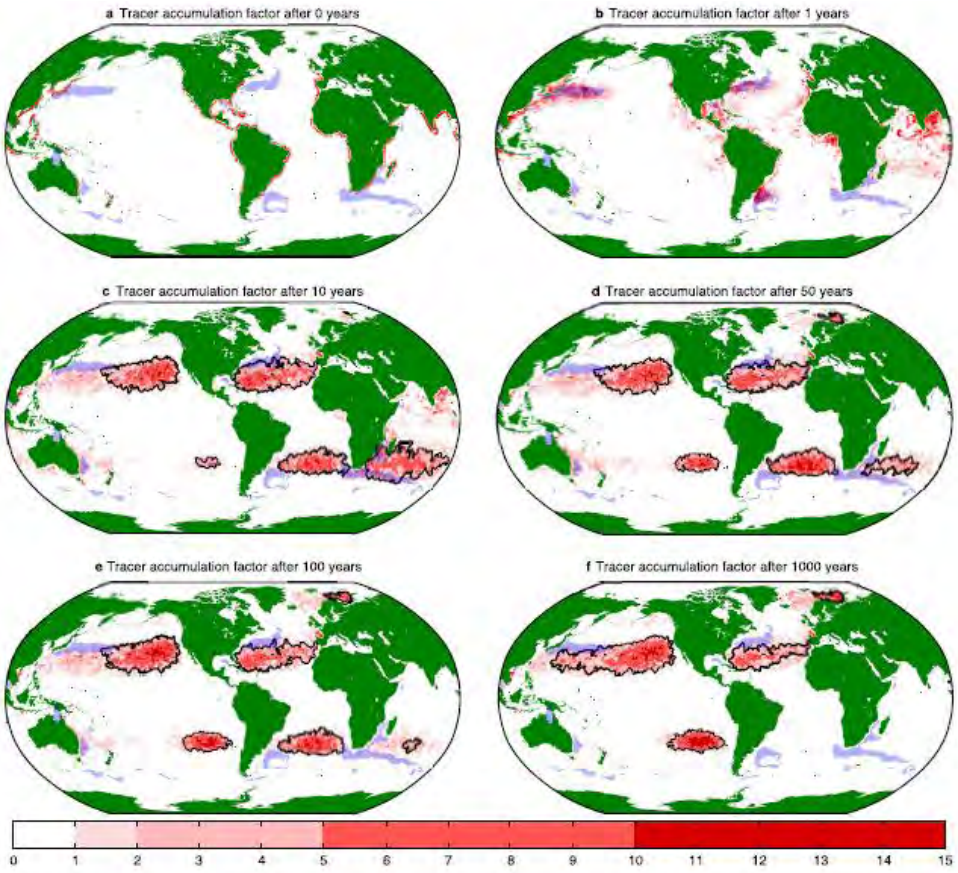


Figure 7: Evolution of the garbage patches in the world's oceans. On the scale bar, the accumulation factors for floating objects, determined by a dispersion model that integrates currents and sources of pollution (Sebille *et al.*, 2012).

1.2 Plastics as a threat to marine life

Once in the ocean, plastic debris becomes available to interaction with marine animals. Plastics, and particularly microplastics, can have adverse effects on all organisms at every scale: from molecular, right up to ecosystem level (Galloway *et al.,* 2017) (Figure 8).

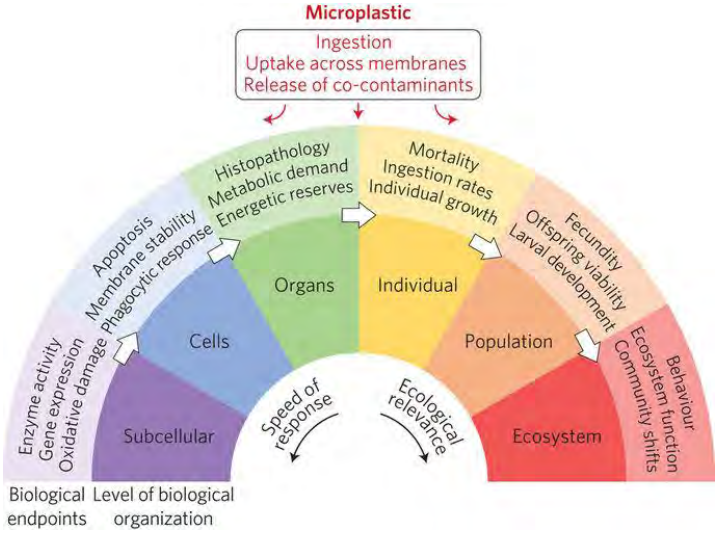


Figure 8: DIfferent impacts that microplastics can have on different biological organization levels (Galloway *et al.*, 2017)

The impact can be mainly of two types: physical or chemical. Entanglement and ingestion of macroplastics can cause physical damage (gastrointestinal blockage, limited movement, drowning etc.), while through the ingestion of smaller pieces, organisms can be exposed to toxic compounds leached by the plastics (Rochman *et al.*, 2013).

These chemicals can cause a vast array of effects: from alterations in the gene´ expression, to changes at ecosystem level due to the reduced fecundity. The effects of toxic compounds associated to plastics, will be discussed more in detail in Section 1.3.

1.2.1 Entanglement

Many marine species have been reported as trapped in floating objects, nets and fishing lines in all the seas and oceans. Fishing gear can be abandoned at sea as the result of accidents or loss. These abandoned nets and fishing tackle can continue to ensnare and threaten marine life for decades, a process commonly called “ghost fishing”. When an

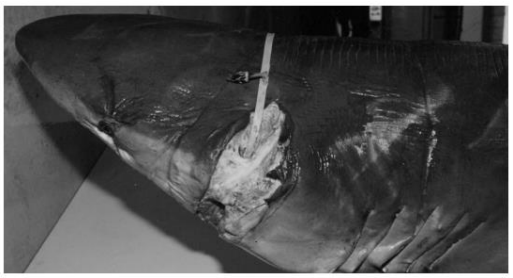


Figure 9: Carcharinus obscurus entangled in plastic, with tissue regenerating around the object (Cliff et al., 2002).

animal gets trapped in plastic objects, it may eventually be able to free itself from the debris, but sometimes ends up carrying parts of the fishing gear or plastic objects for the rest of its life, reducing its fitness and eventually causing its death (Cliff *et al.,* 2002) (Figure 9). Entanglement usually occurs with big objects (macroplastics) and it was the first of such negative impacts on marine life to be identified (Laist, 1987). In recent years there has been rising concern about the impact that ingestion of plastics is having on marine life.

1.2.2 Ingestion

Depending on its size, plastic debris can be available for ingestion by different marine animal taxa. Bigger objects (like bags and packaging) are commonly ingested by turtles and cetaceans (Baulch & Perry, 2014; Seminoff *et al.,* 2002; Williams *et al.,* 2011), while microplastics can enter the food web at a much lower level: in laboratory experiments it was seen that plankton can ingest microplastics (Cole *et al.,* 2013; Setälä *et al.,* 2014) (Figure 10).

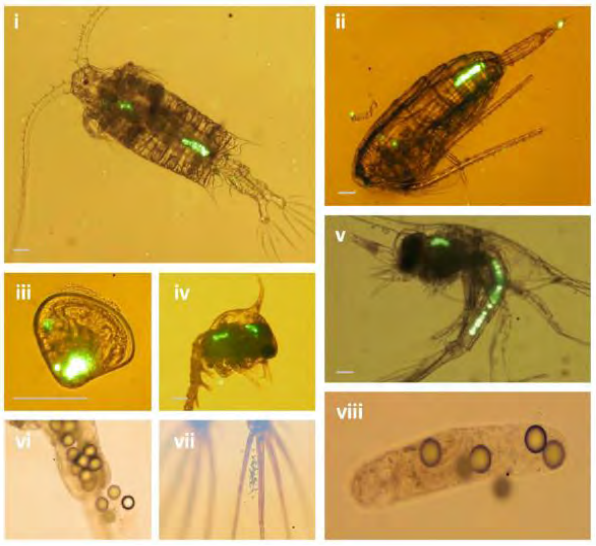


Figure 10: Fluorescence microscope images evidence the ingestion of microplastics in different zooplankton taxa (copepods, bivalvs larvae, decapods etc.) (Cole *et al.*, 2013)

The ingestion of debris can occur in three ways:

- Accidental ingestion usually occurs in filter feeders (bivalves, barnacles, baleen whales, etc.) and in detritivores (sea cucumbers, polychaetae worms, etc.). These animals select their food from the matrix (water, sediment) by size: if plastics are of the same size as the prey, they can't distinguish it and the plastics are also ingested (Fossi *et al.,* 2014; Goldstein & Goodwin, 2013; Graham & Thompson, 2009; Ryan, 2016) (Figure 10). Accidental ingestion can also occur

in predatory animals, when the prey is in proximity to plastics (Rebolledo *et al.,* 2013).

- Direct ingestion happens when the debris is deliberately ingested because is mistaken for prey. This was observed in different groups of marine vertebrates: turtles (Gramentz, 1988; Mrosovsky *et al.,* 2009; Seminoff *et al.,* 2002), fishes (Choy & Drazen, 2013; Jantz *et al.,* 2013; Ory *et al.,* 2017), sharks (Cliff *et al.*, 2002) and marine mammals (Fowler, 1987; Denuncio *et al.*, 2011; Rebolledo *et al.*, 2013; Claro *et al.*, 2019).
- Secondary ingestion occurs when a predator ingests a prey that has previously ingested plastics. It is difficult to distinguish between direct and secondary ingestion, but it can be deduced by comparing the size of the debris with the size of the preys (Eriksson & Burton, 2003).

In any of these cases, the ingestion of big objects can result in digestive tract occlusion, laceration of tissues, starvation and, eventually, death. Recently, more attention is being focused on the less evident consequences that plastic ingestion can have on marine organisms: the toxicity.

1.3 Toxicity

The ingestion of plastics can lead to many negative effects. One of the most hidden and currently studied, is the possibility of the transfer of toxic chemicals from plastics to ingesting animals (Ziccardi *et al.*, 2016; Worm *et al.*, 2017). The toxicity can affect different parts and organs of the animal, depending on the compound leached. The toxic compounds can be derived from the production of plastics, or are adsorbed on the surface of debris during their residency in the environment (Sanborn *et al.*, 1975; Mato *et al.*, 2001; Rios *et al.*, 2007).

1.3.1 Plastic additives

During the industrial production of plastics, the resins are usually added with chemical compounds that enhance the properties and durability of the final plastic product (Thompson *et al.*, 2009). In some cases, plasticizers can constitute up to 50% of the total weight of plastics (Oehlmann *et al.*, 2009). These compounds are not chemically bound to the plastics, so that they can seep out into the environment.

The main groups of additives that are used in the manufacture of plastics, and that are worrying because of their negative effects in the organisms, are:

- Flame retardants: compounds added to the polymers to reduce or inhibit their flammability. These compounds have countless applications in plastics used in construction, textiles, electronics etc. There are three main classes of flame retardants: minerals, organohalogenated and organophosphate compounds. Of the organohalogenated compounds, the polybromodiphenylethers (PBDEs) are of most environmental concern, since their high lipophilicity and resistance to degradation processes make them bioaccumulative and persistent (Kinani *et al.*, 2009). PBDEs have potential endocrine disrupting properties and can induce genetic alterations that eventually lead to cancer (Rahman *et al.*, 2001).
- Stabilizers: these are used to prevent the polymer's oxidation caused, for example, by heat or UV rays (photo-oxidation) and that weakens the plastic structure, making it easily become brittle. The antioxidants that are most

commonly used and are concerning for their negative effects on endocrine functions, are bisphenol A (BPA) and nonylphenol (NP) (Engler, 2012).

- Plasticizers: they are used to enhance flexibility through the reduction of bonding between the monomers that constitute plastics. Phthalates are carcinogenic and recognized endocrine disruptors in aquatic species (Heudorf *et al.*, 2007; Chen *et al.*, 2014). They are used as indicators of plastic ingestion in marine animals (Fossi *et al.*, 2014)

1.3.2 Phthalates

Phthalates, or phthalate esters, are esters of phthalic acid (Figure 11). They were first introduced in the 1920s and are added to plastics to increase their flexibility, transparency, durability, and longevity. They are used primarily to soften polyvinyl chloride (PVC).

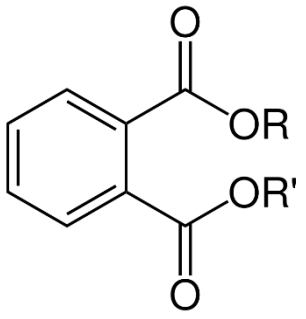


Figure 11: General chemical structure of phthalates (source: (EPA, 2012)

Approximately 8.4 million tonnes of plasticizers are produced globally every year, of which approximately 70% are phthalates (www.plasticisers.org/mediaroom/plasticisers-factsheet). There are more than 30 different phthalates on the market, but di(2-ethylhexyl) phthalate (DEHP) dominates with 51% of production worldwide (Rahman & Brazel, 2004).

Because they are not chemically bonded to the plastics, phthalates are easily leached from plastic items, for example by heat and physical stress. Despite their widespread use, only limited information exists on the consequences of phthalates in the environment or the effect on aquatic organisms that come into contact with them (Stalling *et al.*, 1973). Biodegradation is a critical process affecting the environmental fate of phthalate esters: in natural waters, DEHP has a half-life of 78 days (Staples *et al.*, 1997).

Bioaccumulation refers to the accumulation of contaminants in the tissue of biota via all exposure routes (i.e.: water + diet), whereas bioconcentration refers to accumulation due to aqueous exposure alone. These processes are quantified by the bioaccumulation and bioconcentration factor, respectively (i.e., BAF or BCF). It was shown in laboratory studies, that fish can bioconcentrate phthalates to a BCF of up to 120 (Staples *et al.*, 1997). Because of their chemical properties, exposure to phthalates does not result in bioaccumulation, since they can be metabolized and the monoester can be directly excreted (Heudorf *et al.*, 2007). This ability of biotransformation and metabolization increases in the higher trophic levels.

Phthalates can have various toxic effects on organisms. In particular, they are able to act as endocrine disruptors, even at very low concentrations, interfering with hormone synthesis, altering reproduction, inducing intersex or other physiological and metabolic functions (Zheng *et al.*, 2013). It has also been shown that phthalates can alter the behavior in fish (Barse *et al.*, 2007). For all these reasons, health concerns have been raised regarding the developmental and reproductive toxicity of phthalates, and the US Environmental Protection Agency (US EPA) has listed phthalates among the endocrine disruptors and inhibitors of male fertility (Sparling, 2016).

Recently the presence of phthalates in the tissues of marine animals have been linked to the ingestion of plastics. This has become a useful tool for the indirect determination of plastic ingestion in freeranging protected animals (Fossi *et al.*, 2014; Baini *et al.*, 2017), thanks to the analysis of biopsies of muscle, blubber and skin. Although phthalates can be found in different tissues, they accumulate primarily in fatty tissues such as in the gonads and liver (Savoca *et al.*, 2018).

1.4 Pollutants adsorbed on plastics

Floating plastics have the ability to adsorb the chemical compounds that are present in the environment and in seawater, because of the hydrophobic nature of the plastic surfaces (Mato *et al.*, 2001; Rios *et al.*, 2007). Plastic debris acts like an adsorbant in the sea, concentrating hydrophobic organic compounds that are found in low concentrations in the ocean (Rice & Gold, 1984). Because of their low polarity, these compounds are distributed mainly in the air-water interface and in sediments, where they can interact with plastic surfaces and bond to them. Through oceanic currents, the plastic debris can transport the organic pollutants far away from their source in the environment.

Among the organic pollutants that were found adsorbed on plastics, there are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides (dichlorodiphenyltrichloroethane and its degraded forms (DDD and DDE)), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), lindane etc. Table 1 shows a summary of organic pollutants that have been detected as adsorbed on plastics and microplastics worldwide.

Table 1: Organic pollutants detected adsorbed on plastics and microplastics.

Authors	**Adsorbed pollutants found**
(Mato *et al.*, 2001)	PCBs, DDE
(Rios *et al.*, 2007)	PAHs, PCBs, DDTs
(Ogata *et al.*, 2009)	PCBs, DDTs, HCHs
(Rios *et al.*, 2010)	PCBs, PAHs, DDTs, HCB
(Heskett *et al.*, 2012)	PCBs, DDTs, HCH
(Rios Mendoza & Jones, 2015)	PAHs, PCBs
(Zhang *et al.*, 2015)	PCBs, PAHs, pesticides

These pollutants are recognized as dangerous for the environment due to their persistence, bioaccumulation and toxic effects on living organisms (persistent organic pollutants: POPs). Some of these compounds have been regulated or prohibited since the ‘60s, but because of their low degradation, they are still found in many environments and matrices (Tanabe, 2002). In 2001, 98 countries signed the Stockholm Convention,

prohibiting or restricting the production and use of 12 POPs (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, PCBs, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF)). In spite of the health and environmental risks, some of the POPs are still allowed to be used in some specific circumstances. DDTs, for example, can still be used for the control of diseases such as malaria and dengue (López-Carrillo *et al.*, 1996). A summary of the main regulations and effects of the organic pollutants commonly found associated with plastics is provided in Table 2.

Table 2: Main organic pollutants detected on plastic debris, their uses, main regulations and toxic effects on fish

Compound	Use	Bans	Toxic effects in fish
PCBs	Insulators, lubricants	1970s in Japan and USA, Stockholm convention (2001)	↓ Reproduction, ↓ hormones, immune response, carcinogenesis, lethal
DDTs	Pesticides	1972 USA, Stockholm convention (Annex B: still used for disease control)	↓ Reproduction, nervous system damage, liver and kidney damage
HCB	Pesticide, fungicide	1966 USA, Stockholm convention (2001)	Carcinogenesis, teratogenic effects
HCH	Pesticide	Stockholm convention, still permitted (pure lindane) in USA	Carcinogenesis, endocrine disruptor
PAHs	Products of combustion (natural and anthropogenic)	EPA and European Union established maximum concentrations allowed for some of them	Carcinogenesis

These substances have proven toxic effects on organisms, especially aquatic ones. Research has focused on short term effects on model species in controlled laboratory conditions. The studies on free-ranging animals are much more complex and show many more variables that can add noise to the results. An understanding of long-term effects on wildlife is a challenge to future investigations in ecotoxicology

1.4.1 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are a group of over 100 organic compounds with two or more fused aromatic rings. They are found throughout the environment in the air, water, and soil, deriving from anthropological or natural sources (Eisler, 1987). These compounds are formed during the incomplete burning of coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat. PAHs enter the environment mostly in the air from volcanoes, forest fires, residential wood burning, and exhaust from automobiles and trucks. They can travel long distances before they return to earth in rainfall or particle settling (ATDSR, 1995).

PAHs are nonpolar and lipophilic, they can accumulate in all the tissues of animals that contain fat, bioconcentrating at beyond the environmental levels. PAHs can be stored in kidneys, liver, and fat but in fish they can also be metabolized by the liver (D'Adamo *et al.*, 1997). In all cases where assimilation of ingested PAHs was demonstrated, metabolism and excretion of PAHs were rapid. Thus, little potential exists for food web biomagnification of PAHs (Eisler, 1987).

Studies in controlled conditions have proven that PAHs can have severe effects on organisms, especially on fish. These pollutants can have deleterious effect on reproduction: they cause a reduction in circulating hormones and plasma vitellogenin, estrogenic and antiestrogenic effects, retardation of oocyte maturation and reduction of reproductive success (Nicolas, 1999). PAHs interfere also with the immune system in fish, and can cause cancer. PAHs can be biotransformed to toxic metabolites, which can be boud to DNA and RNA, causing cell damage, mutagenesis, teratogenesis and carcinogenesis (Tuvikene, 1995).

For these reasons, the United States Environmental Protection Agency (EPA) and the World Health Organization (WHO) have identified 16 PAHs as priority pollutants (Figure 12).

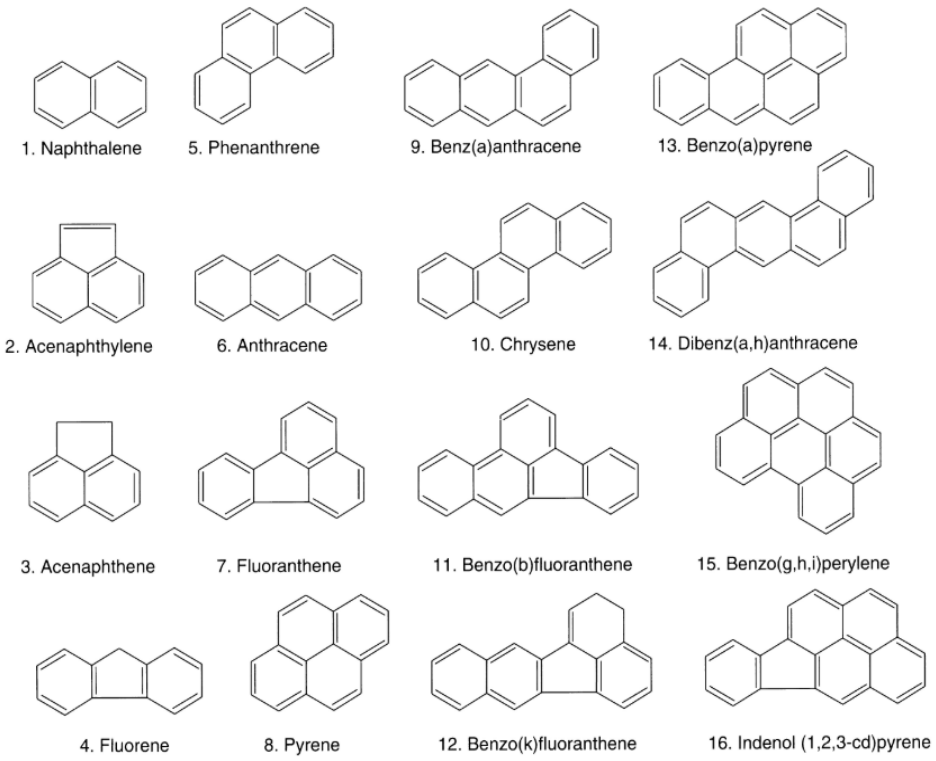


Figure 12: The 16 PAHs considered by the EPA and WHO as priority pollutants (Tuvikene, 1995)

1.4.2 Organochlorine pesticides (OCPs)

Organochlorine pesticides (OCPs) are fat-soluble compounds that persist and bioaccumulate in the environment. Since the discovery of DDT in 1939, numerous pesticides have been developed and used extensively worldwide (Figure 13). They have been used to repel or kill rodents, fungi, insects, and weeds that reduce the productivity of intensive farming. In some countries, they are also used for the control of human disease vectors such as malaria and dengue mosquitoes. However, many first generation pesticides have been found to be harmful to the environment. Some of them are persistent in soils and aquatic sediments, they bioconcentrate in the tissues of invertebrates and vertebrates, biomagnificate in the trophic chains, and affect top predators (Mnif *et al.*, 2011).

Figure 13: A truck sprays Jones Beach in New York with DDT, 1945 (source: Corbis Images).

Rachel Carson's book "Silent Spring", published in 1962, first drew attention to the hazard of the widespread and extensive use of pesticides for the environment and for human health. The book resulted in a national ban on DDT and other pesticides, at first in the USA, and then in many other countries through the Stockholm Convention. In Mexico, production of DDT began in 1959 and grew until the '70s. Nowadays, the use of DDT in Mexico is stricly limited, but still permitted for the prevention of malaria and dengue (López-Carrillo *et al.*, 1996; CEC, 1997).

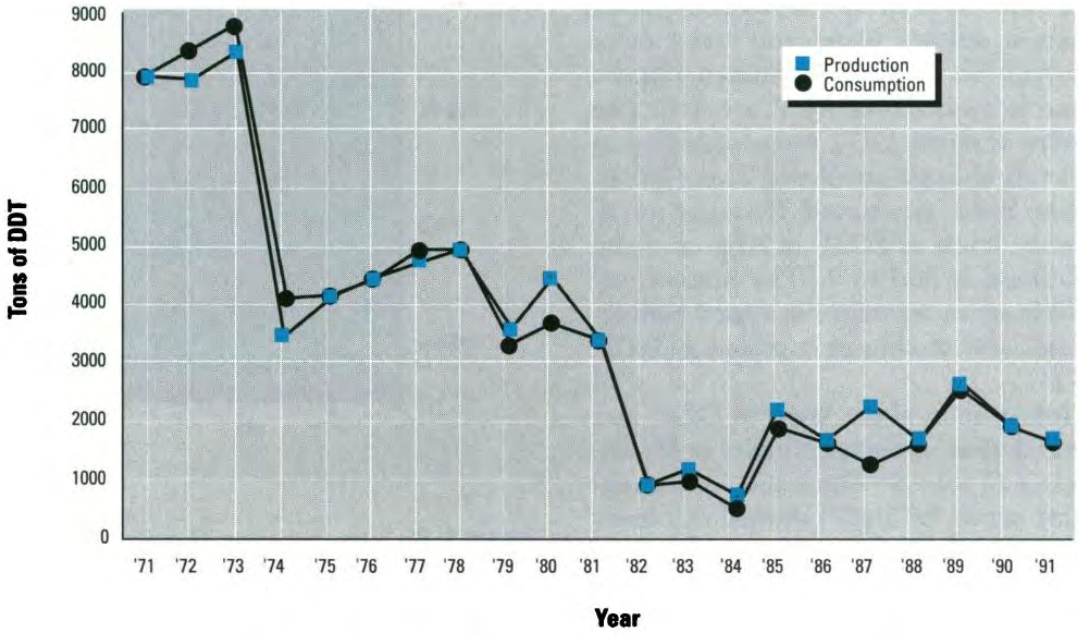


Figure 14: Commercial DDT production and consumption in Mexico, 1975-1991 (López-Carrillo *et al.*, 1996)

OCPs have the potential to affect development, reproduction, and behavior of fish and wildlife (Sapozhnikova *et al.*, 2004). Acute toxicity primarily damages the central nervous system, while chronic effects include damage to the liver and kidneys, reduced reproduction, slowed reaction to external stimuli, loss of appetite, and restricted growth (Johnson, 1968).

1.4.3 PCBs

Polychlorinated biphenyls (PCBs) are organic chlorine compound with the formula $C_{12}H_{10-x}Cl_x$ (Figure 15). There are 209 different chemical compounds in which one to ten chlorine atoms can replace hydrogen atoms. They are hydrophobic, with low water solubilities but high solubilities in most organic solvents, oils, and fats. PCBs have been produced since 1930 and were typically made as mixtures of compounds (Teil *et al.*, 2012), commonly used in electronics manufacture, as vehicles for pesticides, and in building materials (Korrick & Sagiv, 2008).

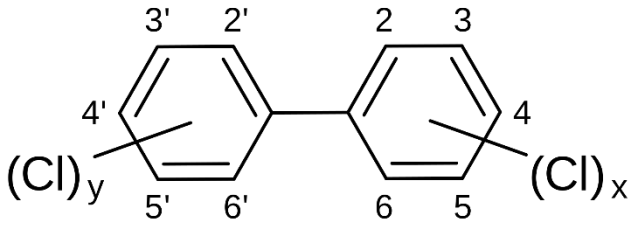


Figure 15: Chemical structure of PCBs (Source: Wikipedia)

Despite restrictive legislation having being in effect since the 1970s in the United States and Europe, PCBs persist in the environment (Teil *et al.*, 2012). In 2001 they were also included in the Stockholm Convention, prohibiting their production and use in the signatory countries. In Mexico, PCBs are considered as hazardous waste, and their disposal is regulated under the NOM-133-SEMARNAT-2000 since 2001.

Although they are regulated in most countries, PCBs can still be detected in many environments and matrices like soil, water and organisms, including remote habitats (Letcher *et al.*, 2010). Exposure to PCBs induces various adverse health effects in animals and humans. In fish, PCBs have been found to cause genotoxic damage (Marabini *et al.*, 2011), interfere with reproduction (Holm *et al.*, 1993; Murphy *et al.*, 2005) and with growth rates (Bengtsson, 1980) in controlled environment experiments.

1.5 Oceanic Manta Rays

Figure 16: Dorsal view of an oceanic manta ray, Mobula birostris. Copyright: Marc Dando

Oceanic manta rays are filter-feeding elasmobranchs that are found circumglobally in tropical and temperate waters (Marshall *et al.*, 2018). They are the biggest of the mobulid family, that comprises 8 species, recently reclassified as one single genus (*Mobula*), combining together the previous two (*Mobula* and *Manta*) (White *et al.*, 2017).

The genus *Manta* was thought to consist of just a single species (*Manta birostris*) but in 2009 Marshall *et al*. found morphological and meristic evidence of the existence of at least one other species (*M.alfredi*, commonly known as the reef manta ray for its prevalence in coastal and reef habitats). A third putative species, *Manta sp. cf. birostris,* in the Atlantic may be distinct from *M. birostris* but more analysis is needed in order to clarify its taxonomic status (Marshall *et al.*, 2009).

The oceanic manta ray can be easily distinguished by its larger size, body coloration (dorsal and ventral), and the presence of a reduced caudal spine (Figure 16). *M.birostris* can grow to > 7m disc width (DW), and live probably more than 30 years (Stewart *et al.*, 2018).

Both manta species show a low reproductive rate, giving birth usually to one single pup after a 12 month gestation period, during which the mother nourishes the embryo through uterine milk (secreted by the trophonemata). The newborn is completely autonomous at birth, measuring approximately 2m DW. It is estimated that oceanic manta ray males mature at about 3.8 m DW, while females at 4.5 m DW (Rambahiniarison *et al.*, 2018) (Table 3).

Table 3: Life parameters of the oceanic manta rays M.birostris. DW= disc width in m, M=males, F= females (Rambahiniarison *et al.*, 2018; Stewart *et al.*, 2018)

Max DW	Max age	DW at birth	DW at maturity (M)	DW at maturity (F)
7.1	>28	2.0	3.8	4.5

1.5.1 Aggregations

Within the oceanic manta rays broad range of distribution, actual populations appear to be sparsely distributed and highly fragmented, with probably a low interchange of individuals between subpopulations (Stewart, Beale, *et al.*, 2016; Marshall *et al.*, 2018)(Figure 17). Although capable of traveling long distances (Hearn *et al.*, 2014; Arauz *et al.*, 2019), oceanic manta rays have shown a high level of residency along productive coastlines with regular upwellings, oceanic islands and offshore seamounts, where they can find food sources and cleaning stations (Marshall *et al.*, 2009; Medeiros *et al.*, 2015; Barr & Abelson, 2019). Although they are usually solitary animals, oceanic manta rays aggregate in these areas and can be found in high numbers throughout the year or seasonally (Stewart et al., 2016).

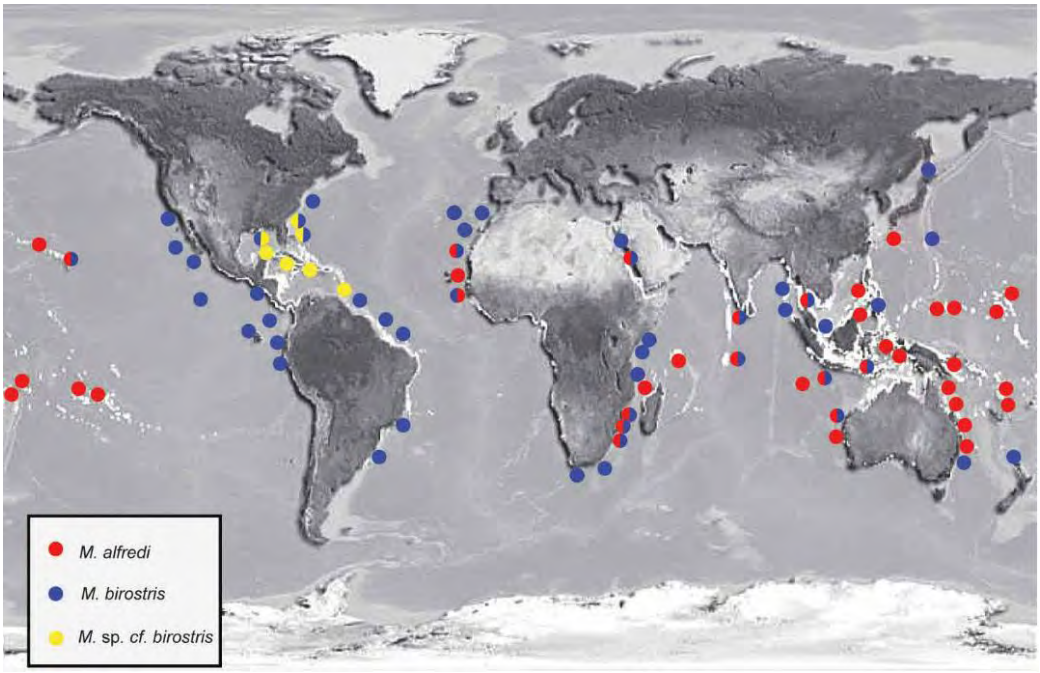


Figure 17: Global distribution of oceanic manta rays (blue spots), reef manta ray (red spots) and putative third species (yellow spot) (Marshall *et al.*, 2009).

In Mexico, oceanic manta rays can be found on the Pacific coast, with two main aggregation sites where they can be found seasonally in abundant numbers: AR and BB. A third area, which used to be seasonally visited by *M.birostris*, is the southern part of the Gulf of California, where mantas were commonly seen during the summer around Cerralvo Island and a seamount called El Bajo, near Espiritu Santo Island. In the late '90s, manta ray sightings became less and less common, as the fishing activity for

mobulid rays in the area was depleting the local population (Stewart et al., 2016, Rubin pers.comm.). Mantas nearly disappeared from the gulf of California for almost 20 years, until the summer of 2018, when the species was frequently sighted again. It is still a mystery where these mantas were coming from, and whether or not they were part of the Revillagigedo/Banderas Bay subpopulations. Healthy populations in nearby areas can be a source for possible future re-colonization of areas where mantas had been fished out, and could eventually be protected from the impact of harvesting.

In Banderas Bay, manta rays are commonly sighted in the southern part of the bay, where a deep canyon provides nutrient-rich waters that support a rich food web. This local population of manta rays is still under-studied, since its presence has only recently been discovered and a local organization, "*Proyecto Manta Pacifico Mexicano*" is leading the studies of the local population (Stewart *et al.*, 2016).

In the Revillagigedo Archipelago, a multi-decadal photo-ID study by the "Pacific Manta Research Group" has revealed that more than 830 individual mantas have visited the islands since the 1980s (Robert Rubin, pers.comm.). In the archipelago, manta rays find favorable conditions: numerous cleaning stations and food sources (Stewart *et al*., 2016). In the archipelago, the mantas are cleaned mainly by an endemic species of angelfish: the Clarion angelfish, *Holacanthus clarionensis* (Figure 18).

Figure 18: Clarion Angelfish cleaning an Oceanic Manta Ray at Revillagigedo Archipelago. Photocredit: Marty Snyderman

1.5.2 Feeding ecology

When feeding, manta rays unfurl their cephalic fins to channel plankton-rich water into their mouths and over the gillrakers, where leaf-like filter lobes strain and capture the food. The only selection that is made through this filtering technique, is one based on particle size, that has recently been described as a novel non-chlogging filtration mechanism called “ricochet” (Divi *et al.*, 2018). Through flow modelling, it was seen that oceanic manta rays can retain food that is slightly smaller (~200 μm) than the pore size of the filtering lobes (340 μm) and they are able to avoid particles getting stuck and clogging the filters, a novel mechanism never reported before.

It was estimated that during feeding activity, oceanic manta rays are capable of filtering large volumes of water: up to 90 m^3/hour (Winters-Mist Paig-Tran, 2012), the equivalent of 10 bathtubs per minute. This makes the energy cost of feeding a variable that must be taken into account when making the decision to start feeding. Actually, manta rays appear to feed on small planktonic organisms when they reach a prey density threshold where foraging efficiency can be maximized (Armstrong *et al.*, 2016). To do so, mantas and devil rays have evolved a wide variety of feeding behaviours. Eight different feeding strategies have been described for mobulid rays (Stevens *et al.*, 2018):

1. Straight feeding
2. Surface feeding
3. Chain feeding
4. Piggyback feeding
5. Somersault feeding
6. Cyclone feeding
7. Sideways feeding
8. Bottom feeding

In some cases, manta rays co-operate with each others during the feeding activity, a complex strategy that requires coordination between the individuals.

Through stomach content analysis, direct feeding observations and inferences from fatty acids, stable isotope analysis and diving profiles, it was found that *M.birostris* shows a certain plasticity in feeding ecology, utilizing both near-surface and mesopelagic zooplankton aggregations.

It was recently found that oceanic manta rays have a preference for the thermocline assemblages and deep scattering layer prey sources (Stewart *et al.*, 2016; Stewart *et al.*, 2018) (Figure 19). It is largely believed that *M.birostris* shares the same food sources with other mobulid species, with euphausiids and copepods dominating the diet while small fishes and fish eggs are occasionally found in their stomach contents (Rohner *et al.*, 2017; Bessey *et al.*, 2019).

Figure 19: Oceanic manta ray feeding on a zooplankton aggregation at 130m depth, recorded in a submarine off Revillagigedo Archipelago (Stewart et al., 2016)

Occasionally, manta rays have been sighted in the Mexican Pacific Ocean feeding on both the surface aggregations of zooplankton (Rubin, pers. comm.) and those in the deep scattering layer at 130m depth (Stewart *et al.*, 2016). It is still not clear, which resources they are targeting more often and at which depth they are foraging more frequently. This aspect might be crucial in the determination of their susceptibility to ingestion of plastics, since debris abundance decreases at depth (Reisser *et al.*, 2015), and manta rays feeding at the surface might be more exposed to this threat.

1.5.3 Threats

Manta ray populations have declined all over the world and both species are listed as "Vulnerable" in the International Union for Conservation of Nature (IUCN) list of threatened species. The reasons for the manta ray's vulnerability to extinction can be found in its own life history: they are slow growing, long living animals and with a very slow reproductive rate. For this reason, they are slow to recover after a decline in their local populations and need effective protection in order to recuperate their numbers.

The main causes of the manta rays decline around the world are:

- Targeted fishery: in the last decades, a growing market of manta rays' gill plates have sustained a hunting for mobulids in many countries, mainly in southeast Asia (Figure 20). The gills are used in traditional Chinese medicine, while meat is consumed locally in some countries (in Mexico manta rays were fished for their meat, to be sold fresh or dried as "*machaca de manta*")(Croll *et al.*, 2016). Mantas are now protected in Mexico, under a permanent fishing ban (NOM-029-PESC-2006), that prohibits its capture, retention and sale throughout the country.

Figure 20: Oceanic manta ray caught by Indonesian fishermen despite the protection that was estabilished since 2014. Photocredit: Shawn Heinrichs

- Bycatch: even when protected by law, manta rays can get caught in different types of fishing gear, such as driftnets, gillnets, purse seines and longlines, which were not intended to target manta rays (Stewart *et al.*, 2018). This can cause serious

damage to the animals, reducing their fitness and eventually killing entangled mantas.

- Boat strikes: while cruising close to the surface, manta rays can accidentally be hit by boats. These injuries can be fatal or leave characteristic scars on the animals (Figure 21) (Stewart *et al.*, 2018).

Figure 21: Propeller's scar on a manta ray in the Maldives, fresh wound and 6 months later scar. Photos by Simon Hilbourne and Flossy Barraud | © Manta Trust

- Bad tourism practices: being charismatic animals, manta rays are a very popular tourist attraction, which has led to both good and bad consequences for the species. The presence of people in the water can, for example, modify the natural behavior of the manta rays, disturbing feeding and cleaning activities, while the prodigious presence of boats increases the probability of strikes when mantas are at the surface (Garrud, 2016; Stewart *et al.*, 2018).
- Pollution: manta rays are exposed to pollutants by ingestion and through contact with their skin and gills. Heavy metals and POPs can accumulate in the body of a manta ray during its long life, being transferred to the offspring through uterine milk. During filter feeding, mantas can ingest plastics that can cause both physical and chemical impacts (Germanov *et al.*, 2018; Stewart *et al.*, 2018). This aspect has been poorly studied, so the long term effect on manta rays is still unknown.

2 Background

Filterfeeders have gained a lot of attention for being particularly susceptible to the ingestion of microplastics, because of their capacity to filter large amounts of water in search of their prey (Cole *et al.*, 2013; Goldstein & Goodwin, 2013; Besseling *et al.*, 2015). Due to the short length of the food chain that leads to filter feeders, the presence of plastics-derived pollutants is most commonly considered to be the consequence of direct ingestion of plastics, or ingestion of zooplankton prey that has ingested plastics (secondary ingestion).

It has been proven in laboratory experiments that the potential prey of manta rays can ingest microplastics: Cole *et al*. in 2013 found that copepods and euphausiids can uptake microplastics from water, and this could lead to a secondary ingestion by secondary consumers, such as filter-feeding megafauna. These results have been confirmed by field observation of microplastics in zooplankton: both copepods and euphausiids in the open Pacific Ocean have been shown to ingest plastics, mainly fibers, with an incidence of 33 and 16%, respectively (Desforges *et al.*, 2015).

The ingestion of plastics by filter feeding megafauna has received a growing attention in recent years. Nevertheless, the detection of debris ingestion in cetaceans largely depends on data collected from the small sample sizes taken from stranded animals, presenting only a snapshot of the impacts occurring at sea, and is often biased by deadly concentrations of plastics in their stomachs (Baulch & Perry, 2014). Data from stranded animals are even less common for elasmobranchs, as they are negatively buoyant and only in rare cases become stranded on land.

Ingestion of plastics has been directly seen in stranded whale sharks (Haetrakul *et al.*, 2009; Sampaio *et al.*, 2018; Abreo *et al.*, 2019) but has not yet been detected in the other filter-feeding elasmobranchs (basking shark, megamouth shark and mobulid rays).

The ingestion of plastics can be determined also indirectly, by using plasticizers as tracers of debris ingestion (Fossi *et al.*, 2014; Baini *et al.*, 2017). This technique can be applied to both stranded/fished and free-ranging animals, through the collection and

chemical analysis of biopsy samples (Fossi *et al.*, 2014; Claro *et al.*, 2019). In this way, it was found that basking sharks in the Mediterranean Sea are filter-feeding on plastics, as are whale sharks in the Bay of La Paz, Baja California (Fossi *et al.*, 2014, 2016). The use of plasticizers as tracers of plastic ingestion is extremely useful when working with species that are protected and the direct analysis of stomach content is not an option. The use of non-lethal techniques, such as skin, blubber or muscle biopsy, is a valid method of determining the concentration of pollutants in free-ranging animals (Marsili *et al.*, 2016; Fossi *et al.*, 2017).

Through a spatial analysis, it has been shown that manta ray distribution overlaps with highly plastic-polluted areas around the world, and a need for further investigation has been underlined by Germanov *et al*. in 2018. Nevertheless, to our knowledge, no reports are available to date about the ingestion of plastics by manta rays.

3 Justification

There is a lack of information about floating plastics in Mexican waters. Large scale studies have detected the presence of plastic debris in the open Pacific Ocean (Law *et al.*, 2014), but no data are available on the seasonality and characterization of floating debris in coastal areas. Baseline information on the background levels of marine plastics in the aggregation areas for oceanic manta rays in the Mexican Pacific Ocean is essential to understand the magnitude of the threat to local vulnerable species populations. There is a lack of information about the possible interaction that plastic debris can have with oceanic manta rays, which is a potential threat to the species in all of its broad range of distribution (Germanov *et al.*, 2018).

The health of the sub-populations of oceanic manta rays in AR and BB is extremely important, in order to avoid and possibly reverse the population decline that happened in the the Gulf of California (Stewart *et al.*, 2016). Manta rays are now protected in Mexico from fishing activity, but the threats deriving from habitat destruction and ocean pollution can also cause severe problems to vulnerable species such as *M.birostris* (Stewart *et al.*, 2018).

Manta ray ecotourism in the Mexican Pacific Ocean is having a great and positive economic impact, with an estimated economic value of 14 million $ US for the Revillagigedo Archipelago population, and a mean value of almost 30’000 $ US per individual manta ray in that population (Ruiz-Sakamoto, 2015). In Banderas Bay, there is no direct ecotourism activity, since the seasonality and distribution of mantas are not yet well known, but random encounters happen when diving in the bay. For this reason, no estimation of economic value exists yet, but responsible and sustainable manta rays ecotourism could potentially become a source of income for the local communities in the future. Losing another population of manta rays, as happened in the Gulf of California, could have a negative economic impact on the tourism activity that is currently happening in the Revillagigedo Archipelago and could also abort any project of sustainable ecotourism in Banderas Bay.

4 Research Hypothesis

There is a seasonality in the floating plastics in Banderas Bay and Revillagigedo Archipelago, and the plastic debris in the areas have POPs adsorbed on its surface. Oceanic manta rays in the Mexican Pacific Ocean are ingesting plastics, and these are leaching plasticizers and POPs into manta ray tissues, including skin and muscle.

5 Objectives

5.1 General Objective

The main objective of this project is to establish a baseline of plastic pollution in the aggregation areas of oceanic manta rays in the Mexican Pacific Ocean, and determine if manta rays have detectable levels of plasticizers and POPs in skin and muscle, using a non-lethal sampling tecnique.

5.2 Specific Objectives

1. Determine a baseline of floating plastics abundance in Revillagigedo Archipelago and the southern part of Banderas Bay by characterizing the floating plastics in both areas and their seasonal fluctuations.

2. Determine if plastics in both areas present POPs adsorbed on their surface.

3. Analyze the concentration of plasticizers (phthalates) in oceanic manta ray biopsies as a tracer of plastic ingestion. Determine the concentration of POPs in oceanic manta ray biopsies that could potentially come from the ingestion of polluted plastics, or from the diet.

6 Study Areas

This thesis is focused on two aggregation sites in the Mexican Pacific Ocean, where *Mobula birostris* is seen regularly: Revillagigedo Archipelago and Banderas Bay (Figure 22).

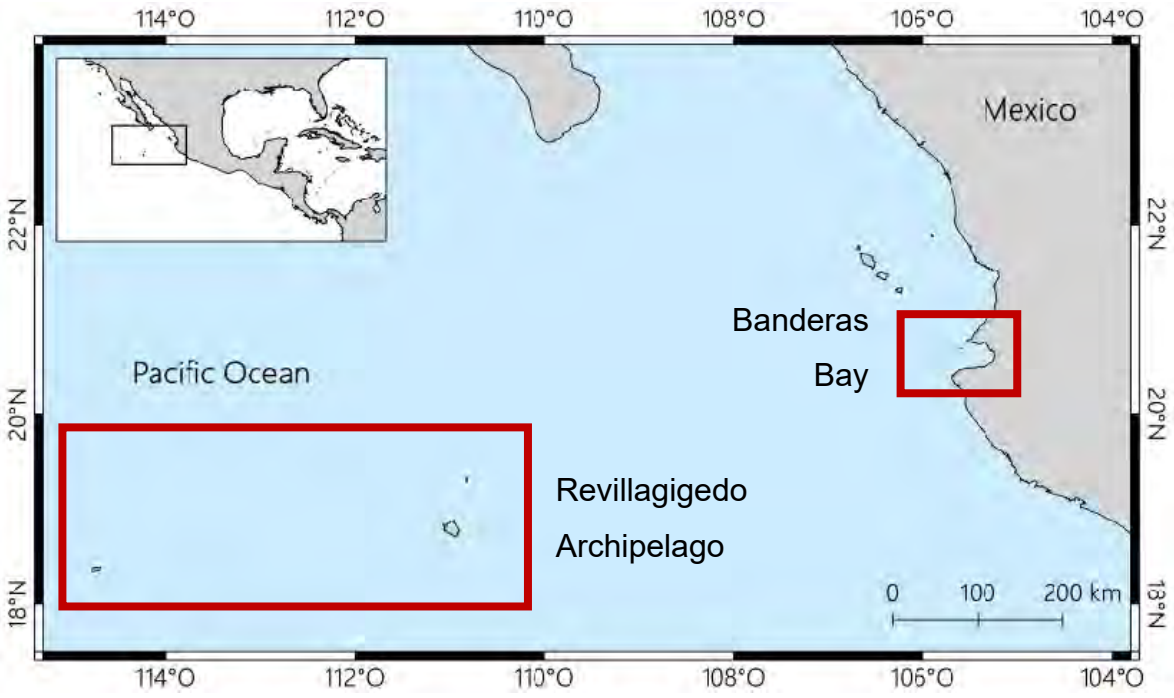


Figure 22: Study Area in the Mexican Pacific Ocean

6.1 Banderas Bay

Banderas Bay is located on the Pacific coast of Mexico and belongs to the states of Jalisco and Nayarit. With its 100 km of coastline, it is one of the largest bays in Mexico (Figure 23).

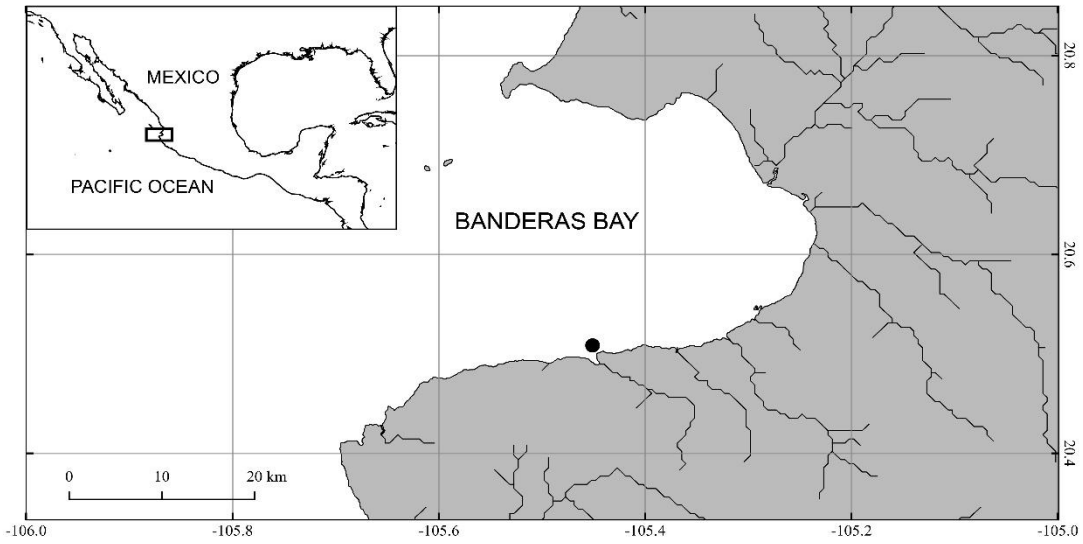


Figure 23: Banderas Bay, and the location of the aggregation area for oceanic manta rays

The bathymetry of the bay is characterized by the presence of a deep canyon (> 2000m depth) located in the southern part of the bay and east-west oriented. The main freshwater input of the bay is the Ameca River, 200 km long and with a drainage basin of >12,000 km^2, while in the southern part of the bay many smaller rivers flow from the adjacent mountainous region (rivers Pitillal, Cuale, Nogalito, Mismaloya, Tomatlán, Quimixto, Tuito, Yelapa) (Cotler Ávalos, 2010). Tropical cyclones are the most important element in the rainfall seasonality of the area, with a rainy season that starts in June and ends in October (García-Oliva *et al.*, 1991).

This bay is characterized by a diverse marine megafauna community, with the presence of 18 different species of marine mammals that are seasonal visitors or residents in the area (Pompa-Mansilla & García-Gutiérrez, 2017), seasonal aggregations of oceanic manta rays (*Mobula birostris*) (Stewart *et al.*, 2016) and visitation by other devil rays (*Mobula* spp.) which are filter feeders that may be particularly impacted by plastic ingestion (Germanov *et al.*, 2018).

6.2 Revillagigedo Archipelago

The Revillagigedo Archipelago is located 250nm south of the Baja California Peninsula, in the Mexican Pacific Ocean, and it comprises three volcanic islands and one islet. The region is characterized by volcanic cones and rift systems with depths of up to 4,856 m (Wilkinson *et al.*, 2009) (Figure 24).

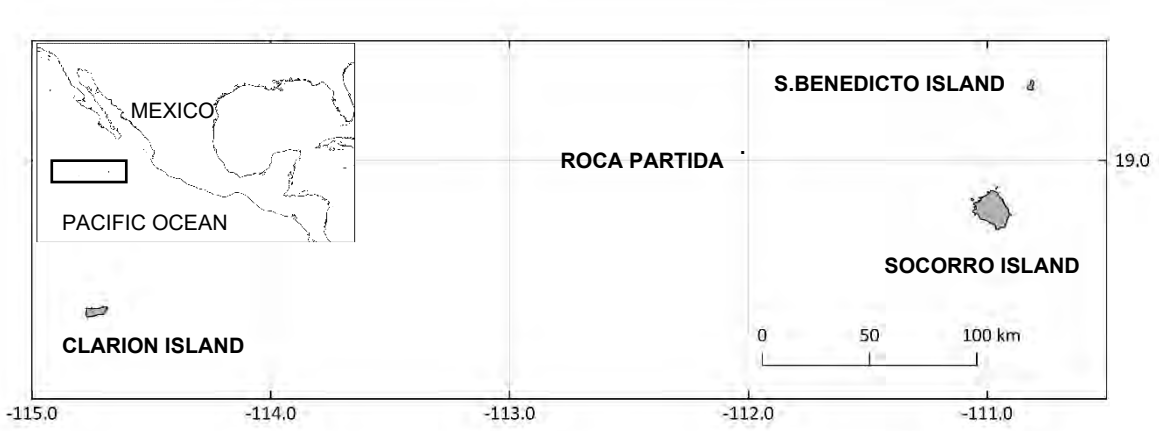


Figure 24: Revillagigedo Archipelago map, with its location in the Mexican Pacific Ocean

In 1994 the archipelago was established as a CONANP Natural Protected Area under the name “Biosphere Reserve”. In 2002 the Mexican government banned commercial fishing within the 9 nautical miles around the four islands and UNESCO declared it world heritage site in 2016. In 2017, the marine protected area was expanded and now comprises 14 million hectares of water surrounding the islands.

The only human presence on the islands is approximately 50 marines to safeguard Mexico's sovereignty of the islands. From November to June, live aboard vessels visit the area taking divers to discover the treasures of the waters surrounding the islands. During summer, the Archipelago is often hit by many hurricanes that form in the Pacific Ocean, so the touristic activities around the islands are forbidden.

These islands host a great terrestrial and marine biodiversity, with 16 endemic species of fish present in the area, sea turtles’ nesting sites, 18 species of marine mammals and a great diversity of elasmobranchs, with 28 species of sharks recorded in the archipelago (CONANP, 2017).

One of the highlights of the Revillagigedo Archipelago is the presence, year-around, of oceanic manta rays, that are particularly friendly with divers. Through photo-identification, the Pacific Manta Research Group has registered more than 830 different individuals in the almost 40 years of investigation in the archipelago (Robert Rubin, pers.comm.).

7 Chapter 1:

Abundance, characterization and seasonality of floating plastics

7.1 Introduction

Plastics are a diverse group of materials derived from oil or gas, and are usually made from these with the addition of various chemical additives (Thompson *et al.*, 2009). Due to their lightweight and durability, plastics are suitable for the manufacture of a very wide range of everyday products. Additionally, they can be a serious hazard in both marine and terrestrial environments (Kühn *et al.*, 2015; De Souza-Machado *et al.*, 2018). It has been estimated that petroleum-based plastic makes up 60–80% of marine debris, the rest being mainly glass and metals (Derraik, 2002).

Understanding the sources of plastic pollution is very important in order to facilitate better debris management and potentially reduce the ingress of plastics into the marine environment (Thompson *et al.*, 2009; Sebille *et al.*, 2012; Eriksen *et al.*, 2014). Every year, an estimated 4.8 to 12.7 million metric tons of plastic debris enters the oceans worldwide from terrestrial sources. But this is only a portion of the total amount of plastics that enters in the marine ecosystem because there is no estimation for other sources of plastic pollution, such as lost fishing gear, inputs from natural disasters (e.g.: tsunamis, hurricanes etc.) and losses from vessels at-sea (Jambeck *et al.*, 2015). Once in the sea, plastic debris has been proven to cause negative impacts at various scales: socio-economic, biological and ecological (Eriksen *et al.*, 2014). These effects are visible not only close to the pollution source, but can also reach remote habitats, due to ocean currents and wind driven transport of floating debris (Barnes *et al.*, 2009; Sebille *et al.*, 2012).

Interactions between megafauna and marine litter have been reported since the 60s (Laist, 1987) through entanglement, ingestion and nest construction, while in the last decades they have also been investigated for potential toxicological effects (Rios *et al.*, 2007; Fossi *et al.*, 2017; Germanov *et al.*, 2018). Through UV degradation and mechanical stress, plastic debris gradually breaks down into smaller particles that adsorb persistent organic pollutants (POPs) (Rios Mendoza & Jones, 2015). These can be transferred to the tissues of the organisms that ingest the plastic and cause toxic effects (Mato *et al.*, 2001; Teuten *et al.*, 2009; Bakir *et al.*, 2014). Smaller pieces enter the food web at a lower level, and the POPs can bioaccumulate and biomagnificate

through the food web, reaching higher concentrations and causing worse effects in long-living and top predator species (Marsili *et al.*, 2016; Germanov *et al.*, 2018; Stewart *et al.*, 2018).

Rivers play a significant role in the offload of land-based plastic debris (Rech *et al.*, 2014) and it has been observed that during the rainy season, increased river flows causes more plastic items to accumulate on beaches close to river sources (Araújo & Costa, 2007; Cheung *et al.*, 2016). Adopting seasonal monitoring in areas with seasonally variable rainfall is important in order to avoid the underestimation of marine plastic debris during the dry season, or overestimation in the rainy season. Many studies have focused on mapping the distribution of plastic debris in the oceans worldwide, mainly in open waters, but less effort has been dedicated to monitoring the floating plastic abundance through time in coastal areas and close to oceanic islands. The high spatial and temporal heterogeneity of plastic debris distribution make trends difficult to discern, both in small and large-scale studies (Goldstein *et al.*, 2013). Understanding the distribution, composition and seasonal abundance of floating debris is prerequisite for the study of its environmental effects on the marine environment (Kang *et al.*, 2015). This study investigates the abundance, seasonality and composition of floating plastics in two sites in the Mexican Pacific Ocean that are important for oceanic manta rays: the southern part of Banderas Bay and the Revillagigedo Archipelago. Due to the high number of protected or endangered species that are present in these areas, an evaluation of the current situation on plastic pollution is important, in order to understand what threats these megafauna species may be facing. The two areas show a very different human presence and ingress of plastic debris: many rivers and creeks discharge into Banderas Bay, which could potentially be a continuous source of plastic pollution in this area, which is exacerbated by hurricane-driven input, while in Revillagigedo the source might be primarly through oceanic currents. Since the removal of plastics from the environment is not a viable way to reduce the current issue of plastic pollution, detecting the main sources of ingress into the environment is the first step in avoiding more plastic debris reaching the ocean, followed by the implementation of effective plastic-reduction solutions (Wessel *et al.*, 2019).

7.2 Materials and Methods

Since for logistic reasons, the sampling methodology in the two areas was different, we will present this case study divided by areas.

7.2.1 Banderas Bay Sampling

Surface sampling of plastic debris took place in the same transect in the southern part of Banderas Bay (BB) (Figure 23) once a week from May 2016 to April 2018, opportunistically during the manta ray monitoring conducted by Proyecto Manta Pacific Mexico (Figure 25). A zooplankton net (0.3 m diameter, 333 µm mesh size) was towed horizontally in the surface waters at a speed of approximately 2 knots, for 5 to 30 min from a small boat. The volume of water filtered through the mesh was determined and standardized using a General Oceanics 2030R mechanical flowmeter attached across the center of the net opening and the area of ocean surface sampled was calculated by multiplying the length of surface water sampled (calculated with the flowmeter) by the width of the net opening (0.3 m). Samples were stored in aluminum foil and kept frozen until analysis.

Figure 25: Activity of search for mantas (left) and the floating plastics collection (right).

7.2.2 Revillagigedo Archipelago Sampling

The floating plastic debris sampling in Revillagigedo Archipelago took place during the non-hurricane season (November-June) from April 2016 to January 2018, opportunistically during 9 citizen science trips onboard the liveaboard vessel Quino el Guardián. A manta net (43.5 cm wide x 14 cm deep, 333 µm mesh size) was towed on the surface of the ocean when sea conditions were calm, at a speed of approximately 2 knots, for 30 minutes from a small dinghy (Figure 26). The sampling was carried out at all of the four islands in the archipelago, in the sites where oceanic manta rays are seen on a regular basis (Roca Partida, Cañon, Boiler, Cabo Pierce, Punta Tosca), and at two sites where the opportunity arose for taking samples during standard naval checks at Socorro Island and Clarion Island. The ocean surface sampled was calculated by multiplying the length of surface water sampled (calculated with a Garmin Etrex 20x GPS) by the width of the net opening (0.435 m), while the volumes of water filtered through the mesh was determined by multiplying the sampled area x the depth of the net (0.14 m). Samples were stored in glass jars and kept frozen until analysis.

Figure 26: Sampling of floating plastics with the use of a manta net towed by a dingy (left), processing of samples and storage (right).

7.2.3 Sample Analysis

Samples collected in both areas followed the same laboratory analysis. Plastic debris was separated from organic material under a Zeiss Stemi DV4 dissecting microscope (8–30× magnifications) and categorized by size increments of 1mm, as well as color

and type (fragment, line, fiber, pellet or film). A Thermo Fisher Scientific microscope Nicolet iN™ 10 FT-IR Spectrometer with attenuated total reflectance (μFTIR-ATR) with germanium crystal (equipped with a liquid nitrogen-cooled mercury cadmium telluride detector) was used to determine composition of the plastic debris identified by visual examination. Particle counts were converted to number of particles per cubic meter of seawater (pp/m^3) by dividing the number of particles found in each sample, by the volume of water filtered. We determined also the number of particles per 1000 square meters of sea surface (pp/1000m^2) by dividing the number of particles found in each sample, by the area of sea surface sampled. Samples were separated into two seasons: dry (November–May) and hurricane season (June–October). In Revillagigedo Archipelago we only took samples from the dry season, so seasonality was not investigated.

7.2.4 Precipitation data

With the aim of determining the possible relationship between floating plastics abundance and rainfalls in Banderas Bay, we used precipitation data measured in a weather station near Puerto Vallarta (29°, 39’40”N, 105°, 14’37”W) as a proxy of fresh water ingress into the Banderas Bay. Data was obtained from the automated weather stations of Mexico’s National Weather Service (Servicio Meteorológico Nacional) at https://smn.cna.gob.mx/es/estaciones-meteorologicas-automaticas. The database included daily measurements of precipitation (mm), evaporation (mm) and minimum and maximum ambient temperature (°C) from January 2007 to December 2017.

7.2.5 Statistical analysis

Tests for potential differences between seasons (dry vs hurricane season) of plastic abundance (pp/m3) were performed. First, the hypothesis that the plastic abundance vector was normally distributed was tested using a Kolmogorov-Smirnov test. This hypothesis was rejected at the 95% confidence level ($D=0.29$; $p<0.05$). Then, a Fligner-Killeen test of homogeneity of variances was applied. We decided to use this test over

the more common tests (such as Levene's or Bartlett's test), because the Fligner-Killeen test is more suitable for non-normally distributed data (Crawley, 2012). Again, the null hypothesis that the variance of plastic abundance in Banderas Bay was equal during dry and hurricane seasons ($FK_{(1,93)}$=18.64; $p<0.05$) was rejected. Since both the normality and homogeneity of variance assumptions were violated, non-parametric tests were applied.

Monthly averages of precipitation and plastic abundance (pp/m^3) were used to test for temporal correlation of the fresh water input proxy and plastic particle abundance in Banderas Bay. A cross-correlation function was used to calculate the linear correlation coefficient of the two time series at different time lags. All statistical analyses were carried out using base functions (ks.test, fligner.test, kruskal.test, ccf) of the R environment (R core team, version 3.3.1, 2016) and Microsoft Office Excel 2016.

7.3 Results Banderas Bay

A total of 94 surface samples were collected in the two years of sampling effort. During March 2017, for logistical reasons no samples were collected. Plastic debris were found in 54 samples, 57% of all tows. We recorded a total of 193 pieces of plastic debris, ranging from 0.5 to 100 mm in length. Most of the plastic pieces (79%) were microplastics (<5mm in length). It was found that the most represented size class was the 1-2mm length (27% of the pieces). The most common type of debris found was film (41%) followed by fragments (40%). No resin pellets or microbeads were detected in any tow.

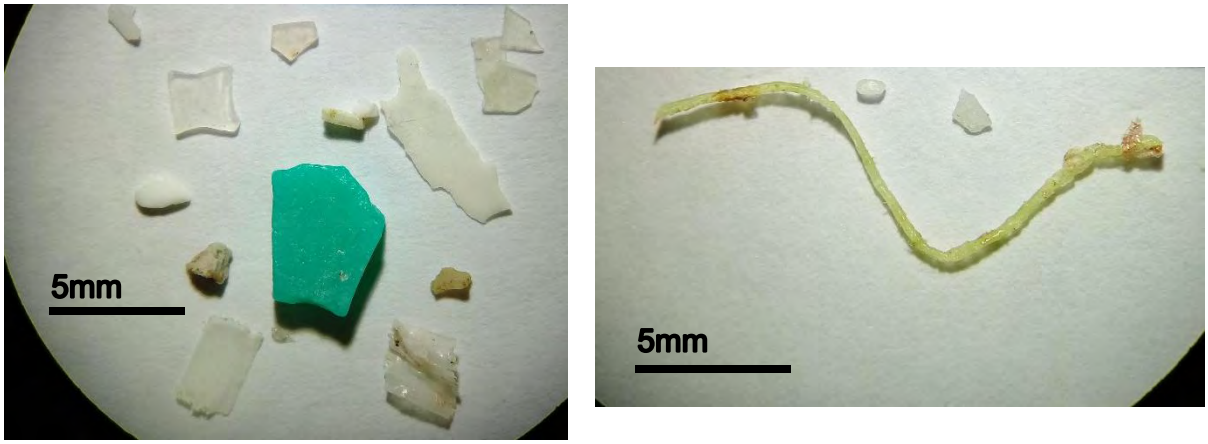


Figure 27: Plastic fragments, film and line found in Revillagigedo (left) and Banderas Bay (right). Scale bar=5mm

All the plastic pieces found had shapes that were far from being spherical (Figure 27), suggesting they resulted from the breakdown of larger items. Most plastics were white (44%), transparent (29%) and blue (11%) while other colors accounted for 16% of all plastic pieces. The μFTIR-ATR revealed that Polypropylene (PP) and Polyethylene (PE) were the most abundant polymers, accounting for 45% and 43% of the pieces, respectively (Figure 28).

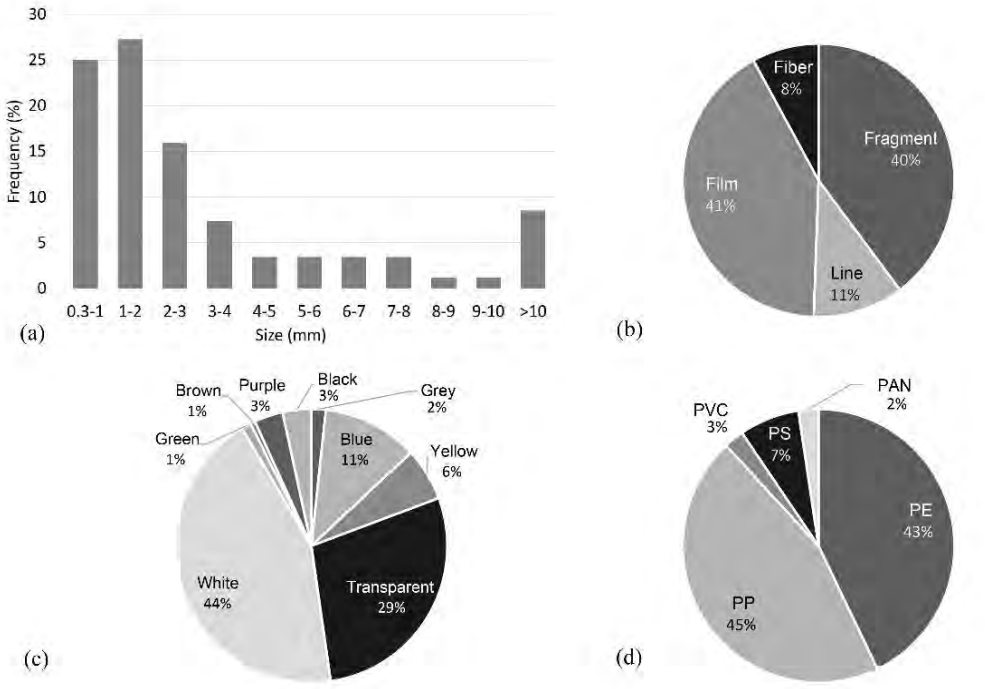


Figure 28:Characterization of floating plastics in Banderas Bay: size classes (a), type (b), color (c) and polymer composition (d).

The highest abundance of pp was found in July 2016, with a maximum of 0.295 pp/m^3 (equivalent to 69 pp/1000 m^2) found in one sample (Figure 29). We found a significantly higher abundance of floating plastics during the hurricane season ($H_{(1,93)}$=14.15;test, p<0.05). The abundance of plastics (mean pp/m^3 ± SD) was 0.013±0.028 in the dry season and 0.044±0.064 in the hurricane season.

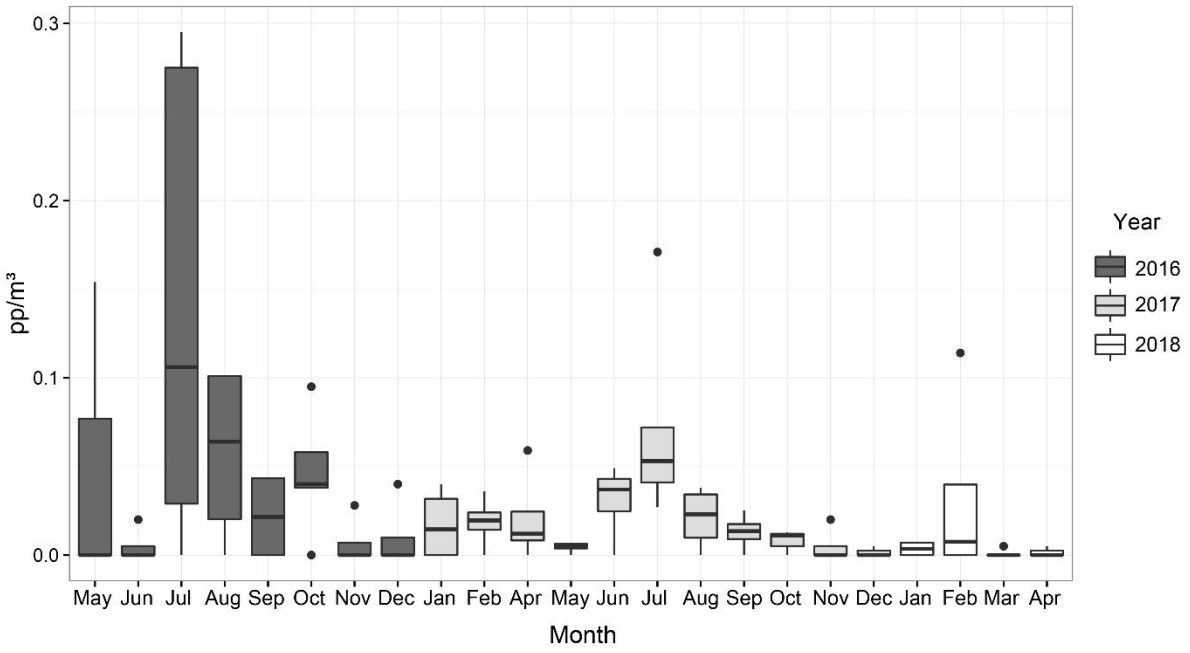


Figure 29: Number of plastic pieces found for every cubic meter in the two years of sampling effort in Banderas Bay

Results of the cross-correlation analysis showed that the correlation between precipitation and plastic abundance time series was higher (~0.50) and statistically significant with +1 month lag, suggesting that higher plastic particle densities in Banderas Bay occur one month after the first peak of precipitation (Figure 30).

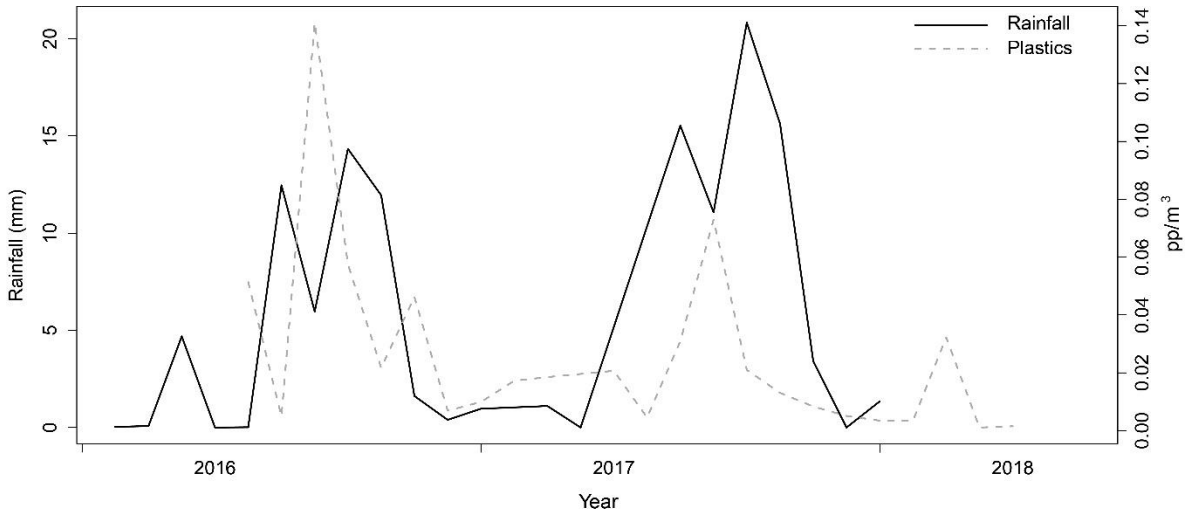


Figure 30: Seasonality of daily rainfalls (mm) and plastic abundance (pp/m^3) in Banderas Bay.

7.4 Results Revillagigedo Archipelago

A total of 47 samples were collected during 9 expeditions to the archipelago. Plastic particles were detected in 42 samples, 89% of all tows. We recorded a total of 180 pieces of plastic debris, ranging from 0.5 to 20 mm of length. Most of the plastic pieces (73%) were microplastics (<5mm in length). The most represented size class was the

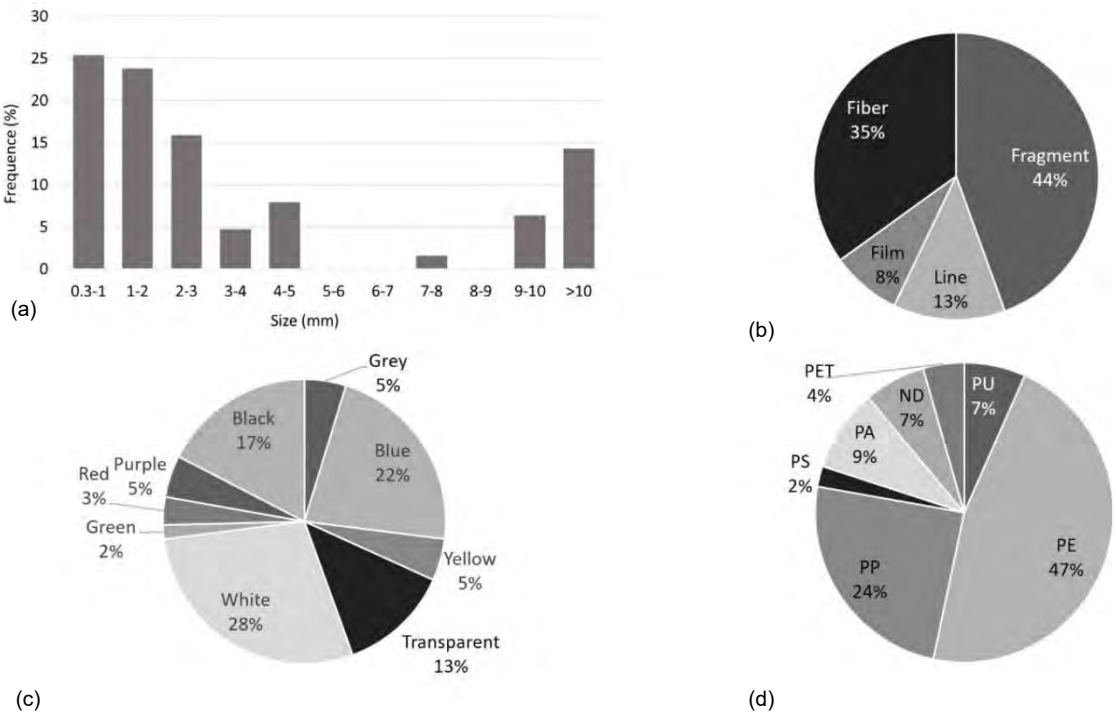


Figure 31: Characterization of floating plastics in Revillagigedo Archipelago: size classes (a), type (b), color (c) and polymer composition (d).

0.3-1mm (26% of the pieces). The most common type of debris found was fragment (44%) followed by fibers (35%). No resin pellets or microbeads were detected in any tow. Most plastics were white (28%), blue (22%) and black (17%) while other colors accounted for 33% of plastic pieces. The µFTIR-ATR revealed that Polyethylene (PE) and Polypropylene (PP) were the most abundant polymers, accounting for 47% and 24% of the pieces, respectively (Figure 31).

The abundance of plastics in volume of water filtered (mean pp/m^3 ± SD) was 0.031± 0.030, while in surface area sampled (mean pp/1000 m^2 ± SD) was 4.768± 4.490.

We looked for a pattern in the floating plastics abundance in the different months sampled, but we found no significant differences between sampled months (November, December, January, March, April, May)($p>0.05$) (Figure 32).

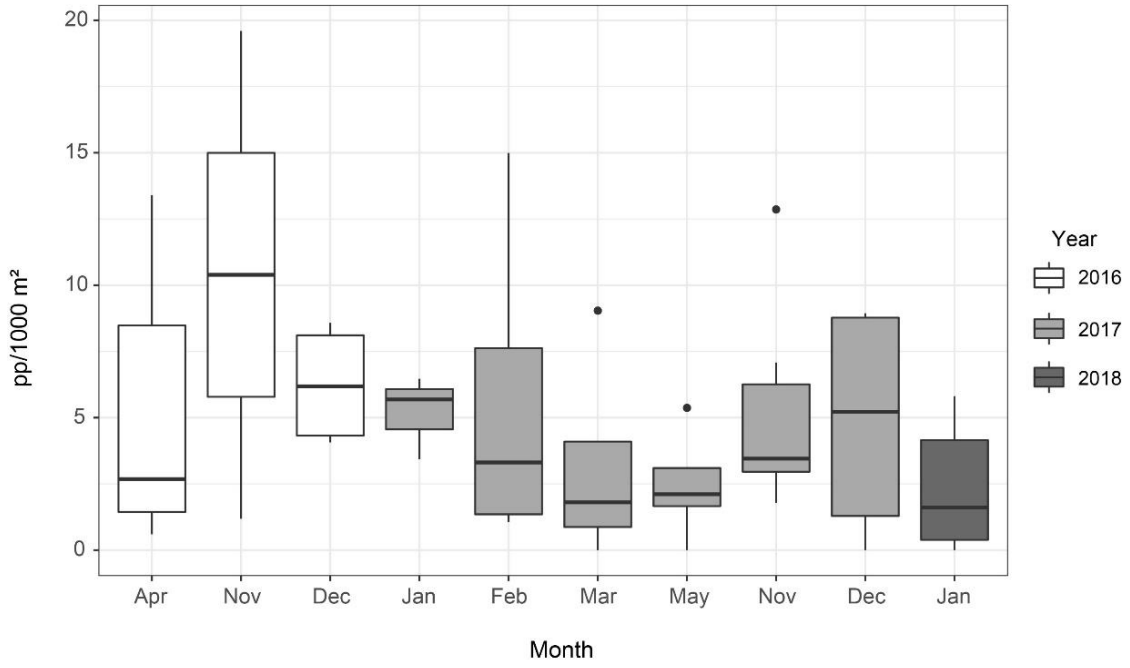


Figure 32: Abundance of floating plastics in the different months and years sampled.

We tested for potential differences in floating plastics abundance at the different islands and sites, but no statistical differences were found ($p>0.05$) (Figure 33, Figure 34).

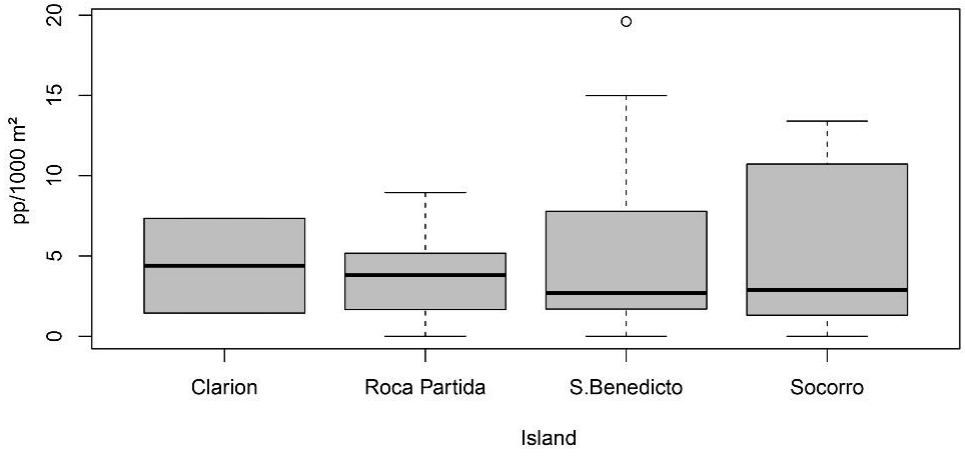


Figure 33: Abundance of floating plastics around the different islands of the Revillagigedo Archipelago. No statistical difference was found ($p>0.05$).

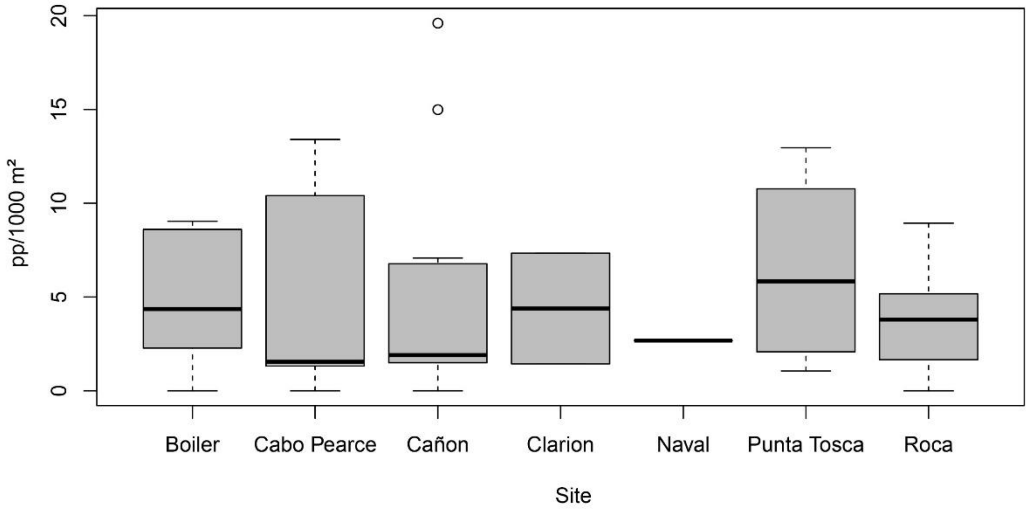


Figure 34: Abundance of floating plastics at the different sites of the Revillagigedo Archipelago. No statistical difference was found (p>0.05).

7.5 Discussion

7.5.1 Banderas Bay

The frequency of plastic debris found in Banderas Bay (57%) was relatively low compared to other studies in more polluted areas. In the Mediterranean Sea, plastic occurs in 90-100% of the samples (Collignon *et al.*, 2012; Suaria *et al.*, 2016), in 96% of the South Pacific Gyre (Eriksen *et al.*, 2013) and in up to 84% of the north-eastern Pacific Ocean (Doyle *et al.*, 2011) (Table 4).

Table 4: Comparison of the occurrence and abundance of floating debris between this study and other coastal areas around the world.

Location	Net/mesh size (μm)	Habitat sampled	Samples with pp (%)	Mean (pp/m^3)	Seasonality	References
S New England	Plankton net/333	Coastal waters	NR	0.01-2.58	ND	(Carpenter, Anderson, Harvet, *et al.*, 1972)
S California	Manta trawl/333	Coastal waters	100*	7.25	Yes	(Moore *et al.*, 2002)
Santa Monica Bay	Manta trawl/333	Coastal waters	100*	3.92	Yes	(Lattin *et al.*, 2004)
NE Pacific Ocean	Manta trawl/505	Coastal, offshore	8-84	0.004–0.19	ND	(Doyle et al., 2011)
SE Bering Sea	Manta trawl/505	Coastal, offshore	25-40	0.017-0.072	ND	(Doyle et al., 2011)
NW Mediterranean	Manta trawl/333	Coastal waters	90	0.116	ND	(Collignon *et al.*, 2012)
Portugal	Neuston net/280	Coastal waters	61	0.002–0.036	ND	(Frias *et al.*, 2014)
Brazil	Neuston net/300	Estuarine waters	NR	0.26*	Yes	(Lima *et al.*, 2014)
SE Coast of Korea	Manta trawl/330	Coastal waters	100*	1.92-5.51	Yes	(Kang, Kwon, *et al.*, 2015)
Mediterranean Sea	Neuston net/200	Coastal, offshore	100	1.25	ND	(Suaria *et al.*, 2016)
S Banderas Bay	Plankton net/333	Coastal waters	57	0.013-0.044	Yes	Present study

**= inferred by the authors, NR= not reported, ND= not determined*

The mean abundance of plastic debris in the present study (0.01-0.04 pp/m^3 in both dry and hurricane seasons, respectively) is low when compared to other floating debris studies (Table 4). This might be due to the relatively low human pressure in the area, compared to the other studies that were conducted in densely populated coastal areas (Mediterranean Sea, Southern California, Korea Sea etc.). Apart ofrom the city of Puerto Vallarta (>300 000 inhabitants plus 5 million tourists visiting every year), the southern part of Banderas Bay has only small rural villages (being part of the Cabo Corrientes municipality with a total of 10 000 habitants) (INEGI, 2015b; a).

The distribution of floating plastics in open ocean environments is very heterogeneous and is driven by different forces (wind, current, tidal flow, source of plastic pollution etc.) (Barnes *et al.*, 2009; Doyle *et al.*, 2011). The waters adjacent to land have been shown to be a zone with elevated plastic abundance, high diversity of polymers and higher proportion of fragments smaller than 2.5 mm (Pedrotti *et al.*, 2016). In Banderas Bay, the most frequent size class of plastic debris was 1-2 mm, followed by 0.3-1 mm, in accordance to what was found by Pedrotti *et al.*, 2016 in the samples collected near the shore in the Mediterranean Sea.

In Banderas Bay, 79% of the total plastics found were smaller than 5mm in length meaning that the plastics in the bay have a much bigger surface area/volume ratio compared to big plastic objects (Ryan, 2015). It means that they can potentially adsorb a much higher quantity of POPs on their surface (Teuten *et al.*, 2009), thus becoming potentially toxic for the many species of wildlife present in the area.

Even though there are more than 5000 different synthetic polymers used in the plastic industry, 80% of all plastic objects are made of polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polystyrene (PS) (Lambert & Wagner, 2018). PE and PP are light polymers that are widely used for packaging, typically single-use disposable products that rapidly pass from the user to the waste and litter stream. PE and PP accounted for 88% of the pp found in Banderas Bay, and the most abundant polymers found in other studies of floating debris (Rios *et al.*, 2010; Reisser *et al.*, 2013; Pedrotti *et al.*, 2016). Due to their prevalence in the environment, PP and PE are also among the most abundant polymers found in the gastrointestinal tracts of Mediterranean megafauna, and the sole fish (Pellini *et al.*, 2018; Claro *et al.*, 2019).

Studies have observed that plastic abundance on the beach is much higher after the rainy season, most likely due to the surface run-off that brings plastic debris from inland areas via rivers and eventually settles on the beaches close to estuaries (Cheung *et al.*, 2016; Brennan *et al.*, 2018; Wessel *et al.*, 2019). The seasonality of floating plastic abundance has been poorly studied. In Table 4, a comparison between the present study and other studies of coastal floating plastics is summarized. Moore *et al.*, 2002

found a greater plastic/plankton ratio in Southern California in the samples collected on the day after a storm and attributed this to land-based runoff.

The authors suggest that the first rain events of the season, might be the source of the highest input of plastics from land-based sources, since during the dry season the debris accumulate and enhance the amount of runoff following the rain events during the hurricane season. This might explain the extraordinarily high amount of floating debris that was found in July 2016 in Banderas Bay. The hurricane season in 2016 started with tropical storm Agatha at the beginning of July and counted a total of eight hurricanes/storms in the Mexican Pacific Ocean during that month. The high abundance of plastics found in July, might be due to the high number of hurricanes and tropical storms that formed in the Mexican Pacific Ocean in that period (CONAGUA, 2016).

Previous studies have reported that the distance from the coast is a proxy of the time spent at sea of plastic objects (Ryan, 2015). Since the samples in Banderas Bay were collected nearshore ($\simeq$ 100m from the coast), we can hypothesize a recent ingress of the plastics found. This is supported also by the low incidence of yellowish plastics (6% of the plastics found), that is considered as an indicator of the oxidation level and time spent in seawater by the plastics (Endo *et al.*, 2005; Ogata *et al.*, 2009). For this reason, we hypothesize that the high abundance of plastic debris in the summer originates from rainfall caused by hurricanes that hit the area and induce the runoff of plastic pollution from adjacent land into the ocean (Lima *et al.*, 2014). Moore *et al.,* 2002 observed that neustonic coastal samples show a higher abundance of small fragments compared to neuston from the North Pacific central gyre, where most of the plastic mass comprised large objects. These small fragments near the coast are attributable to land-based runoff, while the large objects in the open ocean are associated mainly with the fishing and shipping industries.

Since the prevailing currents inside Banderas Bay should spread the debris off-loaded by the Ameca River mainly into the central northern part of the bay (Pantoja, 2017), it is likely that our results are showing only a small percentage of the actual load of debris present in the Bay. The plastics found in the present study may originate from the

smaller communities surrounding the numerous rivers that flow into the bay from the mountainous southern coast, in proximity to the sampling site (rivers Pitillal, Cuale, Nogalito, Mismaloya, Tomatlán, Quimixto, Tuito and Yelapa) (Cotler Ávalos, 2010).

7.5.2 Revillagigedo

The frequency of samples containing plastic debris found in Revillagigedo Archipelago (89%) is consistent with other studies that have analyzed floating plastics in offshore habitats all over the world, and found that they ranged from 50 to 100% (Table 5).

Table 5: Comparison between Revillagigedo Archipelago floating plastics, and other offshore studies

Location	Net/mesh size (μm)	Habitat sampled	Samples with pp(%)	Mean (pp/km^2)	References
Sargasso Sea	Neuston net/ 330	Offshore	100	3,500	(Carpenter *et. al.*, 1972)
Caribbean Sea	Neuston net/947	Offshore	50	1,292	(Colton *et al.*, 1974)
Antilles Sea	Neuston net/947	Offshore	57	2,707	(Colton *et al.*, 1974)
N Pacific Gyre	Manta trawl/333	Offshore	NR	334,271	(Moore *et al.*, 2001)
N Atlantic Gyre	Neuston net/ 335	Offshore	62	20,328	(Lavender *et al*., 2010)
Caribbean	Neuston net/ 335	Offshore	62	1,414	(Lavender *et al*., 2010)
Maine Gulf	Neuston net/ 335	Offshore	62	1,534	(Lavender *et al*., 2010)
S Pacific Gyre	Manta trawl/333	Offshore	96	26,898	(Eriksen *et al.,* 2013)
Australia	Manta,neuston/333	Coastal, offshore	80	1,932	(Reisser *et al*., 2013)
Mediterranean	Neuston net/200	Offshore	100	243,853	(Cózar *et al*., 2015)
Mediterranean	Neuston net/200	Coastal, offshore	100	400,000	(Suaria *et al.*, 2016)
Revillagigedo	Manta trawl/333	Offshore	89	4,769	Present study

NR= not reported

The mean abundance of floating plastics in the waters surrounding the archipelago is comparable with other studies in offshore seas, but is two orders of magnitude lower than convergence zones (gyres) and the heavily polluted Mediterranean basin (Table 5). This might be due to the almost null human activity in the area, that makes local input of plastic debris very low, and the water circulation, which is the main driver of floating plastics distribution in the oceans (Eriksen *et al.*, 2014). We hypothesize that the plastic debris (micro and macro) that we found came to the archipelago through oceanic currents that brings debris from coastal continental areas, and from fishing activities outside of the protected area, as well as illegal fishing that is still happening close to the islands (Mike McGettigan, pers. comm.).

Although the mesh size was the same, we have to be careful when comparing the abundance results obtained in Revillagigedo Archipelago with the ones from Banderas Bay, since we used two different types of net (manta net, 14x43.5 cm/zooplankton net, 30 cm diameter) which could slightly bias the results.

As in Banderas Bay, we found a prevalence of PE and PP in Revillagigedo Waters. This is consistent with other studies (Rios *et al.*, 2010; Reisser *et al.*, 2013; Pedrotti *et al.*, 2016), as PE and PP share the biggest slice of the plastics production industry at world level and find applications in consumer products that are often for single use. These polymers are lighter than sea water and, for this reason, they float and can be easily transported by oceanic currents and winds (Ryan, 2015).

The low incidence of film-like plastics (8%) is characteristic of offshore habitats, since this type of debris has a high surface area/volume ratio, that makes them more easily fouled, and subsequently they sink closer to the source of pollution (Ryan, 2015). In the coastal samples taken in Banderas Bay, for example, the percentage of film was much higher (41%), since the plastic input is recent and from customer products such as packaging and plastic bags, among others.

The small size of the debris found (the <1mm size class was the most represented, at 26%) is in line with what was found in the North Pacific Gyre by Moore *et al.*, 2001, who found that 53% of the plastic pieces were smaller than 1mm. Plastics undergo photooxidation and fragmentation during their permanence in the environment, therefore a greater abundance of smaller particles would be expected in a remote area, such as Revillagigedo Archipelago, far away from the source of plastic pollution. Big plastic objects discarded at sea are more likely to be fouled, and therefore sink to the depths (Moore *et al.*, 2001). For this reason, it is expected that most of the plastics that float in the oceans will be of smaller sizes in the future (Morét-Ferguson *et al.*, 2010). This is particularly alarming, since smaller pieces can be ingested by zooplankton and filter-feeders, that are at the base of the food web. With an increasing amount of small plastic debris in the oceans, we expect a higher incidence of microplastic ingestion from the very first food web levels, all the way up to the top predators.

Due to the impossibility of sampling at Revillagigedo Archipelago during summer, we cannot say if there is a seasonality to the floating plastics abundance, as we found in Banderas Bay. This lack of information does not allow us to make any predictions, and it could be an interesting question to address in future studies in the area. This might have positive or negative consequences for the seasonal visitors of the archipelago, such as humpback whales, which, being filterfeeders, could potentially be exposed to ingesting plastics during their winter visits to the archipelago (Fossi *et al.*, 2014).

This is, to our knowledge, the first study that investigates the abundance of floating plastics in Revillagigedo Archipelago, and it will be a baseline for future studies and, potentially, for an on-going monitoring of the plastic pollution in the area.

8 Chapter 2:

Persistent organic pollutants (POPs) adsorbed on plastics

8.1 Introduction

Plastics have had an exponencial growth in the market, and their popularity can be attributed to some of their physical properties: they are lightweight, corrosion resistant, waterproof and durable (Vegter *et al.*, 2014). Add to that their low production cost, and it easy to understand why plastic production and its applications has grown so fast and continuously over the last decades (Thompson *et al.*, 2009). Their apolarity of plastics makes them useful as containers for liquids, waterproof packaging and many other uses. Nevertheless, because of their apolarity, they also tend to accumulate on their surface all the greasy and non-polar substances like oils and other hydrophobic substances.

During its permanence in the ocean, plastics are exposed to seawater that can contain different toxic compounds, which can be adsorbed on their surface (Rios *et al.*, 2010). Floating plastics in particular, can be transported by currents through very polluted areas, where they can adsorb persistent organic pollutants (POPs) and subsequently be a vector for these pollutants in the marine organisms that ingest them (Bakir *et al.*, 2014; Zhang *et al.*, 2015). Small plastic particles, like virgin resin pellets and microplastics derived from the fragmentation of larger objects, have a higher surface area/volume ratio, and therefore can potentially adsorb many more pollutants than big plastic objects, accumulating toxic compounds up to 10^5-10^6 times than the surrounding seawater (Mato *et al.*, 2001). Plastic debris that has been in the water for a longer time, can also accumulate higher amounts of pollutants, and the concentrations can vary between individual objects by up to three orders of magnitude, depending on the origins and pathway that each piece of plastic has followed with the current (Endo *et al.*, 2005).

These plastics can end up in oceanic gyres, accumulate on beaches or sink to the seafloor. In all of these scenarios, plastics are highly likely to be ingested by organisms, that can selectively feed on them or passively ingest them during filter-feeding activity (Moore, 2008). In laboratory experiments, it was proven that pollutants carried by synthetic polymers can leach under gastric conditions and migrate to the tissues of animals ingesting contaminated debris, especially in warm blooded organisms (Bakir *et al.*, 2014).

For this reason, it is very important to determine which toxic compounds are present on plastics, to be able to understand which pollutants the animals in a determinated area are exposed to. This is particularly so in marine protected areas, where vulnerable species are protected from some human impact, but artificial borders do not stop plastics from entering an area and posing a risk to vulnerable habitats and species. We therefore decided to analyze the toxic chemicals that are adsorbed on plastics in two crucial habitats for oceanic manta rays in the Mexican Pacific Ocean: Banderas Bay and the Revillagigedo Archipelago. This, in order to understand which background levels of pollutants oceanic manta rays (and all the other vulnerable species that inhabit the areas) are exposed to.

8.2 Materials and Methods

Samples of floating microplastics were collected in Revillagigedo Archipelago and Banderas Bay from April 2016 to March 2018, as described in Chapter 1. Due to the small size of the plastics collected, and their low weight, we decided to group together several plastic pieces, in order to have a satisfactory sample weight. The microplastics, after the characterization and polymer determination, were grouped together by area and sampling year (April 2016-March 2017, April 2017-March 2018).

In Banderas Bay, the samples of macroplastics were collected from the beach near the village of Yelapa, which was the starting point of the sampling trips for floating plastics and manta ray monitoring. Samples were collected in May 2018 at 20°29'20.4"N 105°26'55.0"W. Collected macroplastics were stored in pre-cleaned (for 4 hours at 400°C) glass jars and stored at -20°C until analysis. We analyzed a randomly selected sub-sample of each type of plastics (fragments, film, cables, etc.).

In Revillagigedo the samples of beached macroplastics were collected during a sampling trip to the Archipelago in March 2017. We obtained all the permits necessary for landing on the islands (SEGOB oficio n° UG/211/00093/2017, CONANP oficio n° F00.DRPBCPN.DIR.RBAR.-036/2017, expediente sirca: F00.1.DRPBCPN.-00015/2017). During the Citizen Science trip, we landed on Playa el Cañon (San Benedicto Island, 19°18'04.4"N 110°48'33.4"W) and Cabo Pierce Norte (Socorro Island, 18°47'56.3"N 110°55'01.7"W). We collected samples from objects found on the beach (Figure 35), approximately 2 g of plastics from randomly selected objects. The samples were stored in glass jars previously cleaned for 4 hours at 400°C and frozen until analysis, to avoid any contamination of POPs from the samples.

Figure 35: Some of the debris collected from San Benedicto Island (left) and Socorro Island (right).

The synthetic polymer composition of plastic samples was determined through the use of a Thermo Fisher Scientific microscope Nicolet iN™ 10 FT-IR Spectrometer with attenuated total reflectance (μFTIR-ATR) with germanium crystal (equipped with a liquid nitrogen-cooled mercury cadmium telluride detector) (Figure 36), comparing the obtained spectrum with an internal library obtained from virgin plastics, in the laboratories of the University of Wisconsin Superior.

Figure 36: The μFT-IR used for the determination of the polymer of plastic debris.

A mass of ~1g of plastic debris from each sample (in the case of microplastics from net samples, we used ~0.1g) was used to be extracted in a Soxhlet system for 24h in 150mL of dichloromethane (DCM), at a speed of 6cycles/h (Figure 37 left). For every set of 5 samples, a blank was extracted as a quality control. Surrogate standards were used: deuterated standards (1,4-dichlorobenzene-d4, naphthalene-d8, phenanthrene-d10, acenaphthene-d10, perylene-d12, chrysene- d12) for PAHs, tetrachloro-m-xylene (TCMX) and PCB- 209 for PCBs and organochlorine pesticides. The recovery standard was DBOFB (4,4-dibromooctafluorobiphenyl) for PCBs and pesticides, and p-terphenyl-d14 for PAHs.

After the Soxhlet extraction, the extract was concentrated to 1 mL using a water bath (Figure 37 right) and subsequently the samples were cleaned using a liquid chromatography: they were passed through a glass column packed with silica at 5% deactivated with water HPLC grade, in order to separate the toxic compounds to analyze. Three factions were eluted and concentrated to 1mL in a water bath, and a gentle flow of nitrogen gas was used to evaporated the sample to 1 mL or 100 µL.

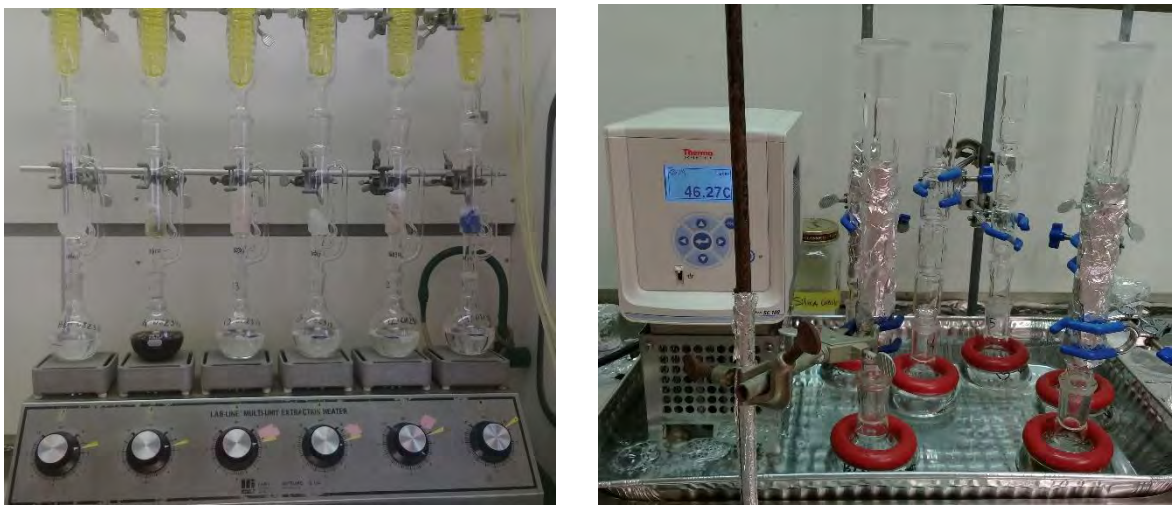


Figure 37: The Soxhlet extraction (left) and the concentration water bath (right).

Samples that showed some solid particles were passed through a GF/F Whatman Filter before injecting the samples into a GCMS. Samples were then injected in an Agilent 7890A GC System equipped with an Agilent 5975C mass spectrometer (with triple axis detector) that was operated under selected ion monitoring mode (SIM), using one ion for quantification and two ions for confirmation for each analyte (Supplementary data: Table 18, Table 19, Table 20).

In order to avoid sample contamination, all glassware was cleaned and baked in a furnace for 4 hours at 400°C prior to use. Volumetric material was washed twice with acetone, hexane and dichloromethane (all HPLC grade). Procedural blanks were analyzed for quality control to check for any sources of contamination during the analytical procedures. For each analyte, a procedural blank value was used for subtraction. The limit of detection (LOD) was set at 3 times the standard deviation of the ration of areas of standards (RAS) noise, while the limit of quantification (LOQ) was set at 10 times the standard deviation of the RAS (Vial & Jardy, 1999)

(Supplementary Data: Table 18, Table 19, Table 20). All PAHs, pesticides and PCBs are reported on ng/g of plastic basis.

The toxic potential of PAHs was analyzed based on the reference values for TEL (Threshold Effect Level) and PEL (Probable Effect Level), for marine sediments (Buchman, 2008) because there are no criteria for plastics yet. The eleven considered compounds for TEL and PEL are naphthalene, 2-methylnaphtalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo [a]anthracene, chrysene, and the ΣPAHs, according to the National Oceanographic Atmospheric Administration (NOAA) guidelines.

In order to determine the possible source of PAHs, different ratios of PAH isomers were calculated (Fisner *et al.*, 2013):

- anthracene/anthracene + phenanthrene (Ant/(Ant + Phe)), where values <0.10 indicate the dominance of petrogenic sources and >0.10 the dominance of pyrolytic sources;
- fluoranthene/fluoranthene + pyrene (Fluo/(Fluo + Py)), where values <0.40 indicate the dominance of petrogenic and >0.40 the dominance of pyrolytic sources;
- benz[a]anthracene/benzo[a]anthracene + chrysene (BaA/(BaA + Ch)), where values <0.20 indicate the dominance of petrogenic sources, 0.20–0.35 a mixture of sources, and >0.35 dominance of pyrolytic sources.

8.3 Results

A total of 15 samples of plastics were collected from both areas (five from Banderas Bay, 10 from Revillagigedo). Of these, three were of microplastics sampled from the sea surface and 12 of macroplastics collected on the beaches from both areas.

Of the 18 PAHs analyzed, 14 different PAHs were detected in the samples. Standard recoveries varied from 56 to 110%, which is acceptable for this type of analysis (Fisner *et al.*, 2013). Only in three samples out of 15, PAHs were not detected at LOD, the concentration of total ΣPAHs varied significantly even in samples collected in the same sites: ΣPAHs ranged from 3 to 7997 ng/g (Figure 38, Table 21). The most abundant PAHs were: Fluorene>Phenanthrene>Acenaphthylene>Napthalene. The percent composition of the different PAHs detected in each plastic sample is summarized in Figure 39. In BB, we found an extremely high concentration (7997 ng/g) of ΣPAHs in one sample of packaging plastics collected on the beach.

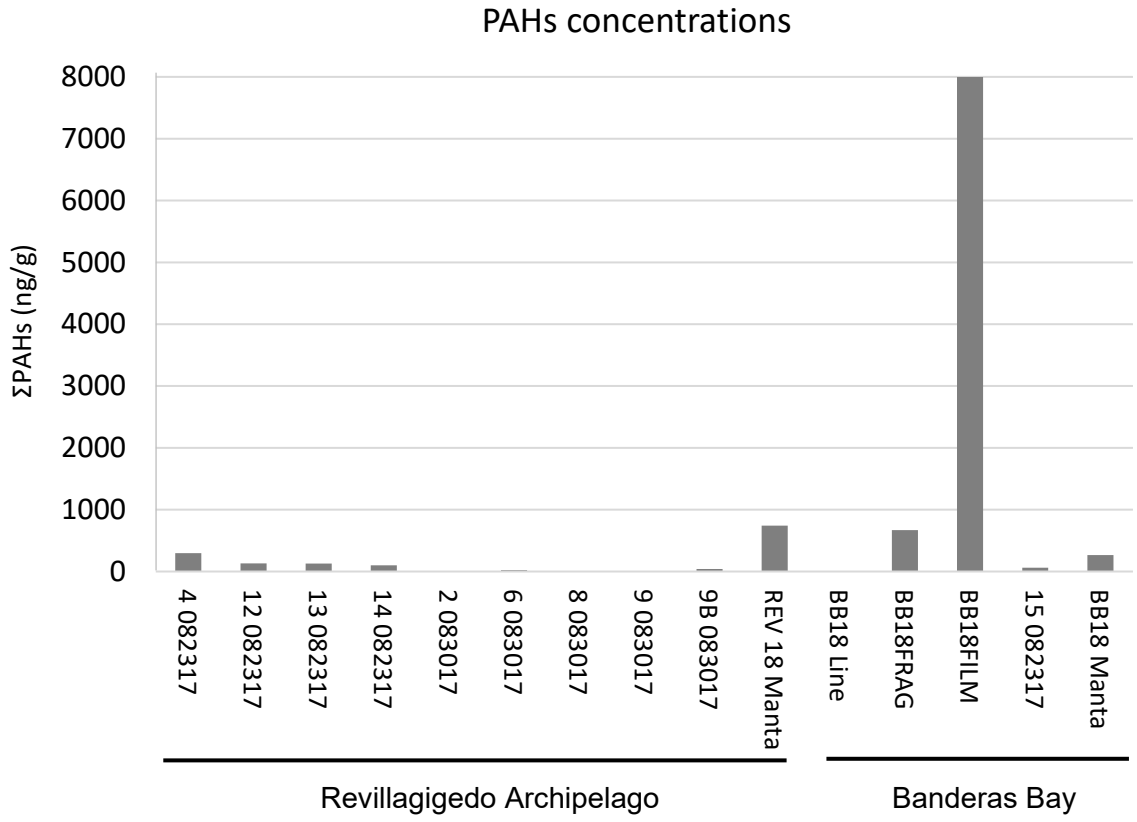


Figure 38: PAHs content in the samples collected on the beaches and floating in the two study areas.

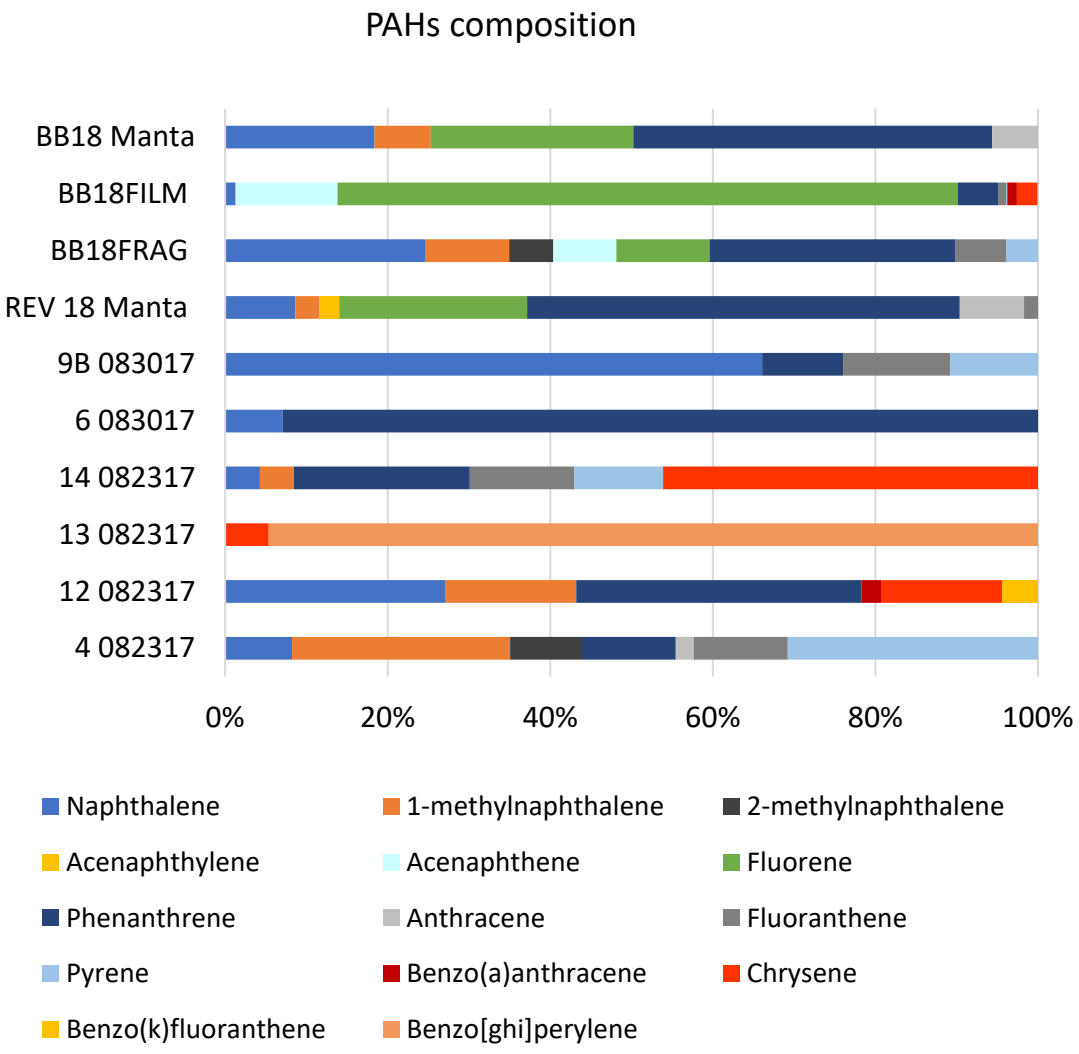


Figure 39: Composition (%) of the different PAHs detected in the samples. Only samples with detectable levels of PAHs are shown. In red, are the three carcenogenic PAHs that were detected in plastic samples (Benzo(a)anthracene, Chrysene and Benzo(k)fluoranthene).

In order to determine the potential sources of PAHs, we calculated the ratios between different PAHs isomers. In Table 6, a summary of the three ratios is provided, with the respective probable origin of the PAHs for each sample.

Table 6: PAHs isomers ratios and probable origin.

	Sample	Ant/(Ant+Phe)	Origin	Fluo/(Fluo+Py)	Origin	BaA/(BaA+Ch)	Origin
AR	*4 082317*	0.16	pyrolytic	0.27	petrogenic	NA	
	12 082317	NA		NA		0.14	petrogenic
	13 082317	NA		NA		NA	
	14 082317	NA		0.54	pyrolytic	NA	
	2 083017	NA		NA		NA	
	6 083017	NA		NA		NA	
	8 083017	NA		NA		NA	
	9 083017	NA		NA		NA	
	9B 083017	NA		0.55	pyrolytic	NA	
	REV 18 Manta	0.13	pyrolytic	1.00	pyrolytic	NA	
BB	*BB18 Line*	NA		NA		NA	
	BB18FRAG	NA		0.62	pyrolytic	NA	
	BB18FILM	NA		0.87	pyrolytic	0.32	pyrolytic
	15 082317	NA		NA		NA	
	BB18 Manta	0.11	pyrolytic	NA		NA	

Ant = anthracene; Phe = phenanthrene; Fluo = fluoranthene; Py = pyrene; BaA = benzo[a]anthracene; Ch = chrysene. NA= isomers not detectable in the sample.

Of the 40 PCB congeners analyzed, we detected only 12 PCBs were detected only in five of the samples collected at Revillagigedo Archipelago, while Banderas Bay samples had no detectable PCBs. Concentrations of ΣPCBs ranged from 0.37 to 127 ng/g (Figure 40, Table 22). The most abundant congeners were PCB-128 and PCB-156. The highest concentration of ΣPCBs was found in the microplastic samples collected from the water at Revillagigedo during the 2017/2018 season. Of the six PCB congeners considered as indicators of PCBs in food by the World Health Organization (WHO), only PCB-28 was detected in 4 out of 15 of our samples. In Figure 41, a summary of the composition (%) of PCBs in the samples with detectable levels of PCBs is provided.

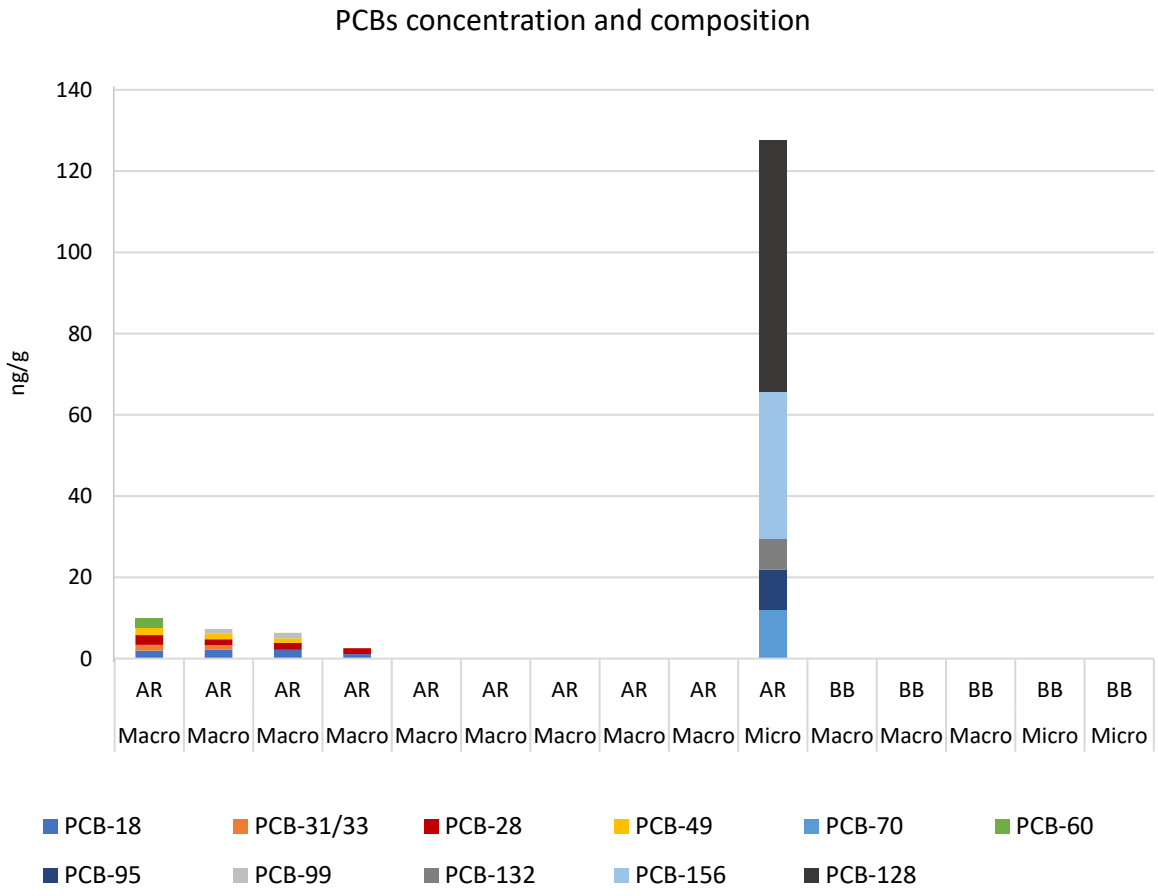


Figure 40: PCBs concentrations and congeners composition. On the x-axis are the individual samples, AR= Revillagigedo, BB= Banderas Bay, Macro= macroplastics sample, Micro= microplastics sample

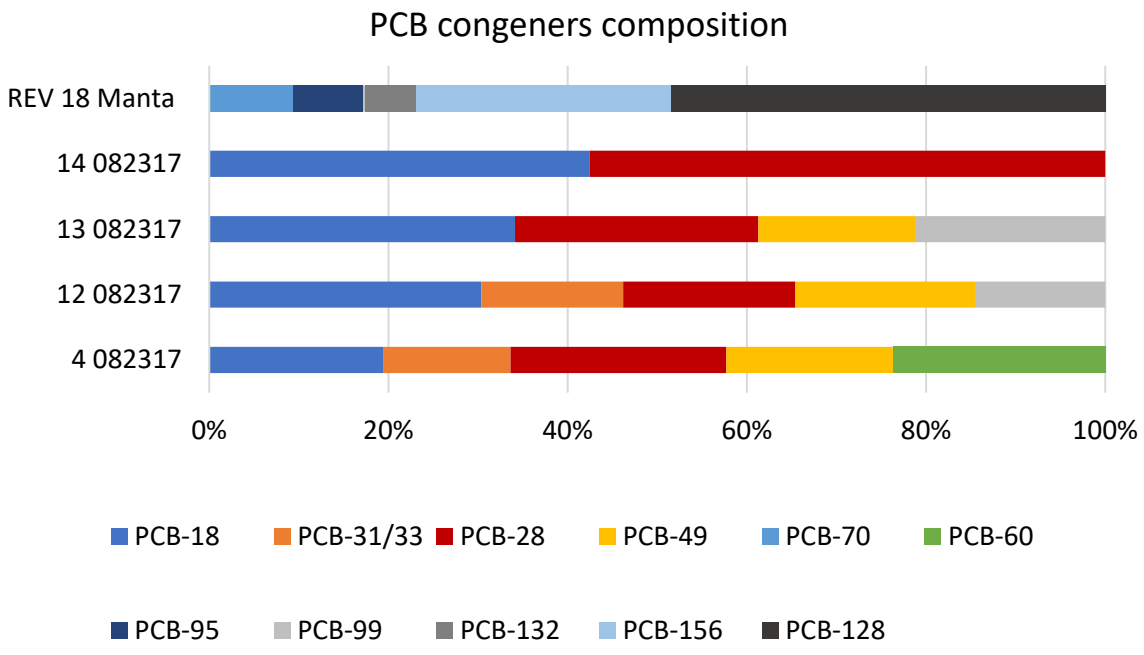


Figure 41: Composition (%) of the PCBs in plastic samples. Only samples with detectable levels of PCBs are shown. In red, is the PCB-28 that is the only PCB indicator found in the samples.

Pesticides were detected in only three samples, but in very high concentrations. Of the pesticides analyzed, only DDTs and chlordanes were detected in the samples. DDTs were the most commonly found pesticides, found in two out of the three samples containing pesticides, at concentrations of 810 and 1061 ng/g. The highest concentration was found in microplastics collected in Banderas Bay (Table 23). Two out of the three samples of microplastics contained pesticides, while only one out of 12 samples of macroplastic showed detectable levels of pesticides. In Revillagigedo, the sample containing DDTs was collected on the beach in San Benedicto Island, while α-chlordan, γ-chlordan, trans and cis-nonachlor were detected in microplastics collected from the sea surface during the 2017/2018 season (Figure 42).

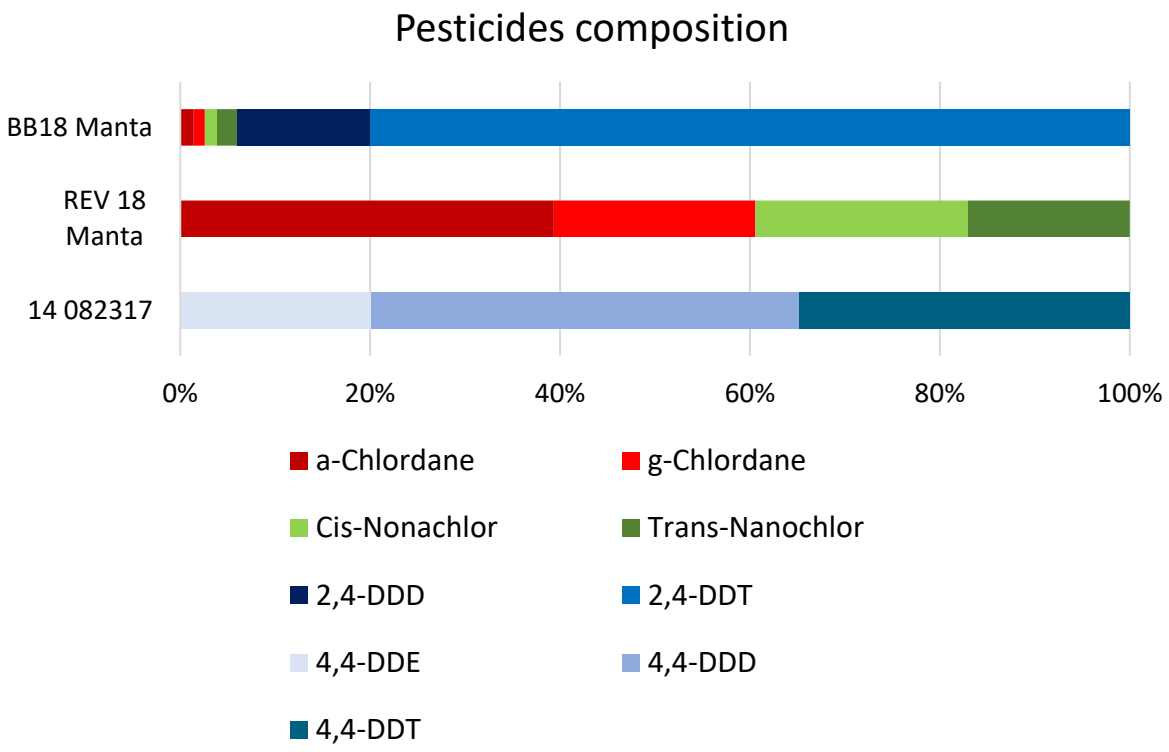


Figure 42: Pesticides composition (%) in the plastic samples. Only samples with detectable levels of pesticides are shown. In red are chlordanes, in green nonachlors and in blue DDTs.

In Figure 43, a summary of the content of the different pollutants analyzed (PAHs, PCBs and organochlorine pesticides) is provided. PAHs were the most abundant pollutants detected in the plastics, followed by pesticides, while PCBs were detected in lower concentrations and in only 5 samples.

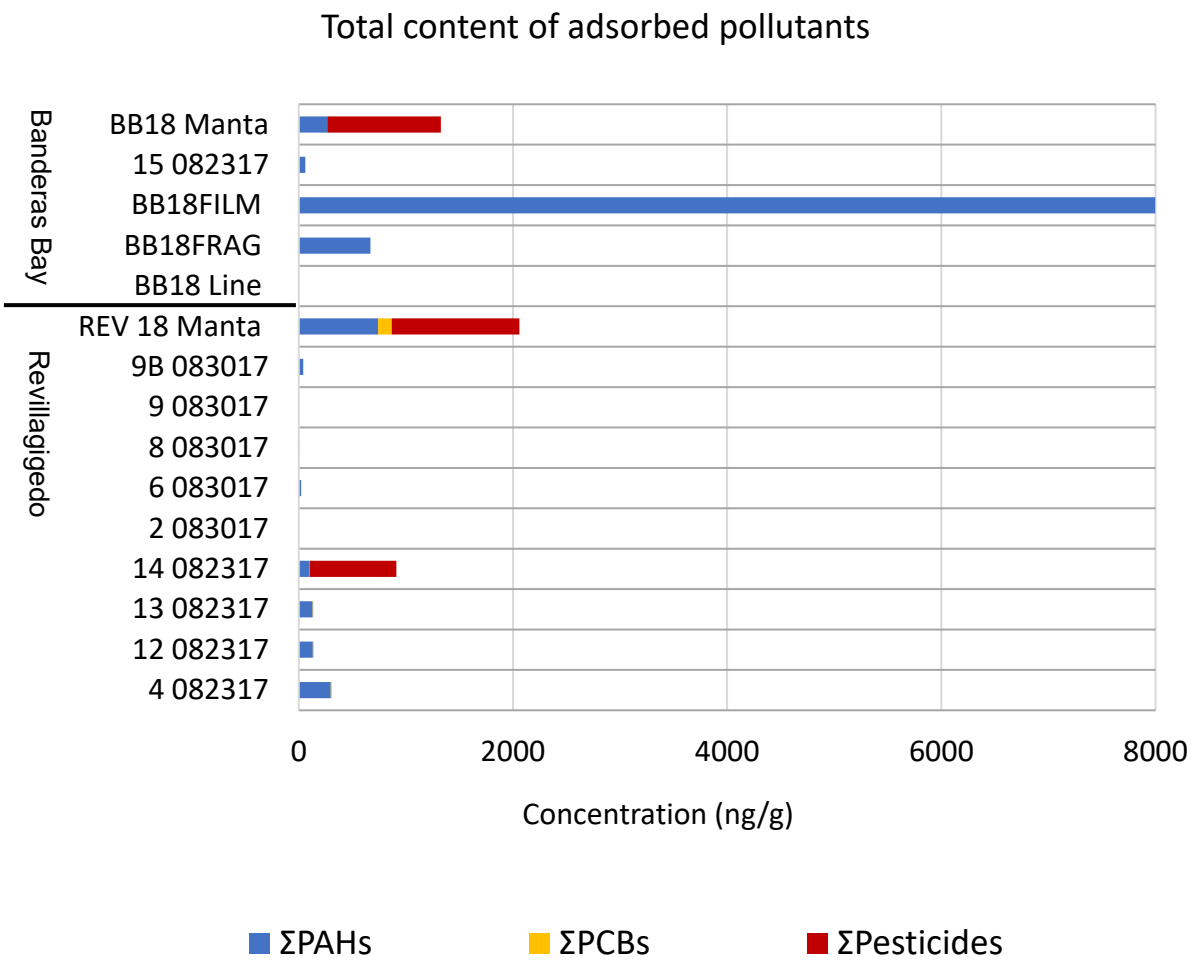


Figure 43: Content of adsorbed pollutants detected in each plastic sample.

8.4 Discussion

Our results are, to our knowledge, the fist data on persistent organic pollutants adsorbed on plastic debris in Mexico. It is interesting to see how our research represents a baseline at national level, when the first reports of this type of pollution were published for the first time in 1972, 47 years ago.

The concentration of adsorbed pollutants in plastic debris can be very variable, since the amount and diversity of pollutants adsorbed depends on the path that the plastic has followed in the ocean. Plastics that have travelled near industrial, agricultural and urban areas are more susceptible to the accumulation of higher amounts of pollutants. Nevertheless, both polluted plastics and “cleaner” plastics can reach common areas, where there can be a high variability in the load of pollutants for each individual plastic object (Endo *et al.*, 2005).

The Environmental protection agency (EPA) has classified the following seven PAH compounds as probable human carcinogens: benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(ah) anthracene, and indeno(1,2,3-cd) pyrene. Of these, three (benz(a)anthracene, chrysene and benzo(k)fluoranthene) were detected in plastics from our study area, at variable concentrations (ranging from below detection limit to 201 ng/g of plastics for chrysene). These carcinogenic compounds were detected in both macro and micro plastics, meaning that the ingestion of the debris could lead to the desorption of toxic PAHs in the ingesting organisms. Genotoxic effects for some PAHs have been demonstrated both in rodents and in vitro tests using mammalian (including human) cell lines. Genotoxicity plays an important role in the carcinogenicity process and could lead also to teratogenicity, with toxic effects on developing embryos (Abdel-Shafy & Mansour, 2016).

In Table 7, a summary of the individual and ΣPAHs concentrations in the present study are compared to the TEL (Threshold Effect Level) and PEL (Probable Effect Level) used by the National Oceanic and Atmospheric Administration (NOAA) for marine sediments, since no reference exists about plastics yet (Fisner *et al.*, 2013). The TEL and PEL are used to identify the following three ranges of chemical concentrations with

regard to biological effects: below the TEL, adverse effects rarely occur; between the TEL and PEL, adverse effects occasionally occur and above the PEL adverse effects frequently occur. Only a few PAH concentrations above the PEL were observed in our study, and are marked in red. The concentrations that are above the TEL, are marked in yellow. Through the TEL and PEL, NOAA identifies potential impacts to coastal resources and habitats likely to be affected by hazardous substances. Therefore, the presence of plastics containing these levels of PAHs may pose a risk to the environment and the local species.

Table 7: Individual and ΣPAH concentrations (ng/g) used for comparison with the TEL and PEL reference values of the NOAA Screen Reference Tables (Buchman, 2008).

	TEL	PEL	4 082317	12 082317	13 082317	14 082317	6 083017	9B 083017	REV 18 Manta	BB18FRAG	BB18FILM	BB18 Manta
Naphthalene	34.6	391	24.5	35.6	ND	4.23	1.5	27.3	63.9	165.2	102.5	48.7
2-methylnaph.	20.2	201	26.3	ND	ND	ND	ND	ND	ND	36.3	ND	ND
Acenaphthyl.	5.8	128	ND	ND	ND	ND	ND	ND	18.2	ND	ND	ND
Acenaphthen.	6.7	88.9	ND	ND	ND	ND	ND	ND	ND	51.8	1003	ND
Fluorene	21.2	144	ND	ND	ND	ND	ND	ND	170.9	76.7	6104	66.0
Phenanthrene	86.7	544	34.3	46.1	ND	21.4	20.9	4.1	394.3	202.6	392.9	116.9
Anthracene	46.9	245	6.6	ND	ND	ND	ND	ND	58.6	ND	ND	14.9
Fluoranthene	113	1494	34.5	ND	ND	12.7	ND	5.4	12.5	41.9	79.9	ND
Pyrene	153	1398	91.7	ND	ND	10.7	ND	4.4	ND	25.9	11.8	ND
Benzo(a)anthrac.	74.8	692	ND	3.1	ND	ND	ND	ND	ND	ND	94.0	ND
Chrysene	108	846	ND	19.7	6.9	45.6	ND	ND	ND	ND	201.5	ND
ΣPAHs	1684	16770	298	131	129	98	22	41	740	669	7997	265

PAHs' solubility in water is low and decreases with increasing molecular weight. They are therefore lipophilic and their concentration in water is very low (Kafilzadeh *et al.*, 2011). PAHs accumulation in coastal habitats is due to both anthropogenic and natural emissions. Among anthropogenic factors, petrogenic and pyrolytic sources are the most predominant. Pyrolytic sources include combustion (fossil fuel combustion, forest fires, etc.), while petrogenic input is related to petroleum products, such as oil spills and asphalt. The PAH composition reflects the source(s) from which the PAHs were derived: analyzing the ratios of PAHs isomers, it is possible to speculate on the source of the pollution (Kafilzadeh *et al.*, 2011). Phenanthrene occurs in greater concentration in oil, whereas anthracene is generally associated with pyrolytic sources. The Ant/(Ant + Phe) ratio ranged between 0.11 to 0.16, therefore suggesting a pyrolytic source in the samples with detectable levels of those isomers. The predominance of fluoranthene over pyrene, is attributed to pyrolytic sources. The Fluo/(Fluo + Py) ratio in the samples ranged between 0.54 to 1.00 suggesting the dominance of pyrolytic sources, with only one sample where the ratio was 0.27, thus suggesting a petrogenic origin. BaA/(BaA + Ch) were determined in only two samples, with ratios of 0.14 and 0.32, suggesting a predominant input from petrogenic and pyrolytic sources, respectively. Overall, a predominance of pyrolytic sources was found in both areas, especially in samples collected in BB where all the samples showed a probable pyrolytic source. That might be due to the fires that occur in the area of BB, sometimes naturally but also through arsons, used to make space for crops and fertilize the soil.

Overall, a predominance of low molecular weight PAHs was found. This might be due to the lower rate in which these compounds tend to sink to the depth through the association with particulate matter. This process is a consequence of the hydrophobic nature of PAHs, and since higher molecular weight PAHs are more hydrophobic, they tend to end up in sediments through this process, while low molecular weight PAHs are more water-soluble and bioavailable (Marsili *et al.*, 2001). Floating plastics might therefore be susceptible to concentrate higher amounts of low molecular weight PAHs.

PCBs were found adsorbed only on plastics from the AR, while plastics from BB didn't have detectable levels of PCBs. This might be due to the origin and time spent in the

water by plastics collected in the two different areas: plastics in BB are thought to have entered the ocean only recently from a nearby source, while the ones collected in AR have probably originated on the mainland and have reached the archipelago after a long journey in the Pacific Ocean. During the permanence in the oceans, plastics accumulate and concentrate the pollutants from marine areas contaminated by these compounds (Rios *et al.*, 2010). Our results of the quantity of PCBs adsorbed support our hypothesis (Chapter 1) that BB plastics originate from the surrounding area and ingressed recently: for this reason they don't have detectable levels of PCBs. The plastics collected in Revillagigedo, on the other hand, entered into the environment further away, and have spent a longer time in the ocean, thus having had more time to adsorb PCBs (Endo *et al.*, 2005).

Although PCBs have been banned since 1970 in Canada, 1976 in the USA and since 1993 in Mexico, they can still be used in completely closed systems, and are contained in old electrical equipment (Rios *et al.*, 2010). In Mexico, their disposal is regulated by the "Norma Oficial Mexicana NOM-CRP-001" since 1993. Nevertheless, these compounds are extremely stable and persistent in the environment, and can still be detected in low concentrations in many matrices like soil, sediment and waters. Being completely synthetic molecules, all detectable levels of PCBs are imputable to anthropogenic sources and human contamination.

The high pollutant concentrations found in the plastic debris may also be due to their permanence in the marine microlayer. Some hydrophobic contaminants such as OCPs and PCBs are known to be hyper-concentrated in the air-sea interface microlayer. Because the plastic we found in the Revillagigedo Archipelago was likely transported on the sea surface, it could have easily adsorbed the contaminants from the contaminant-enriched microlayer (Mato *et al.*, 2001). Plastics from BB, originate locally and therefore have less probability of concentrating pollutants in the shorter time period they spend in the microlayer.

Comparing our results with other adsorbed pollutants studies, we have found lower concentrations of PCBs than most of the studies conducted on plastics that were collected in the Pacific Ocean (Table 8). On the other hand, the content of

organochlorine pesticides was higher than the plastics collected in the other studies, and only comparable with samples collected in California by Rios *et al.,* 2007. PAHs adsorbed on plastics have received less attention and only a few studies have reported their content. Our results are comparable with the concentrations of PAHs reported in those studies. When PAHs and POPs are analyzed, usually the concentration of ΣPAHs is greater than other contaminants, which is in accordance with our findings (ΣPAHs>ΣOCP>ΣPCBs).

Table 8: Range of concentration of pollutants found adsorbed on plastics in literature (concentrations in ng/g).

Area	Source	Type pp	ΣPAHs	ΣPCBs	ΣOCPs	Reference
New England	Water	Pellets	NA	5000	NA	(Carpenter *et al.*, 1972)
Japan	Beach	Pellets	NA	3.97-117	0.16-3.1	(Mato *et al.*, 2001)
Japan	Beach	Pellets	NA	ND-2300	NA	(Endo *et al.,* 2005)
Hawaii	Beach	Fragments, pellets	ND-500	ND-980	ND-22	(Rios *et al.*, 2007)
California	Beach	Fragments, pellets	ND-6200	ND-730	ND-1100	(Rios *et al.*, 2007)
California-industrial sites	Beach	Fragments, pellets	ND-12000	ND	ND-7100	(Rios *et al.*, 2007)
USA	Beach	Pellet	NA	32-605	ND-128	(Ogata *et al.*, 2009)
Portugal	Beach	Pellet	NA	27	1.69	(Ogata *et al.*, 2009)
UK	Beach	Pellet	NA	50-87	ND-2.16	(Ogata *et al.*, 2009)
Netherland	Beach	Pellet	NA	169	ND	(Ogata *et al.*, 2009)
Italy	Beach	Pellet	NA	94	ND	(Ogata *et al.*, 2009)
Greece	Beach	Pellet	NA	5	9.41	(Ogata *et al.*, 2009)
Turkey	Beach	Pellet	NA	53	27.6	(Ogata *et al.*, 2009)
India	Beach	Pellet	NA	20-141	ND-29.8	(Ogata *et al.*, 2009)
Malaysia	Beach	Pellet	NA	8-12	ND	(Ogata *et al.*, 2009)
Thailand	Beach	Pellet	NA	6	25.9	(Ogata *et al.*, 2009)
Indonesia	Beach	Pellet	NA	16	13.7	(Ogata *et al.*, 2009)
Vietnam	Beach	Pellet	NA	26	163	(Ogata *et al.*, 2009)
China	Beach	Pellet	NA	48	ND	(Ogata *et al.*, 2009)
Japan	Beach	Pellet	NA	ND-453	11.7	(Ogata *et al.*, 2009)
Australia	Beach	Pellet	NA	16	6.69	(Ogata *et al.*, 2009)
Mozambique	Beach	Pellet	NA	9	4.49	(Ogata *et al.*, 2009)
South Africa	Beach	Pellet	NA	41	2.43	(Ogata *et al.*, 2009)
Pacific Gyre	Water	Fragments, pellets	ND-14459	ND-2058	ND-454	(Rios *et al.*, 2010)
Pacific, Caribbean	Beach, Water	Fragments, pellets	0-3028	0-436	0-198	(Hirai *et al.*, 2011)

Canary	Beach	Pellets	NA	9.0	2.5	(Heskett *et al.*, 2012)
Hawaii	Beach	Pellets	NA	3.05	1.45	(Heskett *et al.*, 2012)
Barbados	Beach	Pellets	NA	1.7	3.1	(Heskett *et al.*, 2012)
Cocos	Beach	Pellets	NA	6.5	3.4	(Heskett *et al.*, 2012)
St. Helena	Beach	Pellets	NA	7.0	3.4	(Heskett *et al.*, 2012)
San Diego	Beach	Pellets	18-210	3.8-42	0.33-8.2	(Van *et al.*, 2012)
Banderas Bay	Beach	Fragments	ND-7997	ND	ND	Present study
Banderas Bay	Water	Fragments	60-265	ND	ND-1061	Present study
Revillagigedo	Beach	Fragments	ND-298	ND-10	ND-810	Present study
Revillagigedo	Water	Fragments	740	127	1192	Present study

NA= Not analyzed, ND= not detected or below detection limit

Regarding the PCB congeners found adsorbed on plastics in our study, our results on floating plastics are in line with the findings of Mato *et al.*, in 2001. They found a higher proportion of high chlorinated congeners, compared to the low chlorinated, and they suggested that this is because PCBs are adsorbed onto plastics through sorption processes where more hydrophobic components (i.e. higher chlorinated congeners) preferentially partition to nonpolar plastic surfaces. In our samples, high chlorinated congeners (PCB 156, 128) were predominant only in the sample of microplastics collected at the surface of the ocean, while in the other samples found on the beach in AR, lower concentrations of PCBs were found, with a higher proportion of low chlorinated compounds.

Through hydrophobic sorption, microplastics floating in the oceans are more suitable for accumulating high chlorinated congeners, while plastics that reach the beaches are more exposed to higher temperatures and UV rays that cause degradation of many compounds. It is known that the half-life of PCBs is significantly reduced when exposed to higher temperatures and photodegradation (Sinkkonen & Paasivirta, 2000), such as when contaminated plastics reach the beach and are exposed to sunlight and higher temperatures than the ocean. This might explain the high concentration of PCBs that was found in microplastics floating in the Revillagigedo Archipelago.

Regarding OCPs, we found a very high concentration of DDTs in one sample from the Revillagigedo Archipelago, from a blue, weathered and fragmented flask. We are not able to determine if the DDTs were stored directly in the flask, or if they were adsorbed by the plastic during its permanence in the water, that we suppose that we suppose

had been a long time due to the level of oxidation and fragmentation of the flask. Weathering may accelerate the sorption of pollutants on plastic objects and increase the adsorption capacity. Weathering creates cracks and fragments the macroplastic objects, increasing the surface area available for adsorption of toxic compounds (Endo *et al.*, 2005). Since the pollutant content of marine debris reflects their history of exposure to contamination, it is not clear whether or not the DDTs have an origin in waters close to the archipelago. If the plastics are transported over a long distance, the pollutant content reflects not only local pollution in the area where they are collected but also pollution along the entire transport path between the source in the environment and the sampling site (Mato *et al.*, 2001).

Organochlorine pesticides were detected in two samples of microplastics out of the three collected in AR and BB. Microplastics have a higher surface area/volume ratio, meaning that smaller particles have more surface exposed for the adsorption of pollutants from the environment. For this reason, microplastics tend to show higher concentrations of pollutants than bigger debris (Mato *et al.*, 2001; Ogata *et al.*, 2009; Rios Mendoza & Jones, 2015).

Analyzing the composition of different pesticides in the samples, the macroplastic sample with DDTs had the original DDT and its breakdown products DDD and DDE. In the microplastic samples containing OCPs, a higher diversity of pesticides was detected, with DDTs and chlordanes (α-, and γ-chlordanes and trans-, and cis-nonachlor). This might support the hypothesis that the blue flask with a high concentration of DDTs, might have been an old container of DDTs, since no other pesticide was detected on it.

Overall, PAHs were the most widespread pollutants found on plastics in both areas, followed by pesticides, that were predominant only in floating microplastics from both areas (Figure 43). Only 5 samples contained low concentrations of PCBs, that is in contrast with what found by Mato *et al*., 2001 and Ogata et al., 2009 in beached resin pellets, where PCBs were more abundant than DDTs. Rios et al., in 2008 found that PAHs were the most abundant pollutants adsorbed on floating plastics in the North Pacific Gyre, followed by PCBs, and then pesticides as the least common compounds.

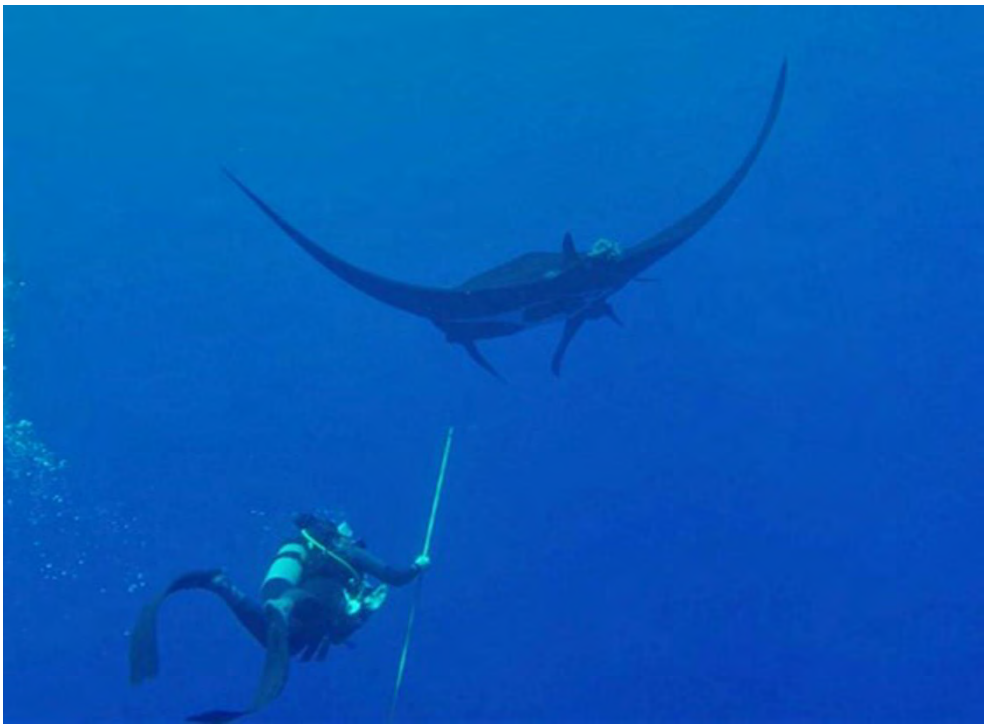
The variability of the results in the different studies, can be aput down to the heterogeneity of sources and the path that the floating plastics can follow during their permanence in the oceans, which implicates exposure and subsequent sorprion of different polluttants.

The ingestion of floating plastics containing PAHs, PCBs and OCPs is likely to be happening in both areas, threating the marine biodiversity in both BB and AR. Filterfeeders can be exposed to an incidental ingestion of contaminated microplastics during their non-selective feeding activity, especially species that feed at the surface of the ocean. Due to the small size of the plastics collected in both areas, it is likely that POPs are entering the food web from the base up, meaning that top predators might show higher concentrations of PCBs and OCPs due to biomagnification processes. On the other hand, PAHs are metabolized more efficiently by vertebrates and do not tend to biomagnificate. For this reason, lower throphic level species might be more susceptible to show higher concentrations of PAHs than top predators.

Beached plastics can also pose a serious threat to terrestrial species, especially birds, that selectively feed on colored objects and are known for ingesting high amounts of plastics (Ryan *et al.*, 2009). Such animals may be exposed to high concentrations of pollutants adsorbed on the debris. This can be particularly dangerous for sensitive ecosystems such as oceanic islands that present endemic species, especially of birds. It is the case at Revillagigedo, that hosts 4 endemic species and 11 endemic subspecies of birds, plus 4 species of reptiles and 9 invertebrates (CONANP, 2017). An integrated plan of action is urgent in order to remove the beached plastics that have accumulated on the islands in the last decades, and that are a potential threat to the fragile ecosystem and biodiversity of the islands.

9 Chapter 3:

Organic pollutants in oceanic manta rays



9.1 Introduction

Plastic pollution is one of the greatest threats faced by wildlife in the current century (Murray & Cowie, 2011). Plastics are produced in immense quantities and are very resistant to degradation. Although the problems associated with large plastic items, such as carrier bags and discarded fishing gear, with regard to large marine animals are well documented, it is only recently that the impacts of smaller plastic fragments has been considered. Filter-feeders have recently been identified as a susceptible category to the ingestion of microplastics, due to their feeding strategy in which they select their food on its size, which in many cases is the same as microplastics. Evidence from the field and in controlled laboratory incubations has revealed ingestion of microplastics by zooplankton, as well as benthic filterfeeders, such as mussels (Murray & Cowie, 2011; Cole *et al.*, 2013; Setälä *et al.*, 2014, 2016; Rochman *et al.*, 2015; Van Cauwenberghe *et al.*, 2015).

Larger filter-feeding animals such as baleen whales and filter-feeding elasmobranchs, have in the last few years gained more attention for their possible susceptibility to the ingestion of plastics and microplastics (Fossi *et al.*, 2014). Filter-feeders are likely to accidentally consume plastic and other marine debris within the size range of their target prey. However, filter-feeding doesn't necessarily result in increased amounts of ingested plastic compared to more selective feeders (Ryan, 2016). Actually, a higher proportion of toothed whale species have been recorded as eating plastic than baleen whales (Kühn *et al.*, 2015). Nevertheless, large bodied filterfeeders whose feeding grounds overlap with high plastic debris concentrations, might be exposed to the ingestion of microplastics, particularly when feeding at the surface, where the largest abundance of plastics accumulates (Reisser *et al.*, 2015).

The classical approach to determine the ingestion of plastic debris in animals is the analysis of stomach content, performed after the death of stranded or fished animals (Lazar & Gračan, 2011; Valente *et al.*, 2019). This methodology can be applied to animals of every size, from small fish to large stranded whales and sharks (Besseling *et al.*, 2015; Abreo *et al.*, 2019; Karbalaei *et al.*, 2019). Some of these species are protected and are therefore not targeted in fisheries, thus their stomach content

analysis is restricted to stranded animals. This is the case for the baleen whales, whale sharks and manta rays in Mexico (NOM-059-SEMARNAT-2010, NOM-029-PESC-2006). In these species, the number of samples available is limited by the accessibility and occurrence of carcasses. In the case of elasmobranchs, there is a very low incidence of strandings because they lack swim bladders, unlike other fish, so once they die they sink to the bottom of the seabed (Bone & Roberts, 1969). For this reason, the direct determination of plastic ingestion through stomach content in these species is often not feasible or is restricted to very rare stranding events (Sampaio *et al.*, 2018; Abreo *et al.*, 2019). Thus, a need has emerged for non-lethal techniques for collecting data about plastic ingestion in vulnerable and protected species. Plastic pollution in living organisms cannot be assessed by the direct quantification of ingested plastics but can be carried out by an indirect route: monitoring plastic additives and biological responses to the exposure to plastic as a proxy for plastic ingestion (Fossi & Panti, 2018).

The use of biopsy samples for ecotoxicological studies has gained much attention in recent years, as it allows the analysis of noumerous different parameters such as feeding ecology and ecotoxicology. Biopsies can be used to determine the concentration of pollutants as well as the biological responses that can be related to the pollutants, such as the gene expression of biomarkers of exposure to contaminants (Marsili *et al.*, 2016; Fossi *et al.*, 2017). Due to advances in ecotoxicology, different compounds can be determined from the same biopsy sample. This reduces the costs of repeat sampling, which are particularly high in remote areas, from elusive and protected species, and where paperwork can be difficult to obtain.

We decided to analyze a broader set of pollutants in oceanic manta ray biopsies, and the targets were phthalates (as indicators of plastic ingestion), PCBs, OCPs and PAHs, that could potentially derive from the ingestion of polluted plastics, from the water or from the diet (Figure 44).

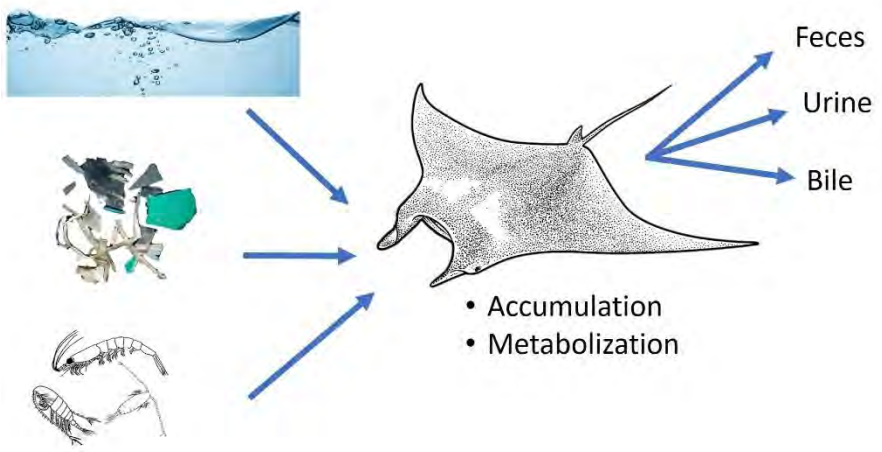


Figure 44: The uptake and elimination mechanisms of pollutants in oceanic manta rays.

Free phthalates are largely degraded naturally in the marine environment, and so the high levels displayed in marine organisms are considered to be related to ingestion of microplastics (Salvaggio *et al.*, 2019), and therefore have been used as a biomarker of exposure to plastic ingestion (Fossi *et al.*, 2014).

9.2 Materials and Methods

Biopsies from free ranging oceanic manta rays (*Mobula birostris*) were collected during several sampling trips between 2016 and 2018, in Revillagigedo Archipelago and Banderas Bay. When possible, a photo/video of the ventral side of each animal was recorded in order to be able to determine the sex and identity of each individual, using its unique ventral spot pattern (Marshall *et al.*, 2011). Subsequently, a small biopsy comprising epidermis, dermis and muscle tissue (when possible) was obtained with the use of a spear pole with a modified stainless steel tip (Figure 45). In Revillagigedo, biopsies were taken while scubadiving from the ventro-posterior part of the pectoral fin. In Banderas Bay biopsies were taken from the dorsal side, while freediving. In both cases, the biopsies were wrapped in aluminium foil and stored at -20°C until analysis.

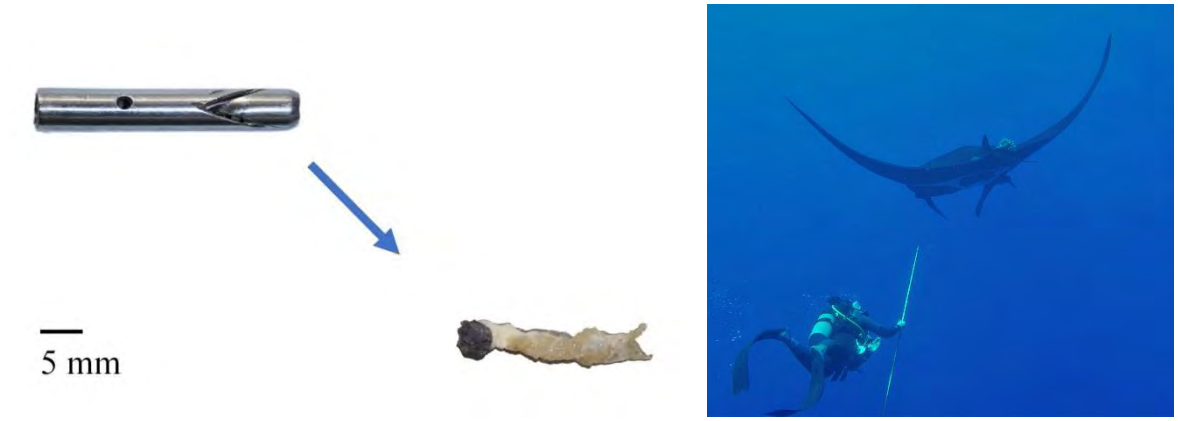


Figure 45: Modified stainless steel tip and obtained tissue samples (left), scuba diving sampling of freeranging oceanic manta ray (right).

Muscle and dermis were then separated and the tissues were freeze-dried for 24 hours until completely dry. Only samples with a dry weight of 10 mg or more were selected for analysis.

A first set of 18 oceanic manta ray samples (4 from Banderas Bay and 14 from Revillagigedo) were analyzed in the Chemistry Faculty of the National Autonomous University of Mexico (UNAM) using the matrix solid phase dispersion (MSPD) for the extraction and determination of phthalates and PAHs content, following the protocol of Olmos-Espejel *et al.*, 2012, with some modifications. The samples were homogenized in a mortar with 120 mg of C18 Silica as a dispersant (Figure 46, left). The mixture was then packed in a glass column with 250 mg of Florisil and 250 mg Si C18, and

acetonitrile was used as eluent. The extract was then concentrated under a gentle nitrogen flow in a 40°C water bath and resuspended in ethyl acetate. The sample was then injected into a gas chromatograph equipped with a simple quadrupole mass spectrometer in both SIM and Scan modes. Perylene-d12 was used as a surrogate standard and n-tridecane as internal standard. The procedure described above was validated for recovery experiment by analyzing spiked samples. With this methodology, phthalates and PAHs were analized in the biopsies and reported as ng/g of dry weight (dw).

Figure 46: The two extraction equipment used to analyze the biopsies: mortar (left) and ultrasound bath (right).

A second set of 20 oceanic manta ray samples (9 from Banderas Bay and 11 from Revillagigedo Archipelago), were analyzed in the Natural Sciences Department of the University of Wisconsin Superior (UWS). The samples were grounded in a mortar and extracted in a ultrasonic bath for 20 mins with 5mL DCM (Figure 46, right) three times, and subsequently concentrated to 1 mL before the cleaning and fractionation process in a glass column packed with 6 mL Silica C18. Two fractions were used for the separation of the different objective compounds: a first fraction (containing PCBs, PAHs and OCPs) was eluted with 30 mL HEX:DCM=75:25, and a second fraction (with phthalates) was eluted with 30 mL DCM 100%. The extracts were then concentrated to 100 µL and injected into an Agilent 7890A GC System equipped with an Agilent 5975C mass spectrometer (with triple axis analyzer) that was operated under both

Scan and SIM mode, using one ion for quantification and two ions for confirmation for each analyte (Supplementary data:Table 18, Table 19, Table 20).

Additionally, four samples of zooplankton were analyzed (2 from Revillagigedo and 2 from Banderas Bay) in order to investigate the baseline levels of contamination that manta rays are exposed to through the ingestion of their preys. The zooplankton samples were collected in both areas using a zooplankton net with 333 µm mesh, to collect the same size of zooplankton as would a manta ray's gill rakers (Divi *et al.*, 2018). The whole bodies of zooplankton were frozen and subsequently freeze-dried and homogenized using a mortar. The zooplankton was analyzed in UWS with the same methodology as used for the second set of manta ray biopsies previously described

In order to avoid sample contamination, all glassware was cleaned and baked in a furnace for 4 hours at 400°C prior to use. Volumetric material was washed twice with acetone, hexane and dichloromethane (all HPLC grade). Procedural blanks were analyzed for quality control to check for any sources of contamination during the analytical procedures. For each analyte, a procedural blank value was used for subtraction. The limit of detection (LOD) was set at 3 times the standard deviation of the ration of areas of standards (RAS) noise, while the limit of quantification (LOQ) was set at 10 times the standard deviation of the RAS (Vial & Jardy, 1999) (Supplementary Data: Table 18, Table 19, Table 20). All PAHs, pesticides and PCBs are reported in ng/g of plastic basis.

Normality of data was verified using a Shapiro–Wilk test, and the homogeneity of variances of the data was checked by a Fligner-Killeen test. All statistical analyses were carried out using functions (shapiro.test, fligner.test, kruskal.test, etc.) of the R environment (R core team, version 3.3.1, 2016) and Microsoft Office Excel 2016. Sex and study area comparisons were conducted for variables such as concentrations of ∑PAHs and ∑PCBs, since no phthalates and pesticides were detected in any sample. Statistical significance was set at $p \leq 0.05$.

9.3 Results

We analyzed a total of 38 biopsies, of which 17 were from female manta rays, 14 from males and 7 fom mantas of undetermined sex (Supplementary data: Table 14). All the biopsies were taken from sub adult and adult oceanic manta rays, at a depth from 0 to 30m. A summary of the number of samples and analytes is reported in Table 9Table 9: Number of biopsies and zooplankton samples analyzed at UNAM and UWS, with pollutants analyzed.

Table 9: Number of biopsies and zooplankton samples analyzed at UNAM and UWS, with pollutants analyzed.

Laboratory	Biopsies Revillagigedo	Biopsies Banderas Bay	Zooplankton	Phthalates	PAHs	PCBs	OCPs
UNAM	14	4		X	X		
UWS	11	9	4	X	X	X	X

The results are divided and presented by class of the analyzed contaminants and by sample set.

9.3.1 Phthalates

In the first set of samples analyzed at UNAM, none of the phthalates that were analyzed were detected in the samples. The recoveries were in a range between 53.05% and 104.88%, that is acceptable for this type of analysis. The detection limits ranged between 10 and 52 ng/g, but no peaks were detected in the 18 manta ray samples analyzed (Supplementary data: Table 15).

In the second set of samples analyzed at the UWS, none of the analyzed phthalates was detected in the samples of oceanic manta rays (Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Benzyl butyl phthalate (BBP), Di-2-ethylhexyl phthalate (DEHP), Di-n-octyl phthalate (DnOP)). Due to a background contamination of DBP that was found in the solvent blanks and procedural blanks, we decided to exclude DBP from our analysis, since the CV% was too high (29.9%) for a blank substraction. The limits of detection of the analyzed phthalates were between 0.1 and 0.6 ng/g (Supplementary data: Table 17), while the recoveries varied between 50 and 60%, which is acceptable

for this methodology, that included many steps of concentration and cleaning procedures.

Phthalates were not detected in any of the 4 zooplankton samples analyzed.

9.3.2 PAHs

In the first set of samples analyzed at UNAM, PAHs were detected in 8 out of the 18 manta ray biopsies. The recoveries for the PAHs varied between 46% and 89%, while the detection limits were between 4 and 20 ng/g (Supplementary data:Table 16).

In the second set of samples analyzed at UWS, we detected PAHs in 19 out of 20 manta ray biopsies. The recoveries for the PAHs varied between 72.3 and 96.4%, while the detection limits ranged between 0.4 and 5.5 ng/g (Supplementary data:Table 18).

We tested for normality and homogeneity of variances in our data, obtaining a p-value <0.05 for both tests, and so the distribution of our data was considered to be non-normal. Thus, we performed non-parametric statistics. We found a statistically significant difference in the ΣPAHs between the two areas: in biopsies from Banderas Bay the mean ΣPAHs was 1185, while in Revillagigedo it was 224 ng/g ($p=0.01$) (Figure 47). No differences were found in the ΣPAHs between sexes ($p=0.9$).

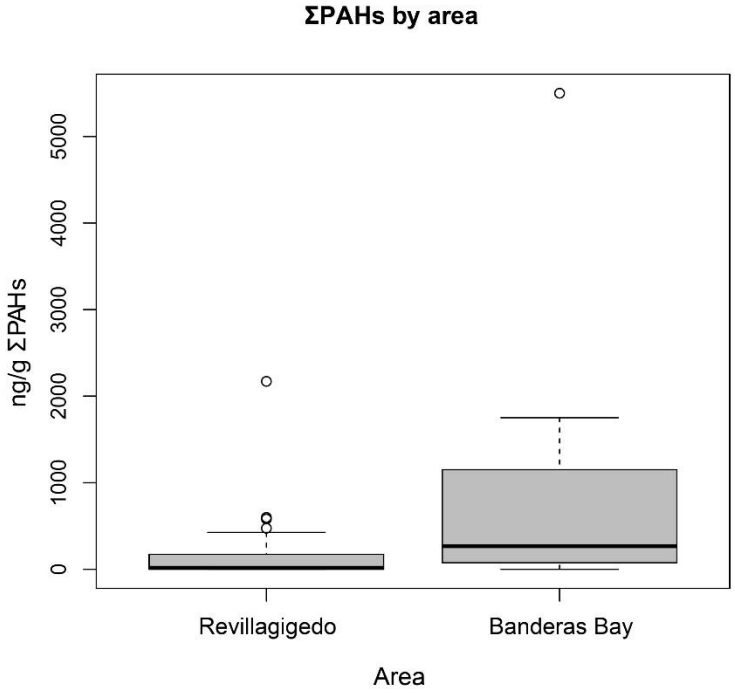


Figure 47: Concentrations of ΣPAHs in the two study areas. In Banderas Bay, oceanic manta rays had higher concentrations of ΣPAHs ($p<0.05$).

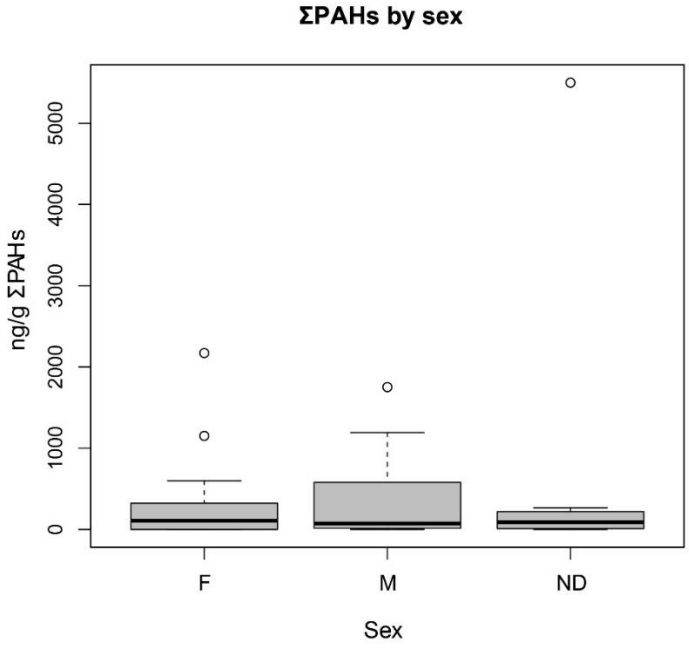


Figure 48: Concentrations of ΣPAHs in the biopsies of oceanic manta rays in both areas. No difference was found between females, males and undetermined sex ($p>0.05$).

Analyzing the PAHs composition, in Revillagigedo we found a prevalence of high molecular weight PAHs compared to low weight PAHS. In Banderas Bay, biopsies showed concentrations of low molecular weight PAHs 10 times higher than of high molecular weight PAHs (Figure 49).

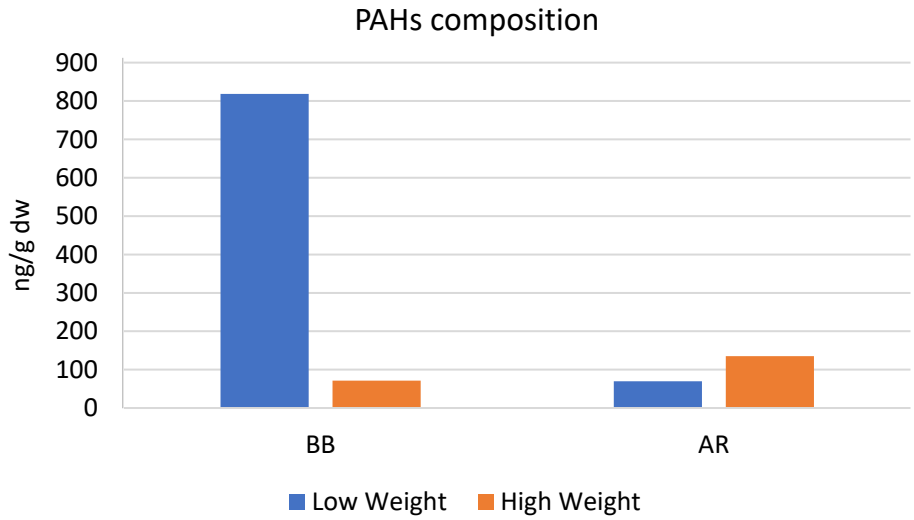


Figure 49: PAHs composition in the two study areas. BB=Banderas Bay, AR= Revillagigedo

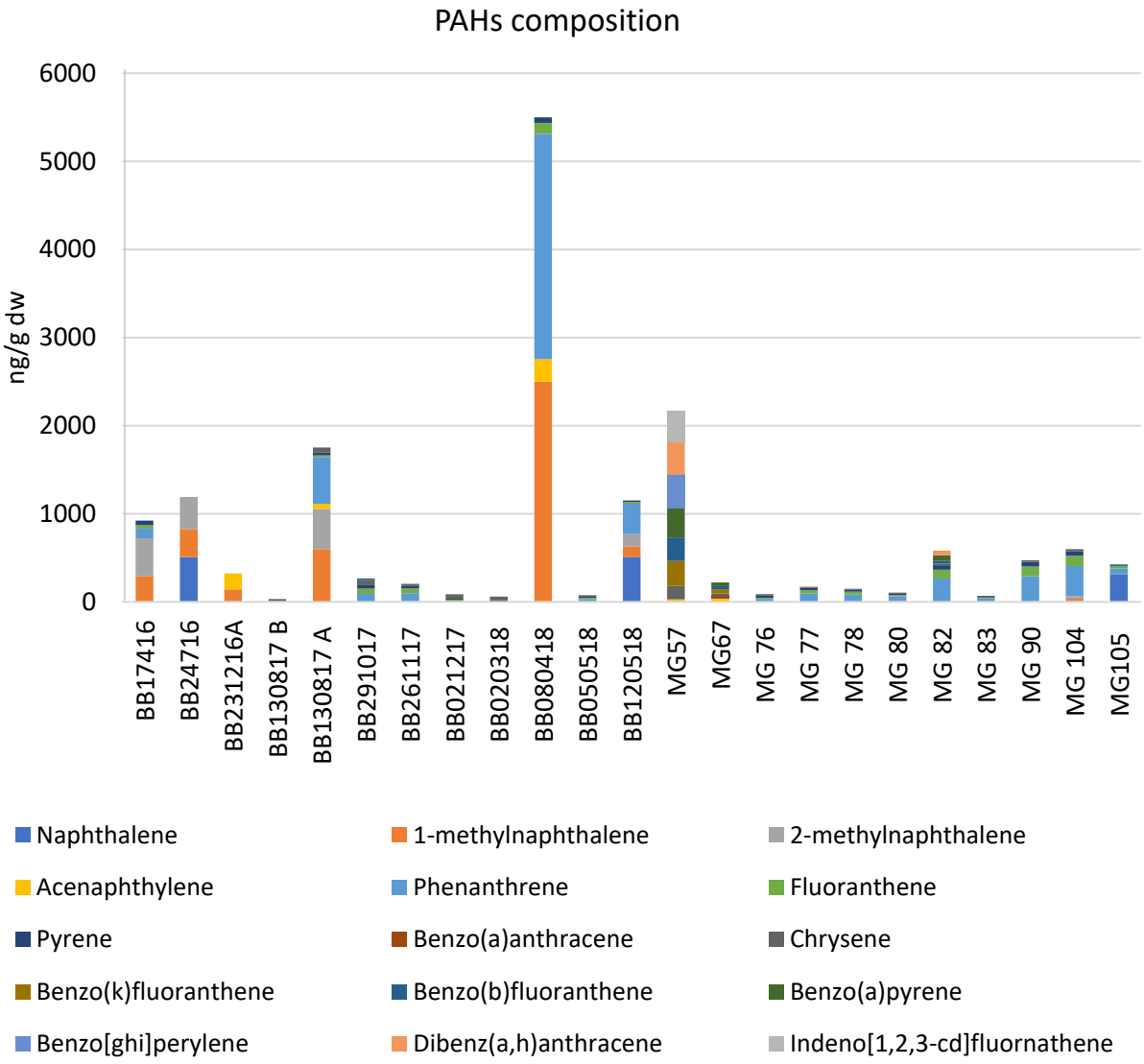


Figure 50: PAHs composition in biopsies of oceanic manta rays. Only biopsies containing detectable levels of PAHs are shown. BB are from Banderas Bay, MG from Revillagigedo Archipelago

PAHs were detected in all 4 samples of zooplankton analyzed. Concentrations of ΣPAHs ranged between 214 and 2316 ng/g (Supplementary data: Table 25). There is no statistical difference in the concentration of ΣPAHs between the two areas. Analyzing the composition of PAHs, we found a prevalence in low molecular weight PAHs in all 4 samples analyzed.

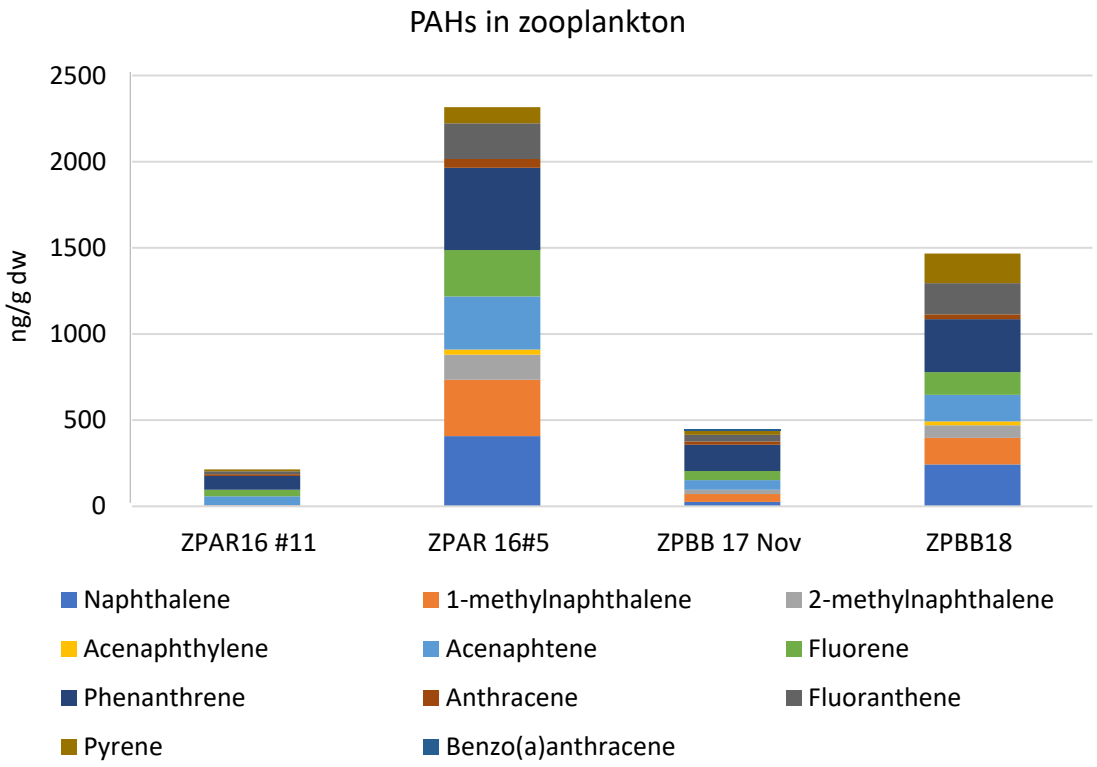


Figure 51: PAHs composition in zooplankton samples from Revillagigedo (ZPAR) and Banderas Bay (ZPBB).

9.3.3 PCBs

PCBs were detected in 6 out of 20 biopsy samples analyzed in the UWS. The ΣPCBs in the samples containing detectable levels of PCBs, they ranged between 3.26 and 21.91. The detection limits ranged between 0.1 and 0.4 ng/g (Table 19), while the recoveries were between 65-86%.

We tested for normality and homogeneity of variances in our data, obtaining a p-value <0.05 and the distribution of our data was considered to be non-normal. Thus, we performed non parametric statistics. We did not find a statistically significant difference in the ΣPCBs between the two areas or between sexes (p=0.6 and 0.9, respectively).

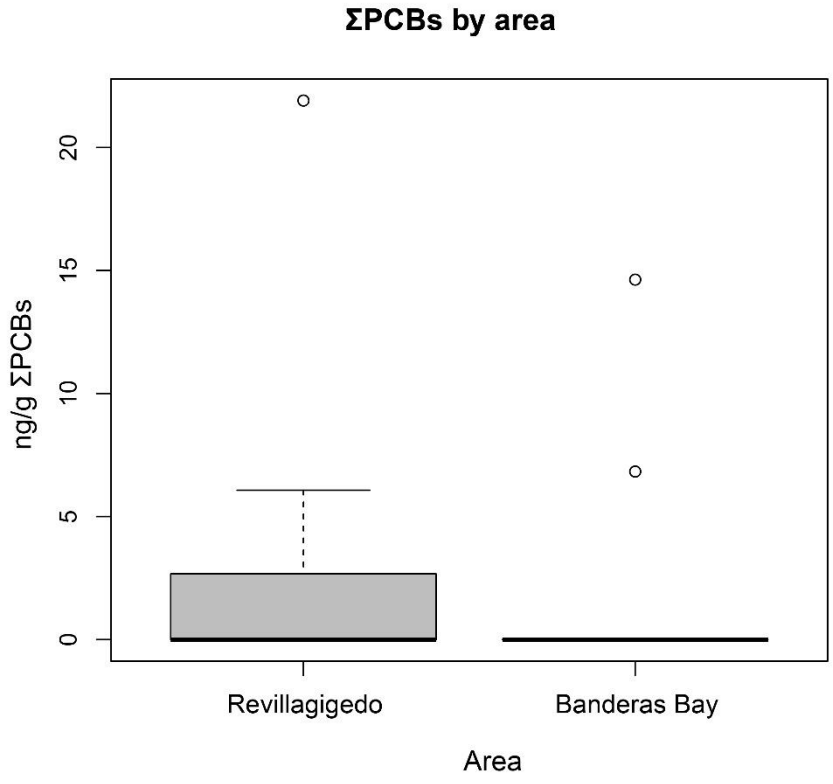


Figure 52: Concentrations of ΣPCBs in the biopsies taken in the two sampling areas.

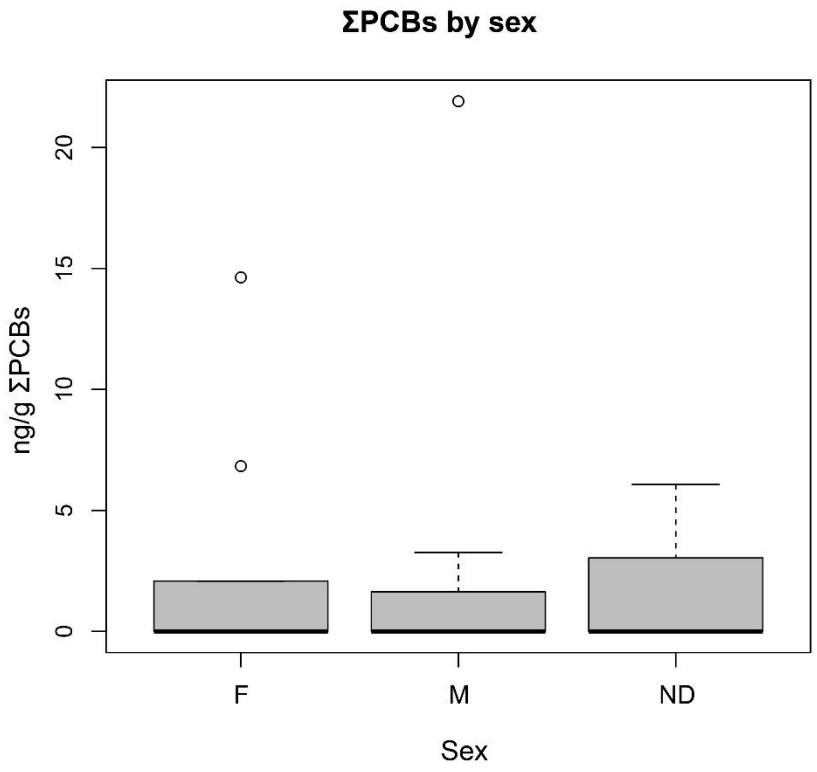


Figure 53: Concentrations of ΣPCBs in females, males and undetermined sex of oceanic manta rays in both areas.

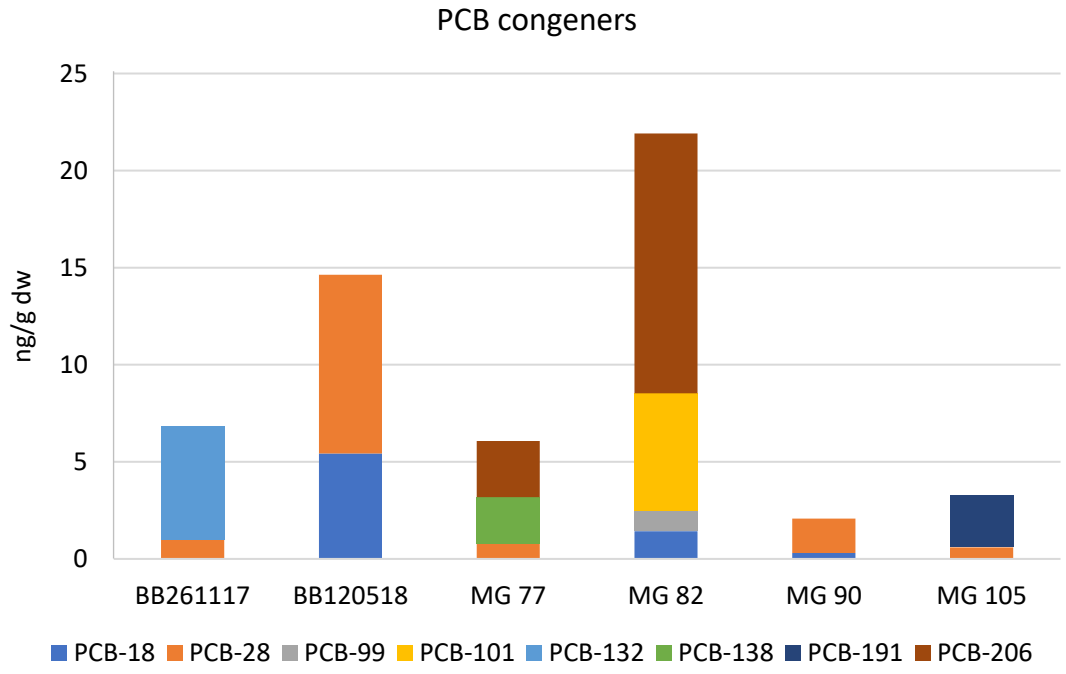


Figure 54: PCB congeners composition in the biopsies of oceanic manta rays. Only biopsies containing detectable levels of PCBs are shown.

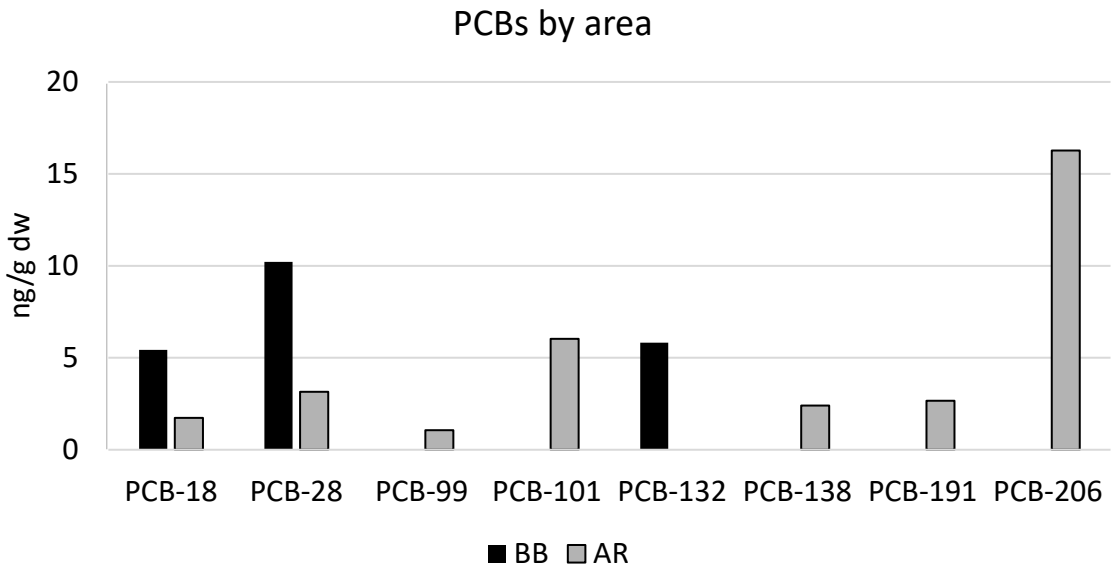


Figure 55: PCB congeners composition by area. In black is the total concentration for Banderas Bay (BB) and in grey is Revillagigedo (AR). Only the congeners detected are shown.

Despite the low LOD obtained, PCBs were not detected in the zooplankton samples from BB or from AR.

9.3.4 OCPs

A summary of the analyzed pesticides, with its analysis parameters, can be found in the Supplementary data: Table 20. Despite the very low detection limits obtained, none of the pesticides and DDTs congeners were detected in the 20 samples analyzed at UWS.

Pesticides were not detected either in any of the four zooplankton samples analyzed.

9.4 Discussion

In some cases, it was not possible to obtain a photo-ID, or positive identification of the sex of the sampled manta rays because of difficulties in the field: the illumination in the photos of the animals sometimes made it difficult to determine the presence/absence of the claspers due to counter-shading, especially in black-morph mantas. In BB, these difficulties were exacerbated by the freediving approach, as manta rays are sometimes scared by freedivers swimming below them (Figure 56).

Figure 56: Comparison between a perfect photo-id picture, with sex visible (left) and a bad photo-id picture, where pelvic fins are not visible and sex can't be determined (right).

BB's biopsies were significantly smaller (mean ± SD: 24.4 ± 11.4 mg) than AR's (51.9 ± 23.6 mg) due to a different size of the sampling tip. Moreover, the samples from BB consisted of epidermal and dermal tissues, while AR's contained mostly muscle. This is due to the different thickness of the skin in the dorsal and ventral sides of the body. In AR, samples were taken from the ventral side, since manta rays usually approach divers from above. On the other hand, in BB the samples were taken by freediving, and therefore manta rays were approached by divers from above, making it easier to take the samples from the dorsal surface. Due to the small size of some samples, only biopsies weighing >10mg dw underwent the extraction and analysis for organic pollutants (Claro *et al.*, 2019).

9.4.1 Phthalates

The analysis of these types of contaminants is very challenging, because of the difficulties in controlling the many sources of contamination with products that contain phthalates. During the development of the methodology to analyze phthalates at the UWS, extra precautions were used from cleaning the glassware to handling the samples. However, the blanks used as quality control were contaminated by DBP during the process (extraction, liquid chromatography, concentration, storage or GC-MS analysis). Before the extraction of our samples, quality controls were performed to check for phthalate contamination, by analyzing the different solvents, Florisil and glass fiber. Phthalates contamination was detected in Florisil, and so it was decided to avoid it (the small size of the samples and low lipid content of skin and muscle tissues allowed the separation and clean-up by the silica alone). A minor DBP contamination was detected also in Hexane, so a newly opened bottle of HPLC grade Hexane was used to reduce this possible source of noise. When the samples and blanks were finally analyzed, variable concentrations of DBP were detected in the blanks. The decision to remove DBP from our analysis was taken, since the concentrations found in the samples were too high and variable to be substracted from the concentrations obtained.

The problem of the laboratory contamination during phthalates analysis has been well known for decades (Belisle *et al.*, 1975; Giam *et al.*, 1975), and some measures have been developed to reduce it (Fankhauser-Noti & Grob, 2007; Marega *et al.*, 2013; Russo *et al.*, 2015). Nevertheless, the most common solution that has been applied is the substraction of the blanks contamination levels from the sample concentration. Due to the high variability of the blanks values, it was decided to remove DBP from the present analysis, in order to avoid any under or over-estimation of DBP concentration in biopsies and zooplankton. It is recommendable for future studies, to report blanks contamination when it occurs, and to check for variability in the different blanks, since the substraction of the blank values is not appropriate if the contamination varies between each blank.

Using two different extraction methods, with acceptable recoveries and low detection limits, none of the phthalates analyzed was detected in the biopsies or zooplankton

from the Mexican Pacific Ocean. The sample size for oceanic manta ray biopsies (n= 38) is greater than the reviewed studies that analyzed phthalates in the wild (Table 10), and therefore we can consider our results representative of phthalate diesters levels for oceanic manta rays in the Mexican Pacific Ocean. To our knowledge, this is the first study to analyze phthalates in mobulids. Thus, we can't compare our results with others from similar studies on the same species or family. In Table 10 we provide a comparison between our results and those of previous studies of phthalate content in wild marine animals.

Table 10: Comparison of our results with other studies where levels of phthalates were determined in aquatic organisms in the wild. Concentration reported as a mean, or range of concentrations found (in ng/g).

Area	Species	Tissue	Analyte	ng/g	Reference
Arctic	*D. leucas* (n=11)	Blubber	DEHP	2.8-4,150 ww	(Morin, 2003)
Arctic	*B. saida* (n=12)	Muscle	DEHP	28.2 ww	(Morin, 2003)
Mediterranean	*B. physalus* (n=5)	Blubber	DEHP	ND	(Fossi *et al.*, 2014)
Mediterranean	*C. maximus* (n=6)	Muscle	DEHP	NR	(Fossi *et al.*, 2014)
Mediterranean	*B. physalus* (n=5)	Blubber	MEHP	176.67 lb	(Fossi *et al.*, 2014)
Mediterranean	*C. maximus* (n=6)	Muscle	MEHP	84.1 lb	(Fossi *et al.*, 2014)
France (river)	*R. rutilus* (n=4)	Muscle	DEHP	523	(Valton *et al.*, 2014)
France (river)	*R. rutilus* (n=4)	Liver	DEHP	3,052	(Valton *et al.*, 2014)
France (river)	*R. rutilus* (n=4)	Muscle	BBP	155	(Valton *et al.*, 2014)
France (river)	*R. rutilus* (n=4)	Liver	BBP	1,082	(Valton *et al.*, 2014)
Mediterranean	*B. physalus* (n= 3)	Blubber	DEHP	7,051 dw	(Baini *et al.*, 2017)
Mediterranean	*T. truncatus* (n= 1)	Blubber	DEHP	26,068 dw	(Baini *et al.*, 2017)
Mediterranean	*G. griseus* (n=1)	Blubber	DEHP	1,130 dw	(Baini *et al.*, 2017)
Mediterranean	*S. coeruleoalba* (n= 2)	Blubber	DEHP	21,460 dw	(Baini *et al.*, 2017)
La Paz Bay	*B. physalus* (n=7)	Blubber	DEHP	ND- 2341	(Olavarrieta-García, 2017)
Mediterranean	*C. caretta* (n=8)	Liver	BBP	700–9,100	(Savoca *et al.*, 2018)
Mediterranean	*C. caretta* (n=3)	Fat	DEHP	2,000-5,500	(Savoca *et al.*, 2018)
Revillagigedo	*M. birostris* (n=25)	Muscle	BBP	ND	Present study
Revillagigedo	*M. birostris* (n=25)	Muscle	DEHP	ND	Present study
Banderas Bay	*M. birostris* (n=13)	Skin	BBP	ND	Present study
Banderas Bay	*M. birostris* (n=13)	Skin	DEHP	ND	Present study
La Paz Bay	Mixed zooplankton/MP	Whole	MEHP	13.08-13.69	(Fossi *et al.*, 2016)
Mediterranean	Mixed zooplankton (n= 21)	Whole	DEHP	34-2,699 dw	(Baini *et al.*, 2017)
Pacific Ocean	Mixed zooplankton (n=4)	Whole	DEHP	ND	Present study
Pacific Ocean	Mixed zooplankton (n=4)	Whole	BBP	ND	Present study

ND= not detected, NR=detected but not reported, lb= lipid base, dw= dry weight, ww=wet weight

The absence of phthalates found in our samples is in discordance with previous studies, where most of the times phthalates were detected. We will discuss the possible causes of our results below.

Increased metabolism and reduced prey concentrations prevents biomagnification of phthalates. In fact, phthalate ester concentrations tend to decrease up the food web (Staples *et al.*, 1997; Mackintosh *et al.*, 2004). For this reason, phthalates are a good indicator of direct plastic ingestion rather than indicators of a contamination of the food web. The absence of phthalates in the zooplankton samples analyzed, might underline the relatively pristine habitat that the Mexican Pacific Ocean represents, and the low incidence of plastics ingestion by the lower levels of the food web.

Phthalate distribution is not homogeneous in the different organs, as found by (Savoca *et al.*, 2018) in Mediterranean sea turtles and Valton *et al.*, 2014 in fish. Different phthalates can be found in different organs, with higher concentrations usually found in fatty tissues. However, chicks fed with a phthalate enriched food, have shown to accumulate phthalates in all tissues sampled, including muscle and skin (Jarosova *et al.*, 2009). In skin and muscle of oceanic manta rays the concentration of phthalates is not detectable, but it is possible that other organs have higher concentrations, thus being possible to detect with the current methodologies. Having access to organs such as the liver, brain and gonads, the overall view might be much clearer. Due to the fishing ban that is currently applied in Mexico, oceanic manta rays are no longer fished. An opportunistic sampling of naturally dead animals or bycatch could allow further investigation of the ecotoxicology in manta rays in Mexico.

The low concentration or absence of phthalates in muscle and skin could also be explained by the strong conversion of these substances to their metabolites after their assimilation. The liver first passage after ingestion convert largely the substances into their metabolites before they reach the other districts (Salvaggio *et al.*, 2019). In fish, phthalates are metabolized to their monoesters (Fossi *et al.*, 2014; Valton *et al.*, 2014). In our study, we only analyzed phthalate diesters, that are the form in which they are applied as plasticizers in the plastics industry. Thus, it is possible that the absence of phthalates diesters that we found, is not representative of the totality of phthalates.

There is the possibility that in oceanic manta rays, phthalate diesters are rapidly metabolized to monoesters, and for this reason we didn't find them in our samples. In the literature, there are some cases in wich phthalate monoesters were found to be present in high concentrations, while the diesters were not detected (Fossi *et al.*, 2014). However, other studies have found high concentrations of diesters in aquatic animals (Table 10). In the future, phthalates monoesters should be anayzed too, in order to determine a possible conversion of some diesters to monoesters, after the ingestion of plastics.

Phthalate diesters were not detected either in the zooplankton. The analysis of zooplankton for the determination of phthalate content has only recently gained interest in the literature, and only two studies were found to have analyzed it in the environment (Table 10). In the Mediterranean sea, the range of concentrations of DEHP is relatively wide (34-2699 ng/g dw) (Baini *et al.*, 2017), while in La Paz Bay, the analysis of MEHP revealed a lower concentration (13 ng/g dw) (Fossi *et al.*, 2016). The Mediterranean is known for being heavily polluted by plastic debris (Chapter 1:Table 4) , and therefore a high rate of ingestion of plastics by zooplankton is expected. In AR and BB, the concentration of plastics in the water column might be too low for determining a high incidence of plastic ingestion by zooplankton. We are aware that the sample size of our study (n=4) might not be representative, so further studies on the incidence and concentration of phthalates (diesters and monoesthers) in zooplankton are needed.

9.4.2 PAHs

PAHs are among the most ubiquitous environmental contaminants in coastal ecosystems and the incomplete combustion, either naturally or anthropogenically derived, has been identified as the largest source of PAHs into the environment (Abdel-Shafy & Mansour, 2016). The atmosphere is the most important vehicle for hydrocarbon dispersial, resulting in PAHs being ubiquitous in the environment.

In BB we found significantly higher concentrations of ΣPAHs than compared to the AR. This might be due to the different anthropogenic impacts to which the two areas are exposed. In Banderas Bay, the main sources of PAHs are probably human-related, since there are more than 400,000 people living in the area, with a lot of terrestrial and marine traffic as possible sources of combustion (vehicle exhaust, agricultural fires, power plants etc.). The presence of lush vegetation in the proximity of the bay, suggests forest fires (natural or man-made) to be another possible source of PAHs. In BB, PAHs can enter the marine environment through direct river runoff or deposition of atmospheric input. Oceanic manta rays are therefore exposed to PAHs through the water, zooplankton and potentially the ingestion of contaminated plastics.

In AR, due to the remoteness of the area and the low human pressure present, PAHs are expected to be mainly from natural local sources (volcanoes, scarce marine traffic, etc.) or transported via the atmosphere. This might explain the prevalence of high molecular weight PAHs in the biopsies from AR. The more hydrophobic PAHs (such as high molecular weight ones) are sorbed to atmospheric particulates more readily than lower molecular weight PAHs (Abdel-Shafy & Mansour, 2016). Thus, high molecular weight PAHs may be transported further away from the source through the atmosphere, and therefore be more available for absorbtion by oceanic manta rays patrolling the remote AR.

The overall concentrations of ΣPAHs found in the present study, are comparable with those of other previous studies of filter-feeding megafauna, sharks and zooplankton in other areas of the world (Table 11). Nevertheless, field measurements of PAHs in these species are scarce.

Table 11: Comparison of our results with other studies where levels of PAHs were determined in elasmobranchs or filterfeeding megafauna in the wild. Concentration reported as range of concentrations found (in ng/g).

Area	Species	Tissue	Analyte	ng/g	Reference
Alaska	*E.robustus* (n=2)	Liver	ΣPAHs	116-161 ww	(Loughlin, 2013)
Alaska	*E.robustus* (n=1)	Blubber	ΣPAHs	467 ww	(Loughlin, 2013)
Alaska	*B.acutorostrata* (n=1)	Liver	ΣPAHs	105 ww	(Loughlin, 2013)
Alaska	*B.acutorostrata* (n=1)	Blubber	ΣPAHs	202 ww	(Loughlin, 2013)
South Africa	*C. carcharias* (n=7)	Skin	ΣPAHs	2,769-7,278 dw	(Marsili *et al.*, 2016)
Banderas Bay	*M. birostris* (n=13)	Skin	ΣPAHs	ND-5,500 dw	Present study
Revillagigedo	*M. birostris* (n=25)	Muscle	ΣPAHs	ND-2,170 dw	Present study
Banderas Bay	Mixed zooplankton (n=2)	Whole	ΣPAHs	449-1,467 dw	Present study
Revillagigedo	Mixed zooplankton (n=2)	Whole	ΣPAHs	214-2,316 dw	Present study

ND= not detected, NR=detected but not reported, lb= lipid base, dw= dry weight, ww=wet weight

PAHs can be divided into two categories: low molecular weight (MW) PAHs that are composed of fewer than four aromatic rings (naphthalene, acenaphthene, fluorene, phenanthrene, etc.), and high MW PAHs, composed of four or more rings (e.g., pyrene, chrysene, benzo[a]pyrene, dibenz[a,h]anthracene, etc.). High MW PAHs are generally less water soluble and partition more readily into organic matter than low MW PAHs. For this reason, high MW PAHs tend to settle in the water column and accumulate in the sediments. Oceanic manta rays patrolling deep waters, or feeding on deeper food sources, may be more exposed to high MW PAHs. This might explain our results in Revillagigedo, where the high MW PAHs dominated the overall PAHs composition in oceanic manta ray biopsies.

PAHs undergo a trophic dilution through the food web, due to the metabolism that vertebrate organisms are capable of, and that can lead to their elimination through urine or faeces (Cullen *et al.*, 2019). Thus, it is normal that the ΣPAHs concentrations in the samples of zooplankton and oceanic manta rays are comparable, since a biomagnification is not expected to occur.

9.4.3 Organochlorine compounds

It is known that large-bodied sharks are long-lived, slow to mature and have low fecundity. These traits contribute to increased individual and population level exposure risks to persistent organic pollutants (Cullen *et al.*, 2019). For this reason, many studies have recently focused their attention on the presence of POPs in sharks and also in marine mammals that share the same life traits (Manta rays, for their lower trophic level and practical difficulties for obtaining samples, are much less studied and ecotoxicological investigations are scarce. To our knowledge, this is the first study to investigate the incidence of OCs in mobulid rays.

Table 12). Manta rays, for their lower trophic level and practical difficulties for obtaining samples, are much less studied and ecotoxicological investigations are scarce. To our knowledge, this is the first study to investigate the incidence of OCs in mobulid rays.

Table 12: Comparison of our results with other studies where levels of PCBs were determined in elasmobranchs or filter-feeding megafauna in the wild. Concentration reported as range of concentrations found (in ng/g).

Area	Species	Tissue	Analyte	ng/g	Reference
California	*M.henlei*	Muscle	ΣPCBs	16.5-148 ww	(Fairey *et al.*, 1997)
China	*C.plagiosum* (n=35)	Muscle	ΣPCBs	1.05-4.77 ww	(Cornish *et al.*, 2007)
Japan	*G.cuvier (n=42)*	Liver	ΣPCBs	72-11,000 lb	(Haraguchi *et al.*, 2009)
Iceland	*S.microcephalus* (n=10)	Muscle	ΣPCBs	4,100 lb	(Strid, 2010)
Iceland	*S.microcephalus* (n=10)	Liver	ΣPCBs	4,400 lb	(Strid, 2010)
Mediterranean	*B. physalus* (n=5)	Blubber	ΣPCBs	8,155-42,778 lb	(Fossi *et al.*, 2014)
Mediterranean	*C. maximus* (n=6)	Muscle	ΣPCBs	ND-1,970 lb	(Fossi *et al.*, 2014)
South Africa	*C. carcharias* (n=15)	Muscle	ΣPCBs	380-11,284 dw	(Marsili *et al.*, 2016)
Atlantic	*P.glauca* (n=20)	Muscle	ΣPCBs	5,020 lb	(Alves *et al.*, 2016)
Atlantic	*P.glauca* (n=20)	Liver	ΣPCBs	637 lb	(Alves *et al.*, 2016)
Gulf California	*R.typus* (n=12)	Skin	ΣPCBs	0.27-41.4 ww	(Fossi *et al.*, 2017)
Banderas Bay	*M. birostris* (n=9)	Skin	ΣPCBs	ND-14.6 dw	Present study
Revillagigedo	*M. birostris* (n=11)	Muscle	ΣPCBs	ND-21.9dw	Present study
NW Atlantic	Mixed zooplankton (n=4)	Whole	ΣPCBs	2,400-260,000 lb	(Risebrough *et al.*, 1972)
Djibouti	Mixed zooplankton (n=4)	Whole	ΣPCBs	109-636 dw	(Boldrocchi *et al.*, 2018)
Banderas Bay	Mixed zooplankton (n=2)	Whole	ΣPCBs	ND	Present study
Revillagigedo	Mixed zooplankton (n=2)	Whole	ΣPCBs	ND	Present study

ND= not detected, NR=detected but not reported, lb= lipid base, dw= dry weight, ww=wet weight

Concentrations of PCBs detected in the biopsies of oceanic manta rays were relatively low when compared with previous studies on sharks (Table 12). Nevertheless, our concentrations are comparable to those found in whale sharks from the Gulf of California (Fossi *et al.*, 2017). It must be taken into account that each study reports the concentrations depending on the methodology used: the lipid base concentration is often reported when comparing tissues with very different lipid content, the dry weight concentration is preferable when the samples are freeze-dried, and wet weight is used mainly in studies that take into account a daily intake for dietary studies. It is therefore challenging to compare the results between studies that report different weights. A standardization of reporting is needed in order to obtain more comparable values in the future.

Many persistent organic pollutants are nowadays forbidden in many countries around the world, and it is expected that their concentrations will reduce gradually in the next decades and centuries. Nevertheless, due to the immense amount used in the past, they are still detectable in most of the environmental matrices at trace concentrations. OCs are accumulated in marine organisms and tend to accumulate in fatty tissues, biomagnificating up the food web. This has been seen in many studies, where a clear correlation was shown between the total organochlorine burden of the animals analyzed and their position within the marine food web (lower trophic levels showed a lower concentration of POPs) (Mössner & Ballschmiter, 1997; Hop *et al.*, 2002). This can explain the absence of organochlorine pesticides and the low incidence of PCBs in oceanic manta rays: due to their low trophic position, the concentration of these pollutants in their tissues is below detection levels. Being filter-feeders, they are at the base of the food web and do not biomagnificate these persistent pollutants as other predatory elasmobranchs do (Table 13).

Table 13: Comparison of our results with other studies where levels of OCPs were determined in elasmobranchs or filterfeeding megafauna in the wild. Concentration reported as range of concentrations found (in ng/g).

Area	Species	Tissue	Analyte	ng/g	Reference
Arctic	*B.mysticetus* (n=1)	Blubber	ΣDDTs	71 lb	(Mössner & Ballschmiter, 1997)
Arctic	*S.microcephalus* (n=17)	Liver	ΣDDTs	7,159 lb	(Fisk *et al.*, 2002)
China	*C.plagiosum* (n=35)	Muscle	ΣDDTs	0.6-23.5 ww	(Cornish *et al.*, 2007)

Mediterranean	*B. physalus* (n=5)	Blubber	ΣDDTs	6,580-26,833 lb	(Fossi *et al.*, 2014)
Mediterranean	*C. maximus* (n=6)	Muscle	ΣDDTs	ND-2,638 lb	(Fossi *et al.*, 2014)
South Africa	*C. carcharias* (n=15)	Muscle	ΣDDTs	86-1,417dw	(Marsili *et al.*, 2016)
Banderas Bay	*M. birostris* (n=13)	Skin	ΣOCPs	ND	Present study
Revillagigedo	*M. birostris* (n=25)	Muscle	ΣOCPs	ND	Present study
Djibouti	Mixed zooplankton (n=4)	Whole	ΣDDTs	21.4-79.2 dw	(Boldrocchi *et al.*, 2018)
Banderas Bay	Mixed zooplankton (n=2)	Whole	ΣOCPs	ND	Present study
Revillagigedo	Mixed zooplankton (n=2)	Whole	ΣOCPs	ND	Present study

ND= not detected, NR=detected but not reported, lb= lipid base, dw= dry weight, ww=wet weight

The presence of pesticides and PCBs showed a high intraspecific variability of concentrations in the muscle of stranded filter-feeding basking shark in the Mediterranean (Fossi *et al.*, 2014). We therefore could also expected to detect OCs in the muscle and dermis of oceanic manta rays in the Mexican Pacific Ocean. Nevertheless, the absence of pesticides and low concentrations of PCBs in their biopsies may indicate a lower baseline contamination level present in our study area, compared to the heavily polluted Mediterranean Sea.

The presence of pesticides adsorbed on plastics in both study areas, and the absence of them in the biopsies of manta rays, is a further indicator that oceanic manta rays are probably not ingesting plastics. If they were, we would have detected pesticides in their biopsies, as suggested by Fossi *et al.*, 2017. The pollutants adsorbed on plastics are desorbed under gastric conditions (Bakir *et al.*, 2014) and therefore enter into the circulatory system, ending up accumulated in the tissues of the organisms. Due to the persistence of OCs in animal tissues, we would expect to be able to detect them also in skin and muscle, as used for ecotoxicological studies in other species (Marsili *et al.*, 2016; Fossi *et al.*, 2017).

Homeotherms tend to accumulate higher concentrations of POPs compared to poikilotherms living in the same environment (Hop *et al.*, 2002). Although it has been suggested that mobulids are capable of brain warming (ALEXANDER, 1996), oceanic manta rays are fish and are therefore considered poikilotherms. This might explain the absence of pesticides in manta ray biopsies and the low concentrations of PCBs. The analysis of OCs in baleen whales, such as the humpback whales *Megaptera*

novaengliae, that are present in both BB and AR, might reveal that not all the filter-feeding megafauna exhibit the same low concentrations of OCs as oceanic manta rays.

Previous studies of OCPs show detectable levels in fatty tissues of filter-feeding megafauna, such as in the blubber of baleen whales (Mössner & Ballschmiter, 1997; Fossi *et al.*, 2014). It is possible that oceanic manta rays in the Mexican Pacific Ocean accumulate detectable levels of pesticides in their fatty tissues, like the liver, brain and gonads. Nevertheless, in Mexico we do not have access to these organs unless a stranding occurs. In that case, it would be extremely interesting to analyze POPs in their fatty tissues to support this hypothesis.

The analysis of OCs in zooplankton revealed that manta rays in the Mexican Pacific Ocean are probably exposed to very low levels of pollutants through their diet. Previous studies in other parts of the world have detected high concentrations of OCs in zooplankton, however no pesticides or PCBs were detected in the four samples analyzed. We recognize that the small sample size of the present study, does not allow us to generalize and more studies are needed in order to determine the levels of OCs that are at the base of the food web.

The next step for the determination of the ecotoxicological status of oceanic manta rays, could be investigating the possibility of a biochemical response to pollutants in their tissues. An analysis of biomarkers of exposure to plasticizers such as antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)) (Zheng *et al.*, 2013), and to POPs such as indicators of oxidative stress (P450 system, ethoxyresorufin-O-deethylase (EROD) activity, oxidized/reduced glutathione (GSH/GSSG) ratio, etc.) could indicate if the PCBs and PAHs that were detected in the manta rays are causing biochemical stress (Sparling, 2016).

10 General Conclusions and Outlooks

Plastic debris contaminates the oceans worldwide and the Mexican Pacific Ocean is not an exception. In coastal areas, the abundance of floating debris seems to be driven by the seasonality of rainfall that brings plastic debris from the mainland to the ocean. These plastics are transported by the currents and winds to remote areas, including the Revillagigedo Archipelago, where the present study reports for the first time this type of contamination. The size of the plastics found suggests that they can easily enter the food chain from the base up, potentially having negative impacts on the whole food web.

Beached and floating plastics in the Mexican Pacific Ocean are a vector for toxic chemicals. Endocrine disruptors and carcinogenic substances have been found adhered to the surface of plastics, chemicals that can potentially be transferred to the animals ingesting them. This is of concern for the possible impact they can have on vulnerable species, both marine and terrestrial, by reducing their reproductive success and their fitness. Some endemic species in the Revillagigedo Archipelago could be in danger of the negative consequences of toxic chemicals found adsorbed on plastics (such as DDTs).

The oceanic manta rays we sampled in the Mexican Pacific Ocean had no detectable levels of pesticides in biopsies of skin and muscle. We detected trace levels of PCBs and high levels of PAHs. These pollutants could have entered the oceanic manta rays' systems through the gills or through ingestion of contaminated preys or plastics. PAHs can have natural or anthropogenic sources in the environment, since they are products of incomplete combustion. On the other hand, PCBs are synthetic compounds that imply an anthropogenic contamination.

In order to identify possible ingestion of plastics by manta rays, phthalates were analyzed in their skin and muscle, and no detectable levels were found of the main plasticizers used in the plastics industry. This could be explained by one or more of the following scenarios:

1. Manta rays are not ingesting plastics, since they are feeding primarly on deep water aggregations of zooplankton. The low concentrations of plastic debris in the area implicates that the probability of ingestion by manta rays is also low.
2. Manta rays are ingesting plastics, but the residence time between ingestion and excretion of plastics is so short that plasticizers don't have enough time to migrate from the debris to the manta rays tissues.
3. Manta rays are ingesting plastics, and plasticizers are accumulated in the fatty tissues (gonads, liver, brain), but we don't have access to these, since manta rays are protected and not targeted in the fisheries, and stranded animals are rarely reported.
4. Manta rays are ingesting plastics and they metabolize phthalate diesters very efficiently and eventually eliminate the monoesters.

These hypotheses are yet to be tested, and further studies in the future should focus on these paths in order to have a clearer idea of the real magnitude of the threat that plastic pollution can represent for manta rays. Similar studies should be done also in other areas of the world, where manta ray populations are present, and where a higher abundance of plastic debris has been found. This can be the case of Hawaii, where manta rays are found in an area that is close to the North Pacific Garbage Patch, and could potentially be in contact with concentrations of orders of magnitude higher than the ones we found in the Mexican Pacific Ocean. South East Asia is one of the most polluted areas of the world, located between some of the main sources of plastic pollution globally. It is also an area of high biodiversity, where reef and oceanic manta rays are commonly found. It could be interesting to compare our results with those from these areas.

The next step could be to determine if there is a biochemical response to pollutants in the tissues of oceanic manta rays from the Mexican Pacific Ocean. An analysis of biomarkers of exposure to plasticizers such as antioxidant enzymes (SOD, CAT, and GPx), and to POPs such as indicators of oxidative stress (P450 system, EROD, GSH/GSSG ratio, etc.), could indicate if the PCBs and PAHs that were detected in the manta rays are causing biochemical stress.

This study provides a baseline for plastic pollution in the Mexican Pacific Ocean, an essential first step towards the improved management of plastic debris and the determination of species that could be more vulnerable to this emerging threat, due to their biology and life history characteristics.

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12 Supplementary Data

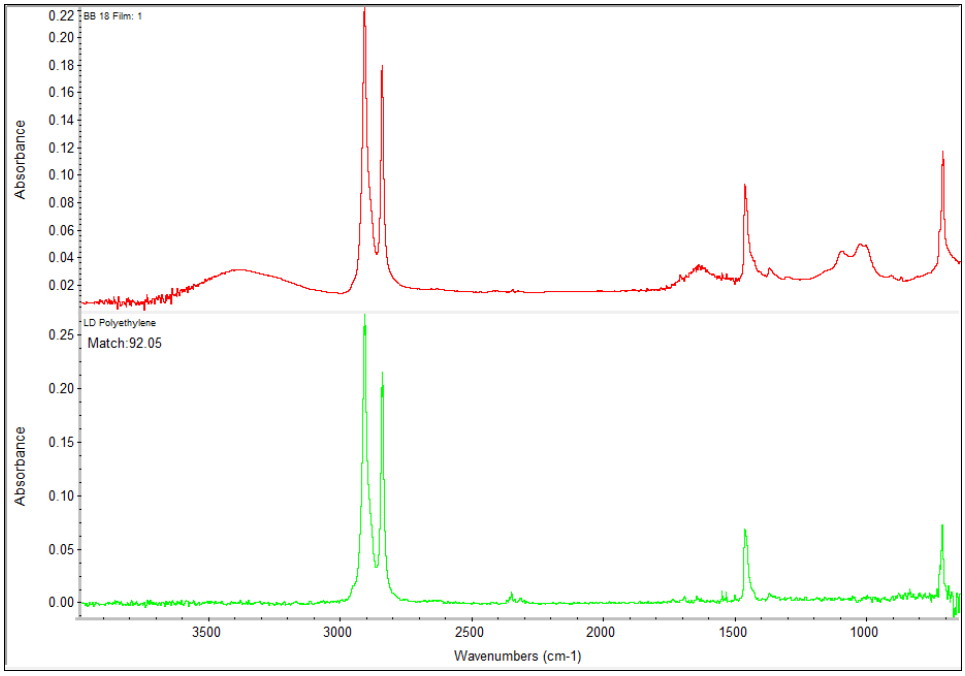

Figure 57: Example of comparation absorbance spectra of sample (above) and internal library sample (below) of Polyethylene (PE), one of the most abundant polymers found in both study areas

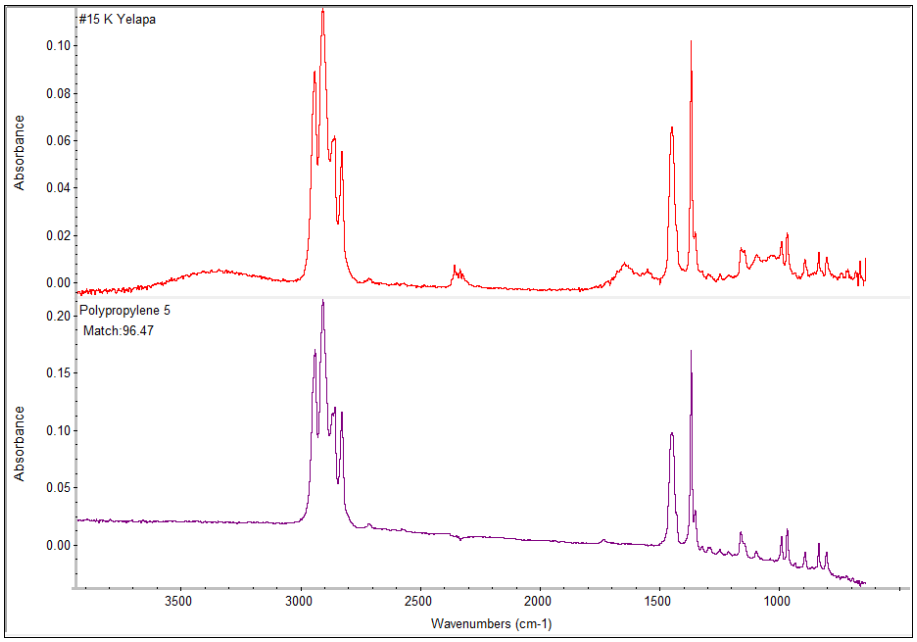

Figure 58: Example of comparation absorbance spectra of sample (above) and internal library sample (below) of Polypropylene (PP), one of the most abundant polymers found in both study areas

Table 14: Information about the biopsy samples analyzed.

Sample ID	Lab	Area	Sex	Tissue	Weight (mg dw)
BB231216A	UNAM	BB	F	D	26.1
BB17416	UNAM	BB	M	D	20.6
BB19716	UNAM	BB	M	D	33.3
BB24716	UNAM	BB	M	D	46
MG44	UNAM	AR	F	M	28.6
MG47	UNAM	AR	F	M	33.9
MG49	UNAM	AR	F	M	26.6
MG51	UNAM	AR	ND	M	44
MG54	UNAM	AR	M	D	35.3
MG57	UNAM	AR	F	M	47.8
MG62	UNAM	AR	M	D	34.9
MG67	UNAM	AR	F	D	50.7
MG69 D	UNAM	AR	M	D	30
MG69 M	UNAM	AR	M	M	58
MG70	UNAM	AR	ND	M	38
MG71	UNAM	AR	ND	M	56
MG73	UNAM	AR	F	M	29
MG74	UNAM	AR	F	M	39
BB08F0418	UWS	BB	ND	D	14.7
BB130817 B	UWS	BB	M	D	34.9
BB130817 A	UWS	BB	M	D	14.5
BB021217	UWS	BB	M	D	15.2
BB050518	UWS	BB	M	D	46.2
BB120518	UWS	BB	F	D	21.3
BB291017	UWS	BB	ND	D	13.4
BB020318	UWS	BB	F	D	16.8
BB261117	UWS	BB	F	D	29.7
MG105	UWS	AR	M	M	124.8
MG 90	UWS	AR	F	M	44.1
MG 76	UWS	AR	ND	M	47
MG 83	UWS	AR	M	M	61.9
MG 91	UWS	AR	F	M	44.2
MG 104	UWS	AR	F	D	44.7
MG 77	UWS	AR	ND	M	111.5
MG 78	UWS	AR	F	M	70.7
MG 79	UWS	AR	F	M	55.7
MG 80	UWS	AR	F	M	55.3
MG 82	UWS	AR	M	M	57

Area: BB= Banderas Bay, AR= Revillagigedo Archipelago; Sex: ND= not determined, M= male, F= female; Tissue: M= muscle, D= dermis, dw= dry weight

Table 15: Phthalates analyzed at the UNAM laboratory, retention time (RT), quantification and confirmation ions, limits of detection (LOD) and quantification (LOQ) in ng/g

Phthalates	RT	Quant. Ion	Conf. Ions	LOD	LOQ
Dimethyl phthalate	8.97	76	77 163	40	132
Diethyl phthalate	10.11	149	150 176	10	32
Dibutyl phthalate	11.04	76	149 150	33	111
Benzyl butyl phthalate	12.73	91	149 206	20	67
Di(2-ethylhexyl) phthlate	15.81	71	149 167	16	52
Di-n-octyl phthalate	16.04	71	149 167	15	50

Table 16:PAHs analyzed at the UNAM laboratory, retention time (RT), quantification and confirmation ions, limits of detection (LOD) and quantification (LOQ) in ng/g

PAHs	RT	Quant. Ion	Conf. Ions	LOD	LOQ
Naphthalene	6.675	102	127 128	20	62
1-Methylnaphthalene	7.741	115	141 142	17	51
2-Methylnaphthalene	7.882	115	141 142	17	51
Acenaphthylene	9.134	151	152 153	7	20
Acenaphtene	9.415	151	152 153	6	18
Fluorene	10.225	163	165 166	4	13
Phenanthrene	11.756	176	178 179	4	12
Anthracene	11.831	176	178 179	4	12
Fluoranthene	13.642	200	202 203	4	12
Pyrene	14.00	200	202 203	4	13
Benzo(a)anthracene	15.905	226	228 229	5	15
Chrysene	15.959	226	228 229	6	19
Benzo(b)fluoranthene	17.526	250	252 253	5	17
Benzo(k)fluoranthene	17.570	250	252 253	6	17
Benzo(a)pyrene	18.167	250	252 253	6	17
Benzo[ghi]perylene	20.388	276	277 278	6	20
Dibenz(a,h)anthracene	20.435	276	277 278	6	18
Indeno[1,2,3-cd]fluornathene	21.04	276	277 278	6	19

Table 17: Phthalates analyzed at the UWS laboratory, retention time (RT), quantification and confirmation ions, limits of detection (LOD) and quantification (LOQ) in ng/g.

Phthalates	RT	Quant. Ion	Conf. Ions	LOD	LOQ
Dimethyl phthalate	5.450	163	194 77	0.4	1.2
Diethyl phthalate	6.130	149	177	0.4	1.2
Benzyl butyl phthalate	9.204	149	206	0.6	2.1
Di(2-ethylhexyl) phthlate	9.824	149	167 279	0.5	1.5
Di-n-octyl phthalate	10.580	149	279	0.1	0.5

Table 18: Polycyclic aromatic hydrocarbons analyzed at UWS, retention time (RT), quantification and confirmation ions, limits of detection (LOD) and quantification (LOQ) in ng/g.

PAHs	RT	Quant. Ion	Conf. Ions	LOD	LOQ
Naphthalene	9.949	102	127 128	0.4	1.4
1-Methylnaphthalene	12.741	115	141 142	0.84	2.8
2-Methylnaphthalene	13.169	115	141 142	4.9	16.6
Acenaphthylene	16.597	151	152 153	0.8	2.8
Acenaphtene	17.456	151	152 153	1.3	4.4
Fluorene	19.757	163	165 166	2.1	7.0
Phenanthrene	24.073	176	178 179	0.9	3.2
Anthracene	24.282	176	178 179	0.7	2.4
Fluoranthene	29.591	200	202 203	0.5	1.7
Pyrene	30.546	200	202 203	0.6	2.3
Benzo(a)anthracene	36.242	226	228 229	0.7	2.4
Chrysene	36.416	226	228 229	2.0	6.6
Benzo(k)fluoranthene	40.977	250	252 253	0.6	2.0
Benzo(b)fluoranthene	41.057	250	252 253	5.5	18.3
Benzo(a)pyrene	42.194	250	252 253	2.2	7.5
Benzo[ghi]perylene	46.292	276	277 278	1.4	4.6
Dibenz(a,h)anthracene	46.487	276	277 278	1.9	6.5
Indeno[1,2,3-cd]fluornathene	47.092	276	277 278	0.7	2.5

Table 19: PCB congeners analyzed (common names), retention time (RT), quantification, confirmation ions and limits of detection (LOD) and quantification (LOQ) in ng/g. In bold, are the six PCB congeners listed as indicators of PCBs in food by the FAO and WHO (FAO/WHO, 2016)

PCB congener	RT	Quant. Ion	Conf. Ions	LOD	LOQ
PCB-18	23.238	256	186 258	0.3	0.9
PCB-17	24.341	256	186 258	0.2	0.7
PCB-31	25.447	256	186 258	0.2	0.8
PCB-33	25.457	256	186 258	0.3	0.9
PCB-28	25.940	256	186 258	0.1	0.4
PCB-49	27.295	292	220 290	0.2	0.8
PCB-52	27.528	292	220 290	0.3	1.1
PCB-44	28.440	292	220 290	0.1	0.3
PCB-74	30.557	292	220 290	0.3	1.0
PCB-70	30.800	292	220 290	0.2	0.8
PCB-95	31.063	326	256 254	0.3	0.8
PCB-99	32.563	326	256 254	0.3	1.0
PCB-118	32.897	326	256 254	0.3	1.2
PCB-87	34.295	326	256 254	0.3	1.0
PCB-101	34.973	326	256 254	0.2	0.7
PCB-105	34.973	326	256 254	0.1	0.4
PCB-82	35.723	326	256 254	0.3	1.1
PCB-132	35.723	360	290 362	0.4	1.3
PCB-149	36.675	360	290 362	0.2	0.7
PCB-110	36.807	326	256 254	0.3	0.9
PCB-156	38.459	360	290 362	0.3	1.1
PCB-128	38.459	360	290 362	0.2	0.8
PCB-138	40.446	360	290 362	0.4	1.4
PCB-158	40.608	360	290 362	0.2	0.8
PCB-191	41.726	394	324 396	0.5	1.7
PCB-177	42.093	394	324 396	0.2	0.6
PCB-153	42.429	360	290 362	0.3	1.1
PCB-171	43.846	394	324 396	0.4	1.3
PCB-183	44.182	394	324 396	0.2	0.5
PCB-169	44.284	360	290 362	0.2	0.8
PCB-187	45.619	394	324 396	0.2	0.7
PCB-180	46.119	394	324 396	0.3	0.9
PCB-151	47.179	360	290 362	0.4	1.2

PCB-170	47.831	394	324 396	0.2	0.8
PCB-199	48.544	430	358 428	0.4	1.3
PCB-194	51.122	430	358 428	0.3	0.9
PCB-206	51.194	464	392 462	0.2	0.6
PCB-205	52.854	430	358 428	0.2	0.8
PCB-195	53.251	430	358 428	0.3	0.9
PCB-208	55.727	464	392 462	0.2	0.8

Table 20: Pesticides analyzed, retention time (RT), quantification and confirmation ions, limits of detection (LOD) and quantification (LOQ) in ng/g.

OCPs	RT	Quant. Ion	Conf. Ions	LOD	LOQ
2,4-DDE	30.634	246	248 318	0.8	2.7
2,4-DDD	32.114	235	237 165	1.1	3.8
2,4-DDT	33.438	235	237 165	1.4	4.6
a-BHC	22.362	183	181 219	1.2	4.0
g-BHC	23.541	183	181 219	0.3	0.9
b-BHC	23.74	109	183 219	0.8	2.6
d-BHC	24.774	109	183 219	0.0	0.0
Heptachlor	26.582	100	274 272	1.4	4.7
Aldrin	27.898	66	263 293	0.6	1.9
Heptachlor Epoxide	29.458	81	353 355	0.7	2.3
a-Chlordane	30.361	373	375 377	1.0	3.4
Endosulfan I	30.803	241	243 195	1.1	3.7
g-Chlordane	30.934	375	373 377	0.6	1.8
Dieldrin	31.124	407	409 411	1.9	6.3
Cis-Nonachlor	31.775	81	263 277	0.6	1.9
p,p'-DDE	31.775	79	263 281	0.4	1.4
Endrin	31.82	246	248 318	1.2	3.9
Endosulfan II	32.912	195	197 241	0.6	2.0
4,4-DDD	33.338	235	237 165	0.4	1.4
Trans-Nanochlor	33.417	409	407 411	0.6	1.9
Endrin Aldehyde	33.615	67	345 250	1.8	5.9
Endosulfan Sulfate	34.321	387	272 274	1.8	5.9
p,p'-DDT	34.678	235	237 165	3.3	11.1
Endrin Ketone	36.102	67	317 319	2.4	8.0
Methoxychlor	35.266	227	228	1.0	3.2

Table 21: PAHs detected in plastic samples collected in Revillagigedo Archipelago (AR) and Banderas Bay (BB). For each sample, the polymer composition, type (macro or micro plastic) and area are shown. Concentrations are in ng/g of plastics.

Sample ID	4 082317	12 082317	13 082317	14 082317	15 082317	2 083017	6 083017	8 083017	9 083017	9B 083017	REV 18 Manta	BB18 Manta	BB18 Line	BB18FRAG	BB18FILM
Polymer	MIX	PS	PE	PE	MIX BB	PE	PE	PE	PE/PP	PE/PP	MIX	MIX	MIX	PE/PP	PE/PP
Type	Macro	Macro	Macro	Macro	Micro	Macro	Macro	Macro	Macro	Macro	Micro	Micro	Macro	Macro	Macro
Area	AR	AR	AR	AR	BB	AR	AR	AR	AR	AR	AR	BB	BB	BB	BB
Naphthalene	24.58	35.66	ND	4.23	ND	ND	1.59	ND	ND	27.37	63.98	48.73	ND	165.29	102.50
1-methylnaphtalene	79.96	21.09	ND	4.16	ND	ND	ND	ND	ND	ND	22.06	18.33	ND	68.76	ND
2-methylnaphthalene	26.35	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	36.37	ND
Acenaphthylene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	18.21	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	51.87	1003.29
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	170.95	66.08	ND	76.76	6104.66
Phenanthrene	34.37	46.11	ND	21.40	ND	ND	20.91	3.76	ND	4.13	394.31	116.99	ND	202.69	392.98
Anthracene	6.60	ND	ND	ND	ND	ND	ND	ND	ND	ND	58.64	14.94	ND	ND	ND
Fluoranthene	34.50	ND	ND	12.75	ND	ND	ND	ND	ND	5.46	12.57	ND	ND	41.92	79.97
Pyrene	91.74	ND	ND	10.79	ND	ND	ND	ND	ND	4.47	ND	ND	ND	25.96	11.88
Benzo(a)anthracene	ND	3.18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	94.04
Chrysene	ND	19.71	6.96	45.65	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	201.51
Benzo(k)fluoranthene	ND	5.64	ND	ND	60.86	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[ghi]perilene	ND	ND	122.90	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.33
ΣPAHs	298.11	131.39	129.87	98.99	60.86	0.00	22.51	3.76	0.00	41.44	740.72	265.07	0.00	669.62	7997.17

Table 22: PCBs detected in plastic samples collected in Revillagigedo Archipelago (AR) and Banderas Bay (BB). For each sample, the polymer composition, type (macro or micro plastic) and area are shown. Concentrations are in ng/g of plastics. In bold, the only PCBs indicator found.

Sample ID	4 082317	12 082317	13 082317	14 082317	15 082317	2 083017	6 083017	8 083017	9 083017	9B 083017	REV 18 Manta	BB18 Manta	BB18 Line	BB18FRAG	BB18FILM
PCB-18	1.95	2.21	2.17	1.09	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB-31/33	1.43	1.15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB-28	**2.42**	**1.40**	**1.72**	**1.47**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
PCB-49	1.87	1.46	1.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB-70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.95	ND	ND	ND	ND
PCB-60	2.38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB-95	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.94	ND	ND	ND	ND
PCB-99	ND	1.06	1.35	ND	ND	ND	ND	ND	ND	ND	0.31	ND	ND	ND	ND
PCB-132	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.26	ND	ND	ND	ND
PCB-156	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	36.27	ND	ND	ND	ND
PCB-128	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	61.81	ND	ND	ND	ND
ΣPCBs	10.06	7.28	6.35	2.56	ND	ND	ND	ND	ND	ND	127.54	ND	ND	ND	ND

Table 23: Pesticides detected in plastic samples collected in Revillagigedo Archipelago (AR) and Banderas Bay (BB). For each sample, the polymer composition, type (macro or micro plastic) and area are shown. Concentrations are in ng/g of plastics. ND= not detected or below detection limit.

Sample ID	4 082317	12 082317	13 082317	14 082317	2 083017	6 083017	8 083017	9 083017	9B 083017	REV 18 Manta	BB18 Manta	15 082317	BB18 Line	BB18FRAG	BB18FILM
Area	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	BB	BB	BB	BB	BB
a-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	234.55	15.87	ND	ND	ND	ND
g-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	126.60	13.38	ND	ND	ND	ND
Cis-Nonachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	133.36	14.47	ND	ND	ND	ND
Trans-Nanochlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	101.70	24.04	ND	ND	ND	ND
2,4-DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	158.15	ND	ND	ND	ND
2,4-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	903.32	ND	ND	ND	ND
4,4-DDE	ND	ND	ND	162.62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DDD	ND	ND	ND	365.47	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DDT	ND	ND	ND	282.03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ΣPesticides	ND	ND	ND	810.11	ND	ND	ND	ND	ND	596.21	1129.23	ND	ND	ND	ND
ΣDDTs	ND	ND	ND	810.11	ND	ND	ND	ND	ND	ND	1061.47	ND	ND	ND	ND

Table 24: PAHs detected in biopsies of oceanic manta rays in the Mexican Pacific Ocean. Concentrations are in ng/g dw.

Sample	*Naphthale.*	*1-methylnaph.*	*2-methylnaph.*	*Acenaphthyl.*	*Phenanthrene*	*Fluoranthe.*	*Pyrene*	*Benzo(a)anthracene*	*Chrysene*	*Benzo(k)fluoranthene*	*Benzo(b)fluoranthene*	*Benzo(a)pyrene*	*Benzo[ghi]perylene*	*Dibenz(a,h)anthracene*	*Indeno[1,2,3-cd]fluornathene*	*ΣPAH*
BB17416	ND	294.2	427.9	ND	109.7	38.2	52.4	ND	ND	ND	ND	ND	ND	ND	ND	922.4
BB19716	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB24716	508.8	318.3	363.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1191.0
BB231216A	ND	141.6	ND	181.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	323.3
BB130817 B	ND	ND	ND	ND	ND	ND	12.0	ND	20.8	ND	ND	ND	ND	ND	ND	32.7
BB130817 A	ND	598.7	456.3	57.3	531.6	19.7	33.9	ND	54.2	ND	ND	ND	ND	ND	ND	1751.7
BB291017	ND	ND	ND	ND	92.1	59.6	36.8	ND	58.2	ND	19.7	ND	ND	ND	ND	266.4
BB261117	ND	6.8	ND	ND	92.2	53.7	26.1	ND	26.6	ND	ND	ND	ND	ND	ND	205.4
BB021217	ND	ND	ND	ND	ND	25.1	10.1	ND	51.8	ND	ND	ND	ND	ND	ND	87.0
BB020318	ND	ND	ND	ND	ND	ND	16.9	ND	43.0	ND	ND	ND	ND	ND	ND	59.9
BB080418	ND	2498.6	ND	256.6	2556.5	125.0	63.0	ND	ND	ND	ND	ND	ND	ND	ND	5499.8
BB050518	ND	ND	ND	ND	32.0	14.9	12.1	ND	16.8	ND	ND	ND	ND	ND	ND	75.8
BB120518	510.8	115.2	142.8	ND	345.9	18.4	18.0	ND	ND	ND	ND	ND	ND	ND	ND	1151.2
MG44	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG47	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG54	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG57	ND	ND	ND	29.0	ND	ND	ND	ND	151.6	284.7	266.1	329.4	380.7	371.2	358.4	2170.9
MG62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG67	ND	ND	ND	34.2	ND	ND	ND	28.9	32.7	48.1	34.9	42.4	ND	ND	ND	221.2
MG69 D	ND	ND	ND	ND	ND	ND	ND	ND	15.1	ND	ND	ND	ND	ND	ND	15.1
MG69 M	ND	ND	ND	ND	ND	ND	ND	6.8	6.6	ND	ND	ND	ND	ND	ND	13.3

MG70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG71	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG73	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 76	ND	ND	ND	ND	33.7	11.8	25.5	ND	17.6	ND	ND	ND	ND	ND	ND	88.7
MG 77	ND	ND	ND	ND	95.3	38.8	24.4	ND	9.2	4.2	ND	ND	ND	ND	ND	171.8
MG 78	ND	4.9	ND	ND	78.7	31.9	20.9	ND	12.0	ND	ND	ND	ND	ND	ND	148.4
MG 79	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 80	ND	14.7	ND	ND	50.5	12.7	14.4	ND	13.7	ND	ND	ND	ND	ND	ND	106.0
MG 82	ND	ND	ND	2.0	264.5	99.5	49.0	ND	20.7	ND	28.4	65.9	ND	50.6	ND	580.6
MG 83	ND	ND	ND	ND	38.9	8.9	9.1	ND	12.0	ND	ND	ND	ND	ND	ND	68.9
MG 90	ND	ND	ND	ND	292.3	109.2	53.8	ND	18.7	ND	ND	ND	ND	ND	ND	473.9
MG 91	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 104	8.7	27.9	28.6	ND	341.0	116.8	53.7	ND	22.4	ND	ND	ND	ND	ND	ND	599.0
MG105	313.8	ND	ND	ND	65.5	29.6	16.3	ND	ND	ND	ND	ND	ND	ND	ND	425.2

ND= not detected or below detection limit

Table 25: Concentration of PAHs detected in zooplankton in the Mexican Pacific Ocean. Concentrations are in ng/g dw.

	Revillagigedo		*Banderas Bay*	
Sample	ZPAR16 #11	ZPAR 16#5	ZPBB 17 Nov	ZPBB18
Naphthalene	ND	407.5	26.1	242.9
1-methylnaphthalene	5.3	326.4	45.4	153.9
2-methylnaphthalene	ND	146.0	25.3	73.5
Acenaphthylene	ND	30.0	ND	22.5
Acenaphtene	53.0	308.1	55.3	154.1
Fluorene	38.4	269.6	53.2	132.1
Phenanthrene	79.8	477.1	152.1	306.7
Anthracene	9.5	50.4	19.1	28.0
Fluoranthene	17.3	207.7	38.4	181.5
Pyrene	10.7	93.7	25.9	171.8
Benzo(a)anthracene	ND	ND	8.5	ND
ΣPAHs	214.1	2316.5	449.3	1467.0

ND= not detected or below detection limit

Table 26: Concentrations of PCBs found in the biopsies of oceanic manta rays in the Mexican Pacific Ocean. Concentrations are in ng/g dw. ND= not detected. Only PCBs detected are shown.

Sample	*PCB 18*	*PCB 28*	*PCB 99*	*PCB 101*	*PCB 132*	*PCB 138*	*PCB 191*	*PCB 206*	*ΣPCBs*
BB130817 B	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB130817 A	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB291017	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB261117	ND	1.00	ND	ND	5.83	ND	ND	ND	6.83
BB021217	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB020318	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB080418	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB050518	ND	ND	ND	ND	ND	ND	ND	ND	ND
BB120518	5.43	9.21	ND	ND	ND	ND	ND	ND	14.63
MG76	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 77	ND	0.78	ND	ND	ND	2.40	ND	2.89	6.07
MG 78	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG79	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 80	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 82	1.43	ND	1.07	6.03	ND	ND	ND	13.38	21.91
MG 83	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 90	0.30	1.77	ND	ND	ND	ND	ND	ND	2.08
MG 91	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 104	ND	ND	ND	ND	ND	ND	ND	ND	ND
MG 105	ND	0.59	ND	ND	ND	ND	2.67	ND	3.26

ND= not detected or below detection limit