INSTITUTO POLITÉCNICO NACIONAL

CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

Organochlorine Contaminants (OCs) in white sharks (*Carcharodon carcharias*) and Northern elephant seals (*Mirounga angustirostris*) in Guadalupe Island, Baja California, Mexico.

TESIS

QUE PARA OBTENER EL GRADO DE

MAESTRÍA EN CIENCIAS EN MANEJO DE RECURSOS MARINOS

PRESENTA

GÁDOR MUNTANER LÓPEZ

LA PAZ, B.C.S, MAYO DE 2019

INSTITUTO POLITÉCNICO NACIONAL
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En la Ciudad de La Paz, B.C.S., siendo las 12:00 horas del día 07 del mes de Mayo 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:

"ORGANOCHLORINE CONTAMINANTS (OCs) IN WHITE SHARKS (*Carcharodon carcharias*) AND NORTHERN ELEPHANT SEALS (*Mirounga angustirostris*) IN GUADALUPE ISLAND, BAJA CALIFORNIA, MÉXICO"

Presentada por el alumno:

MUNTANER	LÓPEZ	GÁDOR
Apellido paterno	materno	nombre(s)

Con registro: | A | 1 | 7 | 0 | 8 | 8 | 2 |

Aspirante de:

MAESTRIA EN CIENCIAS EN MANEJO DE RECURSOS MARINOS

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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Abstract

The presence of Organochlorine Contaminants (OCs) such as Organochlorinated Pesticides (OCPs) and Polychlorinated biphenyls (PCBs) in animal tissues can affect their reproduction and development. Top predators are particularly susceptible to accumulate high levels of OCs due to their high trophic position, longevity and inability to process and excrete certain components. Understanding the degree of exposure to contaminants is a critical first step for the management and conservation of the species. The present study determines the exposure level of white sharks (WS) and Northern elephant seals (NES) to OCs in Guadalupe Island, one of the most important aggregation sites for both species, and compares the bioaccumulation patterns among different age classes and sexes. Biopsy samples were taken from 32 free-ranging WS and 35 NES and its lipid was extracted via Soxhlet to be analyzed for DDT and its metabolites (n = 6), Chlordanes and 54 congeners of PCBs, using GCMS (Gas Chromatography – Mass Spectrometry). The chromatograms indicated the presence of 5 different compounds OCPs and 10 PCB congeners in WS, and 7 OCPs and 22 PCB congener in NES. The average concentrations and ranges for WS expressed in ng/g d.w. basis were 97.7(13.4-291) of tDDTs, 3.09 (0-16.5) of tCHLs and 31.1 (0-219) of tPCBs, and the average concentrations for NES were 3827 (429-28744) of tDDTs, 184 (0-703) of tCHLs, and 587 (0-4487) of tPCBs. The levels of tDDTs were significantly higher in adults than in juveniles of both target species, suggesting an age class-based bioaccumulation, and confirming the differences described in literature in between the feeding behavior of adults and juveniles of both species. Although no significant differences in tDDT, tCHL and tPCB concentrations were found, a higher variability was observed in females NES, which could be explained by the diet variation between adult males and females described in literature using stable isotope analysis. WS and NES showed a similar OC average abundance pattern, suggesting the NES as a potential source of contamination for the WS, its main predator in Guadalupe Island.

Resumen

La presencia de compuestos orgánicos persistentes (COPs) tales como los Pesticidas Organoclorados (OCPs) y los Bifenilos Policlorados (PCBs) en tejidos animales puede afectar su reproducción y desarrollo. Los depredadores tope son particularmente susceptibles a acumular altos niveles de COPs debido a su alta posición trófica, longevidad e incapacidad para procesar y excretar ciertos compuestos. Comprender el grado de exposición a los contaminantes es un primer paso crítico para el manejo y la conservación de la especie. El presente estudio determina el nivel de exposición de los tiburones blancos (TB) y los elefantes marinos del norte (EMN) a los COPs en Isla Guadalupe, uno de los sitios de agregación más importantes para ambas especies, y compara los patrones de bioacumulación entre diferentes clases de edad y sexos. Se tomaron biopsias de 32 TB y 35 EMN, cuyo contenido lipídico fue extraído mediante Soxhlet para analizar DDT y sus metabolitos (n=6), Clordanos totales y 54 congéneres de PCBs, mediante Cromatografía de gases-Espectrometría de masas. Los cromatogramas indicaron la presencia de 5 OCPs diferentes y 10 congéneres de PCBs en TB, y 7 OCPs y 23 congéneres de PCB en EMN. Las concentraciones promedio y los rangos para TB (ng/g p.s.) fueron 97.7(13.4-291) de tDDTs, 3.09 (0-16.5) de tCHLs and 31.1 (0-219) de tPCBs, mientras que las concentraciones promedio para EMN fueron 3827 (429-28744) de tDDTs, 184 (0-703) de tCHLs, y 587 (0-4487) of tPCBs. Los niveles de tDDT fueron significativamente más altos en adultos que en juveniles de ambas especies, sugiriendo la existencia de bioacumulación, así como las diferencias entre hábitos alimentarios de adultos y juveniles de ambas especies, descritas previamente en la literatura. Aunque no se encontraron diferencias significativas en las concentraciones de tDDT, tCHL y tPCB entre sexos, se observó una mayor variabilidad en las concentraciones de tDDT y tPCB en las hembras de EMN, que podría explicarse por la variación de la dieta entre machos y hembras adultas descrita en la literatura usando análisis de isótopos estables. Los TB y los EMN mostraron un patrón de abundancia promedio de COPs similar, lo que sugiere al

EMN como una fuente potencial de contaminación para el TB, su principal depredador en Isla Guadalupe.

INTRODUCTION

Contaminants in the marine environment, such as Organochlorine Contaminants (OCs), are currently a global concern in reference to ecosystems, wildlife and human health (Mull et al., 2013). Most of the contaminants discharged into the environment end up in aquatic ecosystems, where they can remain in sediments or enter the food chain through their assimilation by living organisms, reaching levels that can be potentially harmful to higher trophic level organisms or even to human (Sumpter 2009). OCs include a range of very stable compounds that are typically soluble in lipids and have very low solubility in water. Among them, the polychlorinated biphenyls (PCBs), whose toxic effects can affect the endocrine and nervous system (Storelli et al., 2003), and the organochlorinated pesticide Dichlorodiphenyltrichloroethane (DDT), which presents neurotoxic and agonist activity of estrogen (responsible for numerous effects on reproduction), are of special importance.

In the northern hemisphere, environmental levels of OCs began to decline from the 1970s and stabilized in the 1980s due to industrial regulations and prohibitions (Weber et al., 2003). However, the export of banned pesticides to developing countries continued at least until 1999 (Smith, 2001). It is estimated that, in southern California, 110 tons of DDT and 11 tons of PCBs remain in the marine sediments of the Palos Verdes peninsula, despite the prohibition of discharge of these organic contaminants in the 1970s (Lee et al., 2002). The persistence of these contaminants in the area and their continuous redistribution due to physical and biological processes affects the health status of marine organisms that inhabit southern California, especially predators of high trophic level (Hose et al., 1989; Connolly et al., 2002) such as certain killer whale (*Orcinus orca*) populations that are close from collapse because of these factors (Desforges et al., 2018).

Depending on tides, marine currents and atmospheric transport, contaminants can travel long distances through different trophic levels and increase their concentration in the passage through the food chain. Because of this, the higher the trophic level of the organism, the greater the concentration of contaminants in its tissues (Reindjers et al., 1994). In addition, upper trophic level organisms have a limited ability to metabolize these compounds and therefore these compounds tend to bioaccumulate in certain tissues (Skoch, 1990).

The White Shark (WS) (*Carcharodon carcharias*) is an elasmobranch from the family Lamnidae which plays a fundamental role as top predator inhabiting the oceanic and coastal waters around the world. As juveniles they feed on benthic prey such as fish, invertebrates and other sharks, while as adults they feed on larger pelagic and marine mammals. Adults may reach up to an estimated age of 73 years old and more than 6 m of total length (LT). The sexual maturity size is 3.5 meters for males and 4.5 meters for females (Compagno, 2001). This species is currently classified as vulnerable on the Red List of the International Union of Conservation of Nature (IUCN). In Mexican waters, the presence of this highly migratory species is in the Gulf of California and the western coast of the states of Baja California and Baja California Sur, and Guadalupe Island Biosphere Reserve.

Guadalupe Island is considered one of the most important aggregation sites for the WS populations of the Eastern Pacific, with a high site fidelity. WS can be observed throughout the year, with a seasonal peak between autumn and winter, which coincides with an increase in the densities of its prey, such as the yellowfin tuna (*Thunnus albacares*) and the NES (*Mirounga angustirostris*) (Weng et al.,2007, Gallo-Reynoso et al., 2005, Domeier and Nasby-Lucas, 2008, Hoyos-Padilla, 2009, Jorgensen et al., 2010, Hoyos-Padilla et al., 2016).

The northern elephant seal (NES) (*Mirounga angustirostris)* is an important prey item for WS in Guadalupe Island (Rivera *et al.,* 2013; Hoyos-Padilla *et al.,* 2016). This species is currently categorized in the IUCN as Minor Concern (Reeves, 2005). NES forage offshore of the North Pacific, in the Gulf of Alaska, and near the Aleutian Islands (Stewart et al., 1995; Robinson et al., 2012).The breeding season occurs in winter and pups remain on land for more than two months (i.e. the post-weaning fast); in spring adult females and juveniles molt. Adult males molt in summer; and in autumn juveniles haul-out (Le Boeuf et al., 1994). The breeding and haul-out sites are located in in the subtropical eastern Pacific, particularly in Baja California and California, (Le Boeuf et al., 1994). Guadalupe island is one of the most important breeding areas for the species, representing 59% of the total births of Baja California (García-Aguilar et al., 2018). Because pinnipeds have a high body fat content and occur high trophically , they are useful sentinel species for monitoring lipophilic contaminants (Fossi et al., 2003, Kannan et al., 2004). The accumulation of these contaminants also makes pinnipeds highly susceptible to a variety of cancers, and the impairment of immune, reproductive, developmental, and endocrine systems (Swart et al., 1996, Ylitalo et al., 2005 , Blasius et al., 2008,).

It is well known that long-lived marine predators such as tuna, billfishes and sharks accumulate high levels of bioavailable contaminants through the trophic chain (Gelsleichter and Walker, 2010). High levels of contaminants have been shown to have adverse effects on the reproduction, growth and immune system of aquatic vertebrates (Gelsleichter et al., 2006).

Numerous studies have quantifed OC contaminants in the lipid-rich livers of elasmobranchs (Serrano et al., 2000; Storelli and Marcotrigiano, 2001; Fisk et al., 2002; Storelli et al., 2003; Gelsleichter et al., 2005, 2006, 2008; Schlenk et al., 2005; Cornish et al., 2007; Strid et al., 2007; Gelsleichter and Walker, 2010). Mull et al. (2012) and Marsili et al. (2016) demonstrated the presence of OCs in WS from California and South Africa, respectively. However, little is known about the contaminant levels accumulated in certain tissues of elasmobranches and pinnipeds from Mexican waters. At present, there are no studies that determine the presence of persistent organic contaminants in the WS and NES populations of the Mexican Pacific, nor the effects they produce on these individuals.

Previous studies have demonstrated that OCs can be carcinogenic, cause reproductive impairment, endocrine disruption, and immunotoxicity in many vertebrate species (Fry and Toone, 1981; Guillette et al., 1995; Nebert and Dalton, 2006). Marine mammal studies have shown relationships between OC exposure and immunotoxic consequences such as changes in cell proliferation and phagocytosis

(Lahvis et al., 1995; Van Loveren et al., 2000; Levin et al., 2004, 2005; Schwacke et al., 2012). Also, studies have related fatal epizootics in marine mammals with high OC concentrations (Hall et al., 1992; Aguilar and Borrell, 1996; Jepson et al., 1999). Furthermore, Sawyna et al (2017) provided evidence of OC correlated immunostimulation, primary driven by PCBs, in multiple elasmobranch tissues.

The presence of OCs in the tissues of WS and NES could affect certain physiological functions, and therefore the populations of both species in the area. Thus, understanding the degree of exposure to contaminants is a critical first step for a continuous monitoring of the contaminant loads and their possible physiological effects in the target species, as a tool for their management and conservation.

GOALS

General goal

The main goal of the present study is to determine Organic Contaminants in WS and NES from Guadalupe island.

Particular goals

1. Characterize the contaminant concentrations in axial muscle of WS and blubber of NES from Guadalupe Island.
2. Assess whether bioaccumulation is occurring in WS and NES by examining the relationships between tissue contaminant concentrations by age classes and sex.
3. Determine if there is a relationship between prey and predator by examining the contaminants found in each species and their abundance patterns
4. Compare contaminant levels with WS and NES sampled from other locations to determine the relative degree of exposure.

METHODS

Study Area

Guadalupe Island is located approximately 260 kilometers off the Baja California peninsula (García-Gutiérrez et al., 2005; CONANP-SEMARNAT, 2009) on the geographic coordinates of 29 ° 11 'N and 118 ° 16' W (Anonymous, 1979), constituting the last frontier of the Mexican Republic at its most western and northern end (**Fig.1**).

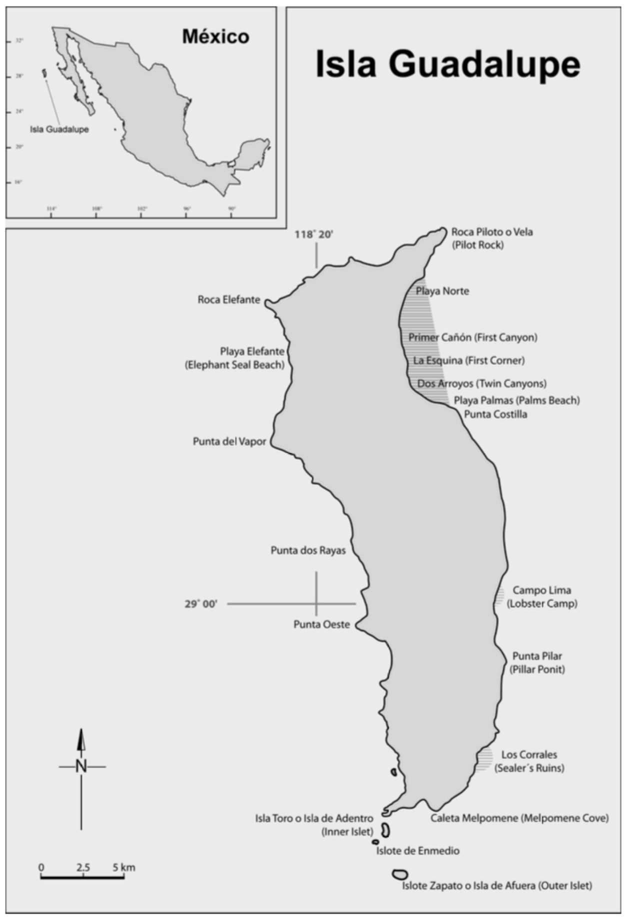

Figure 1. Location of the study area

The island is a 1,300 m volcanic peak surrounded by a narrow shelf, except for the southern tip, where the shelf extends away from the island with depths of 200 m between GI and its closest island, Inner Islet, and farthest, Outer Islet (**Fig. 1**). The offshore waters reach depths of 3600 m. Its north-south orientation and its elongated shape (35 km long and 6.5-9.5 km wide) make Guadalupe Island act as a barrier against the California Current, which results in a series of updrafts or upwellings and eddies in different areas and at different depths, providing cold waters rich in nutrients (Pierson 1987). Northwesterly winds predominate, and the surrounding waters have an average sea surface temperature of 18 °C, ranging from 16 °C in spring to 20°C in summer (Lynn and Simpson, 1987).

Sample collection

Biopsy samples were taken from axial muscle of 32 different free-ranging WS and blubber of 35 NES in Guadalupe Island on August- October 2016 and 2017. Target individuals were chosen randomly.

White Sharks

Each specimen was attracted near the boat by using fresh bait. The size of the individuals was estimated by using the boat as reference. All the sharks were recorded prior to obtaining the sample with a GoProH4 camera attached to an extendable pole, in order to determine their gender, based on the presence or absence of claspers, and to identify the different individuals by their marks and pigmentation patterns. **Table 1** shows the characteristics of each sampled shark.

The measurement accuracy only allowed us to classify WS and NES in a 50 cm resolution. Therefore, all individuals were assigned age classes according to the groups described by Bruce and Bradford (2012) (young of the year, <1.75 m total length (TL); juvenile, 1.75–3 m TL; subadult male, 3–3.6 m TL; subadult female, 3–4.8 m TL; adult male, >3.6 m TL; and adult female, >4.8 m TL). Biopsies (0.04-0.23 g d.w) were taken with a Hawaiian sport fishing pole spear with modified stainless steel tips (**Fig. 2a**), equipped with tissue retention hooks and capable of penetrating the shark's thick skin (**Fig. 4a),** as described by Jaime-Rivera et *al.* (2013). All tips were sterilized with alcohol and under flame in order to prevent any possible contamination.

To be able to obtain white muscle tissue without damaging the individuals, the best penetration zone for the extraction was the area under the first dorsal fin of the shark (**Fig. 2b).** All samples were wrapped in aluminum foil in order to avoid any contact with plastic materials that could contaminate the sample and were placed in a freezer (-20ºC).

Table 1. Summary of the number, ID, sex and age class of the sampled WS and Northern elephant seals; F = female, M = male, J = juvenile, SA = subadult, A = adult

White sharks

N	ID	Sex	Age Class
1	8-M-16	F	SA
2	9-M-16	M	J
3	12-M-16	M	SA
4	17-M-16	F	SA
5	19-M-16	M	SA
6	21-M-16	M	SA
7	22-M-16	M	SA
8	28-M-16	F	SA
9	30-M-16	F	SA
10	32-M-16	F	A
11	39-M-16	M	J
12	2-M-17	M	A
13	6-M-17	F	J
14	7-M-17	F	SA
15	8-M-17	F	J
16	9-M-17	F	A
17	10-M-17	F	J
18	11-M-17	M	SA
19	13-M-17	M	J
20	18-M-17	M	A
21	19-M-17	M	J
22	21-M-17	M	SA
23	22-M-17	F	J
24	24-M-17	F	SA
25	25-M-17	F	J
26	26-M-17	M	SA
27	28-M-17	F	J
28	29-M-17	M	J
29	30-M-17	M	J
30	40-M-16	F	A
31	42-M-16	F	A
32	43-M-16	F	SA

Northern elephant seals

N	ID	Sex	Age class
1	E-1	N/A	J
2	E-2	N/A	J
3	E-3	N/A	J
4	E-4	N/A	J
5	E-5	N/A	J
6	E-6	N/A	J
7	E-7	N/A	J
8	E-8	N/A	J
9	E-9	N/A	J
10	E-10	N/A	J
11	E-11	N/A	J
12	E-12	N/A	J
13	E-13	N/A	J
14	E-14	N/A	J
15	E-15	N/A	J
16	E-16	N/A	J
17	E-17	N/A	J
18	NE-4	F	A
19	NE-5	M	A
20	NE-8	M	A
21	NE-12	M	A
22	NE-15	F	A
23	NE-18	F	A
24	NE-1	M	A
25	NE-3	M	A
26	NE-6	M	A
27	NE-7	F	A
28	NE-9	M	A
29	NE-11	M	A
30	NE-13	M	A
31	NE-14	F	A
32	NE-16	M	A
33	NE-17	F	A
34	NE-19	F	A
35	NE-21	F	A

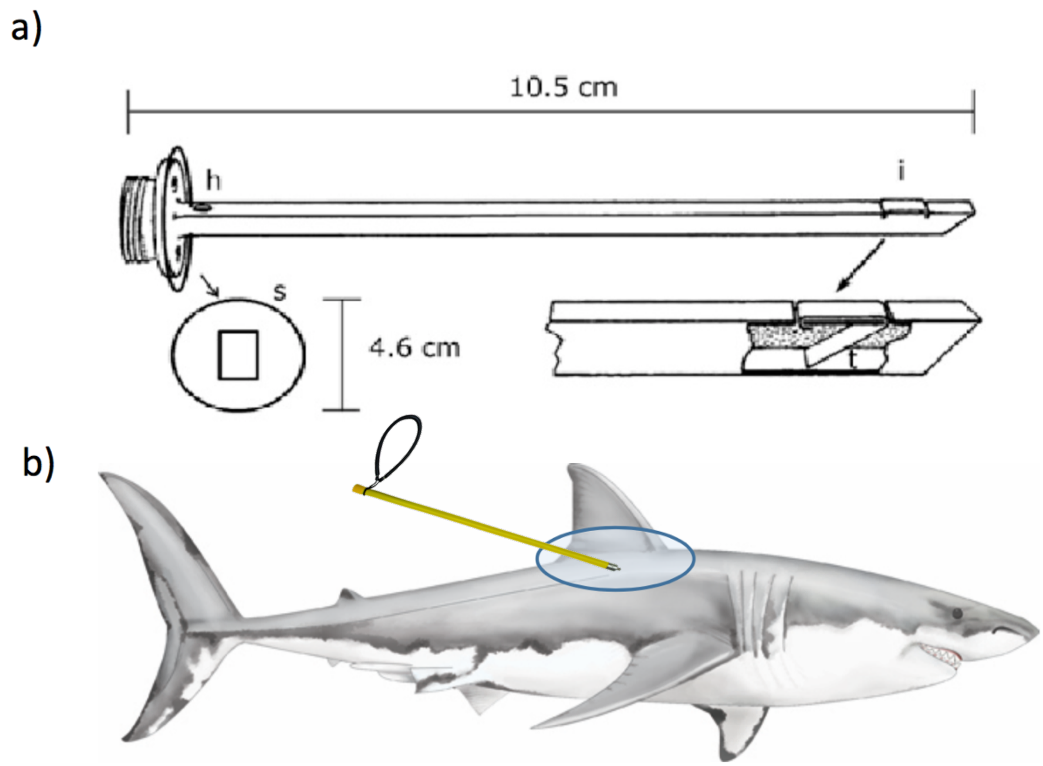

Figure 2. (a) Diagram of the biopsy dart used for WS sampling, (b) Sampling area

Northern elephant seals

A total of 35 individuals of NES individuals were sampled. Biopsy samples were taken on land, using a Hawaiian slang with type B darts (**Fig. 3**) adapted to be able to penetrate the skin of the seals and extract samples of blubber, which were taken from lower back of the individual (**Fig. 4b**), avoiding any contact with delicate organs or tissues. After each shot, the tip was sterilized with fire and the samples were stored in aluminum foil inside a glass container, and were stored in a freezer. Sampled individuals were classified in two age groups: Juveniles (sexually immatures) and adults (sexually matures). Due to the difficulty to observe the ventral part of the individuals in the colonies, sex could only be identified in adults, based on the presence or absence of proboscis (Laws, 1993; Le Boeuf and Laws, 1994).

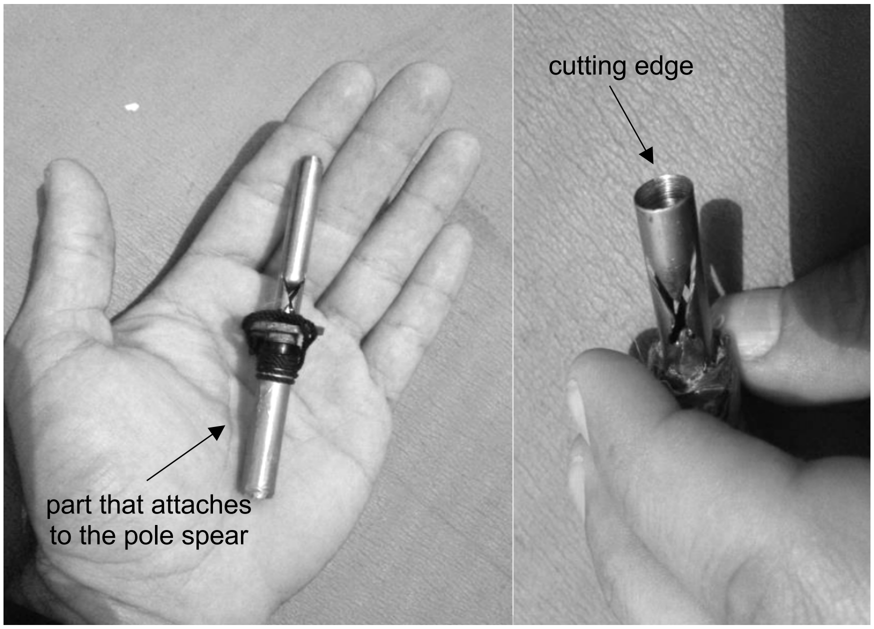

Figure 3. Stainless steel biopsy darts used for NES sampling

Figure 4. Biopsy sampling method used for (a) WS (b) NES.

<u>Statistical analysis</u>

Data of OC concentrations were processed with Shapiro-Wilks test to evaluate the distribution using R software. The Shapiro–Wilk test utilizes the null hypothesis principle: the null-hypothesis is that the population is normally distributed ($p>0.05$). All the investigated groups analyzed with Shapiro-Wilks test were non–normal distributed. Therefore, Mann-Whitney and Kruskall-Wallis test were used to study the differences between different sexes and size groups. In case of significant differences, Dunn's post hoc analysis was applied in order to know between which groups these differences were presented.

OCs analysis

All tissue extractions and contaminant quantifications were performed at CSULB's Institute for Integrated Research on Materials, Environment and Society, in Long Beach, California. Samples were taken from Mexico to California using a CITES permit.

Samples were extracted for 14−16 h via a Soxhlet apparatus in 100% methylene chloride (DCM). Prior to extraction, samples were spiked with a known quantity of recovery surrogates (TCMX, PCB 30, 112, and 198) in order to measure efficiency of preparative and analytical procedures (target recovery of 70− 130%). Sodium sulfate was added to shark and seal samples to eliminate any possible water remaining in the tissue. After the lipid extraction, samples were concentrated by rotary evaporator and lipid content was determined gravimetrically from split aliquots. The remaining lipid extracts underwent Alumina-B/Silica Gel chromatography by sequential elution with hexane, 30% DCM in n-hexane, and DCM. This further purified the apolar phase of lipids that could not be saponified, such as steroids like cholesterol. The samples were concentrated again by rotovap transferred to an autosampler vial, and internal standards (4,49-Dibromobiphenyl and 2,29,5,59-Tetrabromobiphenyl) were added prior to chemical analysis.

Extracted samples were injected onto an Agilent gas chromatograph (GC; 6890N series) equipped with a mass selective detector (MSD; Agilent 5973 inert series) using an autosampler (7683B series, Agilent Technologies). The GC column used was a ZB-5 (JandW Scientific; Santa Clara, California) fused silica capillary (0.25 mm ID660 m) with 0.25 mm film thickness. The temperature profile of the GC oven was programmed from 45°C to 125°C at 20°C/min, then to 295°C at 2.5°C/min and held for 10 min. Injector and transfer line temperatures were set at 285uC and 300uC, respectively. The source and quadrupole temperatures were set at 230°C and 150°C, respectively. Helium was used as carrier gas, at a flow velocity of 40 cm/sec. The MSD was used in the Electron Ionization (EI) mode, scanning from 45–500 amu at a rate of 1.66 scans/sec. Data was obtained by the software in the GCMS system.

Each sample lipid extract was analyzed for DDT and its metabolites (n = 6), chlordanes (oxychlordane, gamma-, alpha-, trans-, cis- chlordane), and 54 congeners of PCBs and summed to obtain the values of total DDTs (“tDDTs”), chlordanes (“tCHLs”), and PCBs (“tPCBs”).

Table 2. Recovery percentages of the OCs in the Certified Reference Material

PESTICIDE	Certified value (ng/g)	Mesured Value (ng/g)	% Recovery
Hexaclorobenzene	7.48 ± 0.66	8.88	118.7%
Heptachlor epoxide	13.4 ± 0.8	11.37	86.5%
Oxichlordane	23.6 ± 1.5	24.52	103.9%
2,4’-DDE	3.39 ± 0.28	3.58	105%
Trans-nonachlor	127 ± 6	124.24	97.8%
4,4’-DDE	720 ± 43	739.49	110.2%
Dieldrin	80.8 ± 3.8	84.55	104.6%
2,4’-DDD	3.31 ± 0.16	3.54	106.9%
4,4’-DDD	45.9 ± 3.6	45.46	99%
2,4'-DDT	15.7 ± 0.89	17.76	113.1%
Cis-nonchlor	54.1 ± 7.3	54.06	99.9%
4’4-DDT	59.5 ± 6.7	56.59	95.1%
Mirex	5.09 ± 0.73	5.06	99.4%

Certified reference material (CRM Lake Michigan 1947) with known pesticide concentrations was used to calculate the percentage of recovery and validate the method (**Table 2**).

RESULTS

OCs analysis

The chromatograms indicated the presence of 5 different compounds OCPs and 10 PCB congeners in sharks, and 7 OCPs and 22 PCB congener in NES. **Table 3** summarizes OCs concentrations detected in WS muscle biopsies and NES blubber biopsies, respectively, expressed in ng/g dry weight (d.w.) basis.

Table 3. Average, median, range for OC contaminants in WS muscle and NES blubber biopsies. Values expressed in ng/g d.w. basis.

	WS (n=32)			NES (n=35)		
	Average	**Median**	**Range**	**Average**	**Median**	**Range**
tDDTs	97.71	83.35	13.39-290.83	3827.35	2304.1	428.95-28744.44
tCHLs	3.09	0	0-16.52	183.61	149.74	0-703.3
tPCBs	31.07	0	0-219.72	587.40	331.38	0-4487.56

The average abundance pattern for the target contaminants in shark muscle and NES blubber was *DDT> PCBs > CHLs*, and *DDT > PCBs > CHLs >HCB*, respectively **(Fig. 5).** The DDT:PCB ratio calculated (mean ± SD) was 2.3 ± 1.6 for WS males, and 5.2 ± 2.1 for WS females, and 6.6 ± 3.1 for NES males and 8.5 ± 3.2 for NES females.

Individuals with zero values for the totality of the analyzed compounds, or below the detection limit, where excluded from the statistical analysis since it was not possible to distinguish between an absence of contamination or a detection problem due to insufficient tissue. Although the lipid content was determined in all samples and the lipid weight (ng/g lipid) was calculated to equal the units between the two species and to compare values with other studies, the dry weight basis was used for most calculations, since it was considered the safest value considering the low weight of the samples.

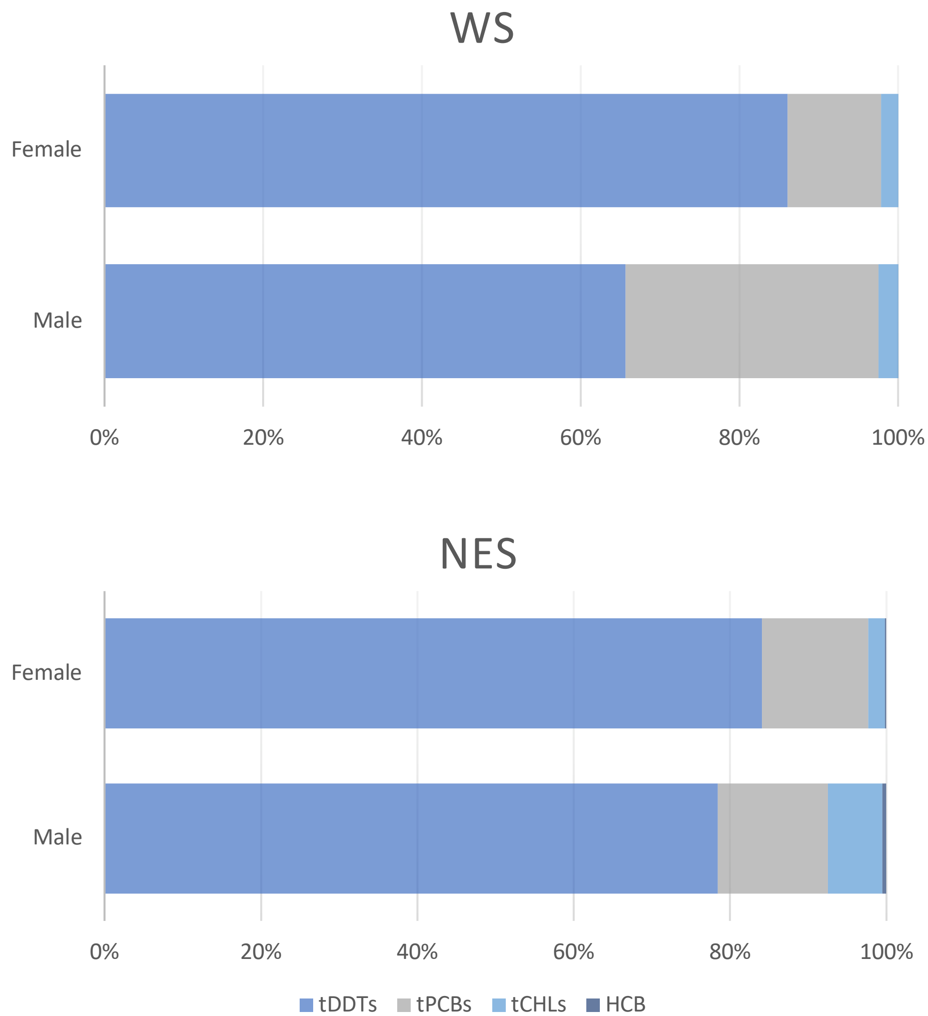

Figure 5. Percentage comparison of levels of tDDTs and tCHLs, tPCBs and HCB on total analyzed contaminants in (a) WS and (b) NES biopsy samples.

<u>Organochlorinated Pesticides (OCPs)</u>

Northern Elephant Seals

The average abundance pattern for the target OCPs in NES blubber was 4,4-DDE>Trans-Nonachlor > Cis-Nonachlor > Hexachlorobencene> Chlordane-alpha > Oxychlordane > 2,4'-DDT.

All the analyzed blubber biopsies from NES contained 4,4'-DDE. Only one sample presented 2,4'-DDT. tDDTs and tCHLs concentrations were significantly higher in adult individuals than in juveniles (U=76, n_1=19, n_2=18, p=0.004; U=80, n_1=18, n_2=19, p=0.005, respectively) (**Fig. 6**). Seventeen out of nineteen adult NES had presence of HCB (19.9 ng/g dw $\pm$ 21.5; mean$\pm$ sd). No HCB was found in any of the sampled juvenile individuals.

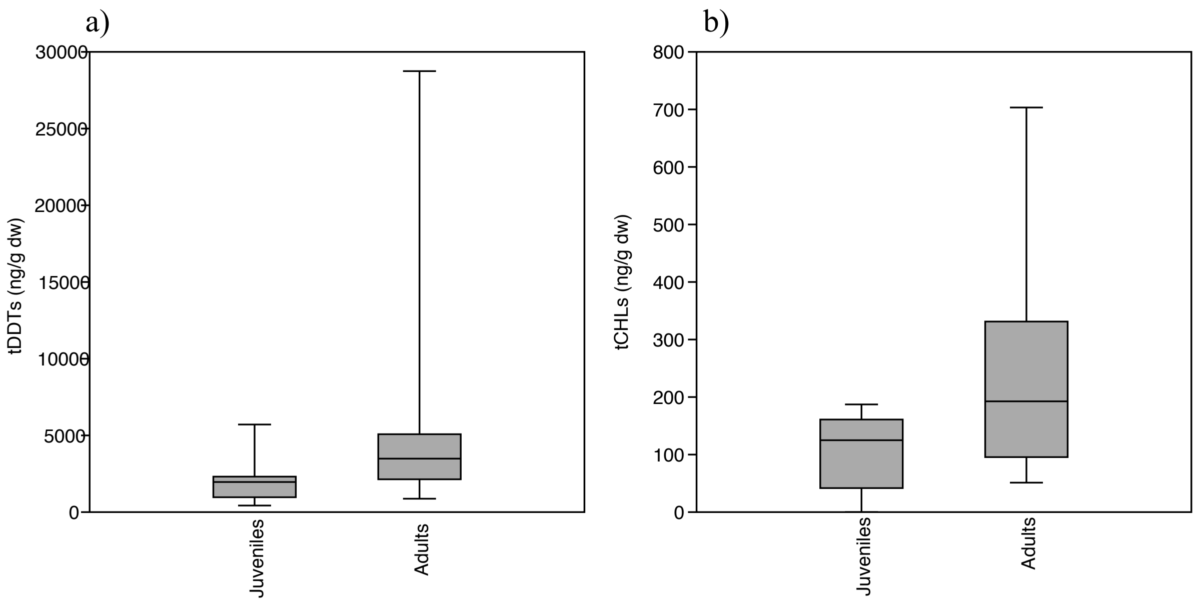

Figure 6. Mean and standard error for (a) tDDT and (b) tCHL concentrations (ng/g, dry weight) in juvenile and adult NES.

No significant differences in tDDT concentrations were found between male and female NES (Mann-Whitney U test, U=28, N_1=8, N_2=11, p=0.2) (**Fig. 7a**). However, a noticeable difference can be observed when calculating the coefficient of variation (CV_{Males}= 0.4 ; $CV_{Females}$=1.1), showing more variability in females than in males. There were no significant differences in tCHL concentrations between male and female NES (Mann-Whitney U test, U=38, N_1=8, N_2=11, p=0.6) (**Fig. 7b**).

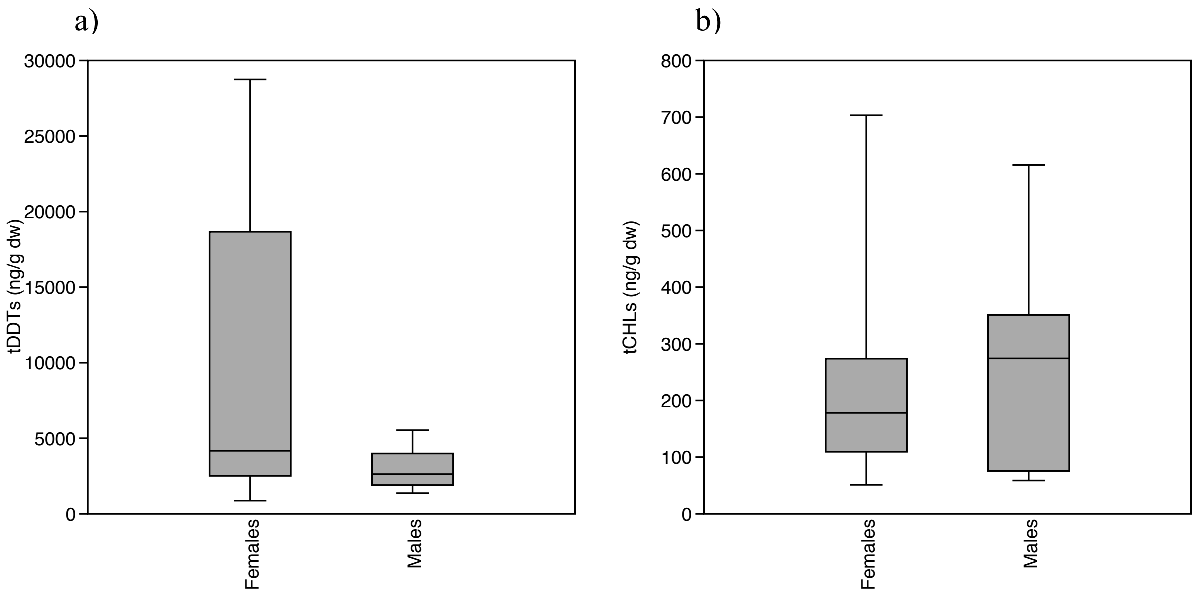

Figure 7. Mean and standard error for (a) tDDT and (b) tCHL concentrations (ng/g, dry weight) in female and male NES.

White Sharks

The average abundance pattern for the target OCPs in WS muscle was 4,4-DDE>Trans-Nonachlor > Cis-Nonachlor > Chlordane-alpha > Chlordane-gamma. The tDDTs concentration was significantly different between WS age groups (Kruskal-Wallis $X2$= 6.099, $p =0.047$) (**Fig. 8a**). Dunn's post hoc test confirmed that the difference occurred between juvenile and adult individuals. From the 32 WS containing DDTs, only 15 presented CHLs. Despite a visible tendency of increasing concentration with age can be observed, there were no significant differences in the concentration of tCHLs among the different age groups (Kruskal-Wallis $X2$= 2.765, $p =0.197$) (**Fig. 8b**) No significant differences in tDDT (Mann-Whitney U test, U=105, N_1=17, N_2=15, p=0.400) and tCHLs (Mann-Whitney U test, U=110, N_1=17, N_2=15, p=0.4) concentrations were found between male and female WS (**Fig. 9**).

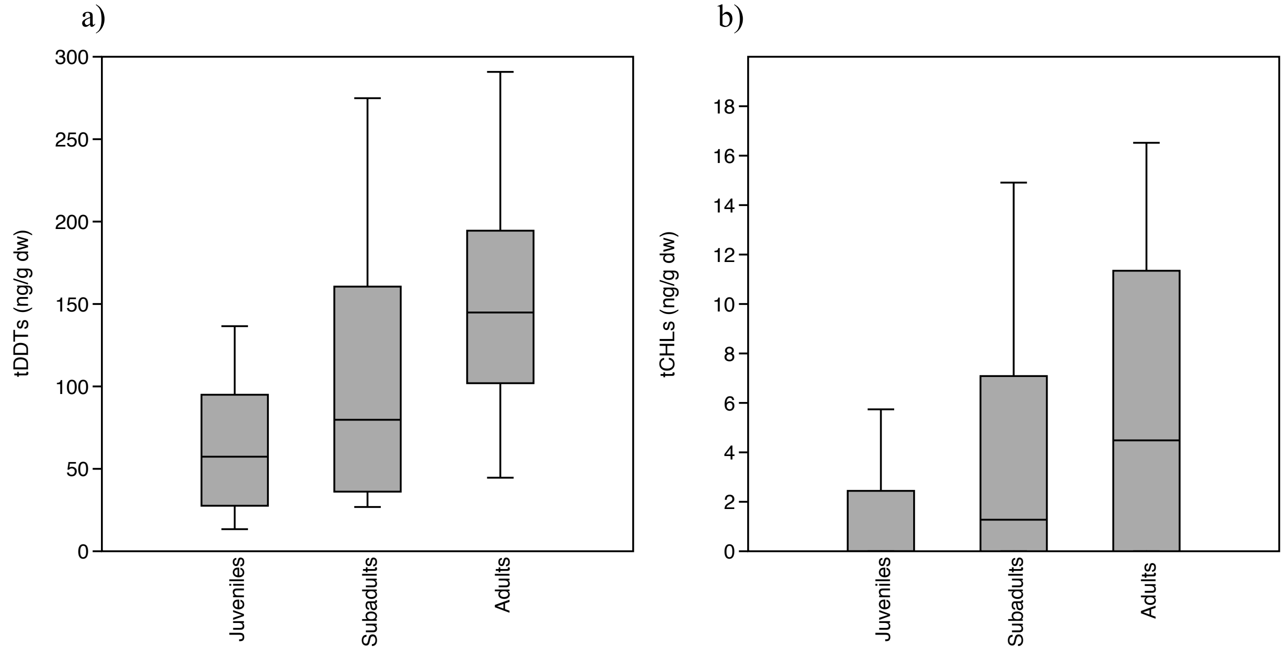

Figure 8. Mean and standard error for (a) tDDT and (b) tCHL concentrations (ng/g, dry weight) in juvenile, subadult and adult WS.

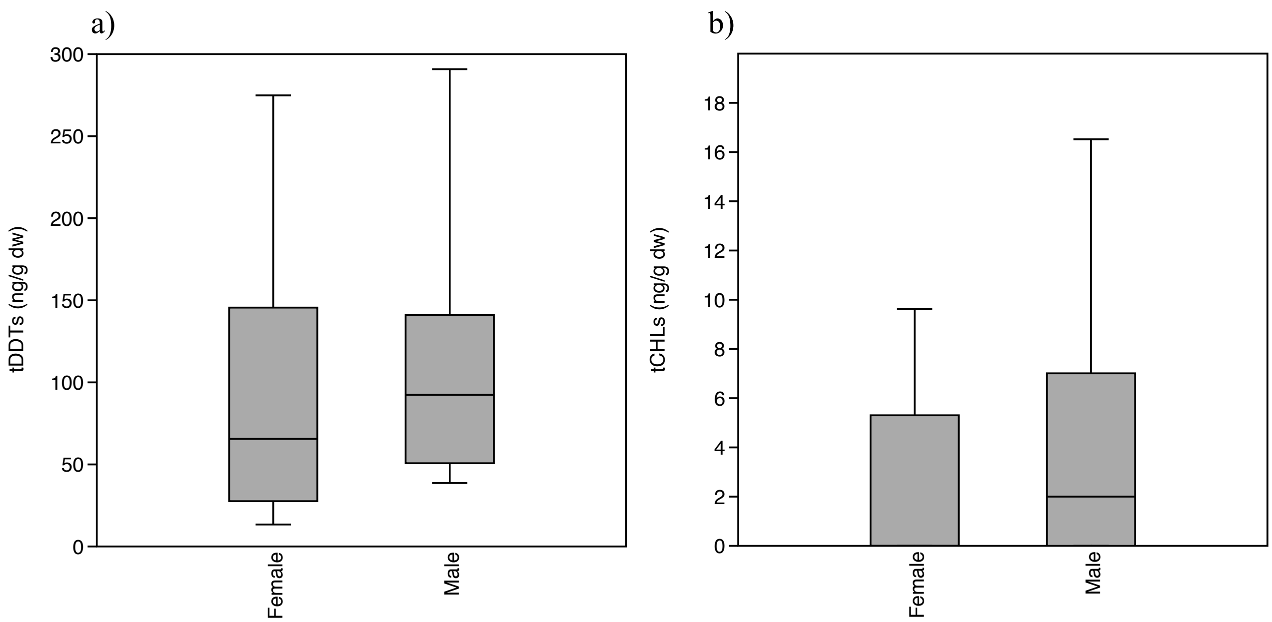

Figure 9. Mean and standard error for (a) tDDT and (b) tCHL concentrations (ng/g, dry weight) in male and female WS.

Polychlorinated biphenyls (PCBs)

Northern elephant seals

A total of 23 PCB congeners were detected in blubber samples of NES. However, there were differences in the variety of congeners found among the different age classes and sexes. All the 23 congeners were detected in adult individuals, while juveniles presented only 5 of the 23 congeners. Also, females presented all the 23 total congeners, while males had only 12 congeners. Therefore, the variety of PCBs was bigger in adult and female individuals of NES (**Fig. 10**).

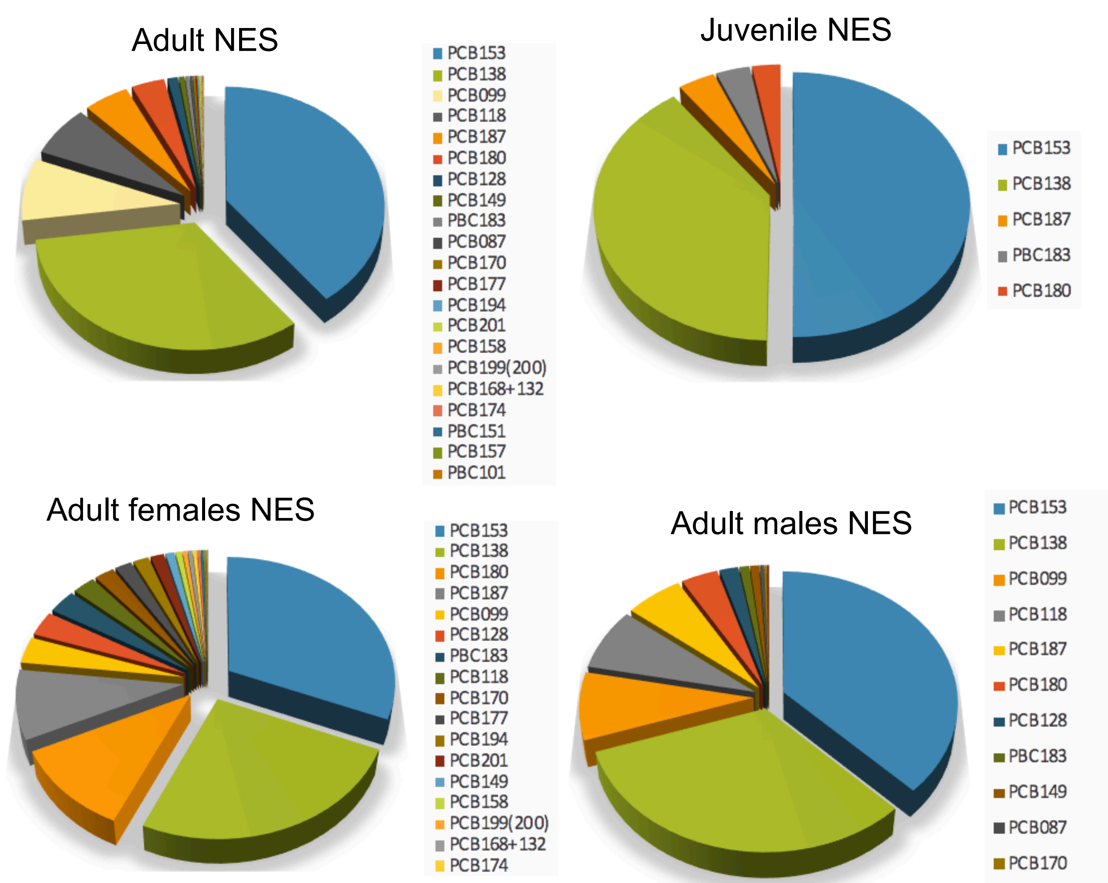

Figure 10. Variety of PCB congeners in (a) adult and (b) juvenile individuals of NES

The tPCBs concentrations were significantly higher in adult individuals than in juveniles of NES (Mann-Whitney U test, U=74, N_1=18, N_2=9, p=0.003) (**Fig. 11a**). No significant differences in tPCBs concentrations were found between males and females (Mann-Whitney U test, U=42, N_1=8, N_2=11, p=0.904) (**Fig 11b**). However, a noticeable difference can be observed in the calculated coefficient of variation (CV_{Males}= 0,6 ; $CV_{Females}$=1,2), indicating more variability in the tPCBs concentrations in female NES than in males.

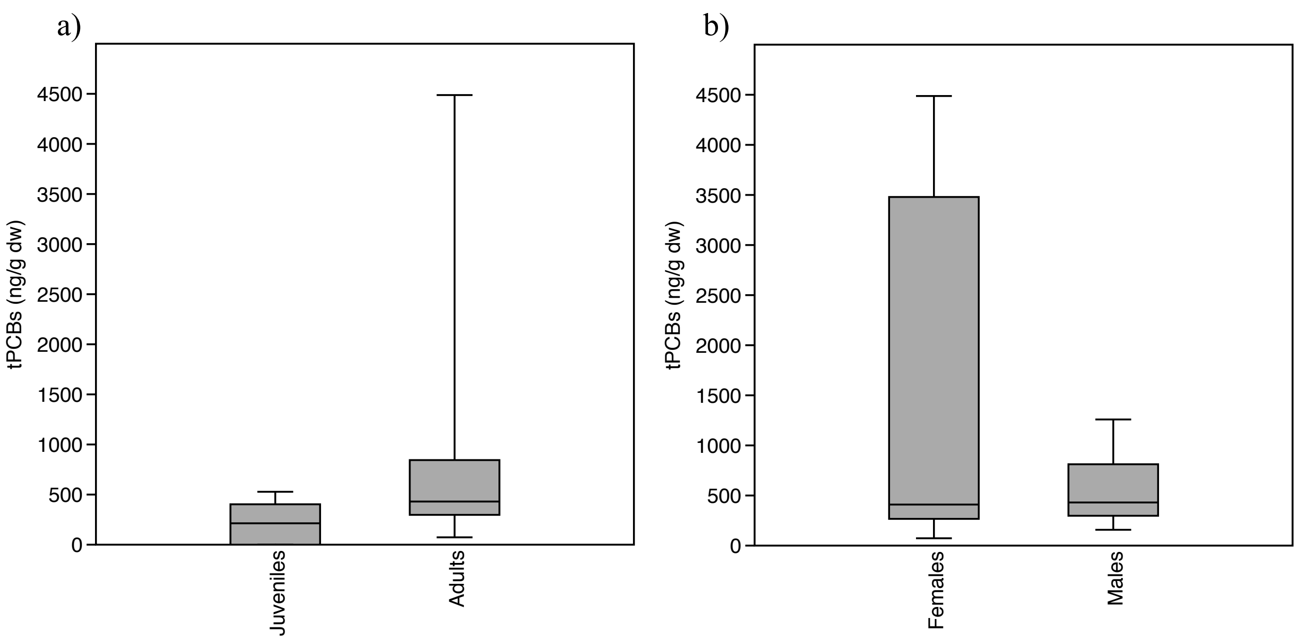

Figure 11. Mean and standard error tPCB concentrations (ng/g, dry weight) in (a)juveniles and adults and (b)females and males of NES.

White sharks

A total of 10 PCB congeners were found in the WS muscle samples. From the 32 individuals sampled containing OCPs, only 15 individuals (6 females and 9 males) had any measurable level of PCB congeners. The average abundance pattern of the PCB congeners in sharks was PCB153 > PCB138 > PCB180 > PCB187 > PCB149 > PCB 58 > PCB183 > PCB151 > PCB174 > PCB177. No significant differences were found among the different age classes (Kruskal-Wallis

$X2$= 3.921, p =0.103) and between sexes *(Mann-Whitney U test, U=77, N_1=16, N_2=15, p=0.069)* of sampled WS (**Fig. 12**)

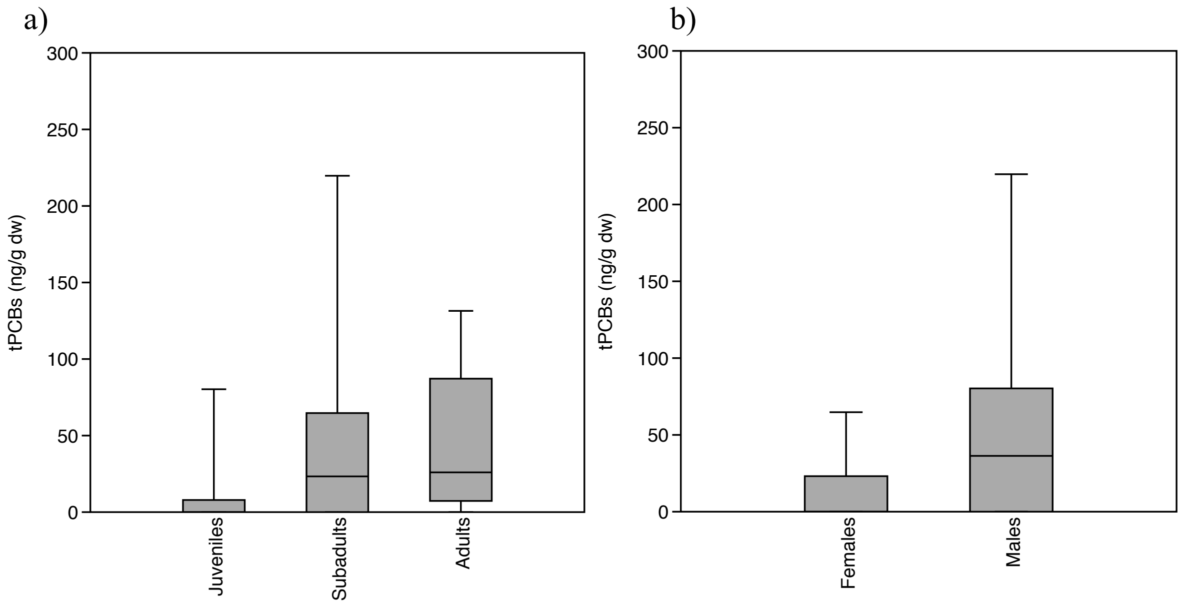

Figure 12. Mean and standard error tPCB concentrations (ng/g, dry weight) in (a) juveniles, subadults and adults and (b) females and males of WS.

PCB congener composition

In terms of congener composition, the PCBs found in the muscle of WS and the blubber of NES specimens were reported in **Figure 13**. In NES, Hexa-CBs (33.6%) were the most abundant, followed by hepta-CBs (26.9%), penta-CBs (21.2%), and octa-CBs (18.3%). In WS, Hexa-CBs (77.4%) were also the most abundant, followed by hepta-CBs (22.6%), Penta-CBs and octa-CBs were not detected in WS.

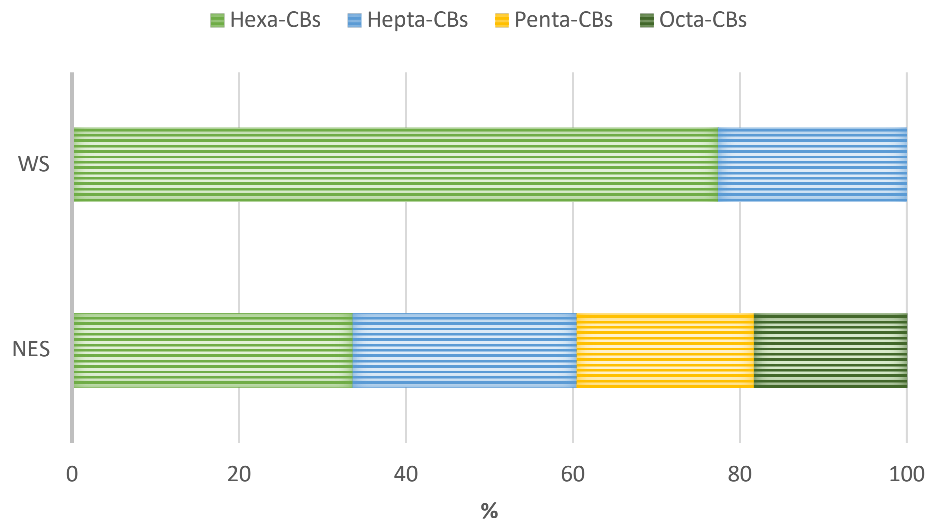

Figure 13. Percentage composition of PCBs divided by chlorine content (penta-CBs, hexa-CBs, hepta-CBs, octa-CBs,) on tPCBs, analyzed in all WS and NES.

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DISCUSSION

The higher OC concentrations found in this study for both WS and NES correspond to the compound 4,4-DDE, which is the major and persistent DDT metabolite, known to be an endocrine disrupter (ED) that has the ability to block the androgen activity (Kelce et al., 1995). 4,4-DDE has been shown to affect some metabolism pathways in fish (Thibaut et al., 2004) and marine mammals (De Swart et al., 1996), followed by PCBs, CHLs.

Size class bioaccumulation in WS

The tDDT concentrations found in WS, which were significantly higher in adults than in juveniles, suggesting the occurrence of bioaccumulation between age classes, whereby the concentration of contaminants increases as individuals grow, since they accumulate more, and contaminant loads in its tissues with time. Also, it is well known that there is a change in diet between juvenile and adult WS, related to an ontogenetic change in the dentition (Tricas and McCosker, 1984; Hubbell, 1996; Hoyos-Padilla et al, 2016). The differences in the diet of adult and juvenile sharks could partially explain the differences found between both age classes. While adults feed mainly on marine mammals (Tricas and McCosker, 1984, Klimley, 1985, Casey and Pratt, 1985), juveniles feed on invertebrates, demersal teleost fishes, sharks, squid and epipelagic fish. Additionally, WS tagged at Isla Guadalupe are known to move offshore to the Shark Offshore Feeding Area (SOFA), near Hawaii (Weng et al., 2007; Domeier el al., 2008) to the pacific coast of California and Baja California, and to some areas in Gulf of California (Domeier, 2012; Hoyos et al., 2016). Therefore, the feeding in these different locations could lead to differences in the OC levels too. Moreover, sharks may also acquire organic contaminants prior to birth through a maternal offloading, whereby females transfer a portion of their accumulated lipophilic contaminants to their offspring during reproduction (Lyons and Lowe 2013). Maternal offloading has been documented in several elasmobranch species for organic contaminants (Butler et al. 1979; Mull et al. 2013, Lyons and Lowe 2013, Lyons and Lowe 2015).

Jaime-Rivera et al (2013) did not find significant isotopic differences between males and females in their study about WS feeding habits in Guadalupe Island, which is consistent with the results found in the present study, which showed no significant differences in the levels of OCPs and PCBs between WS males and females. However, further studies with a bigger sample size would give more accurate information.

<u>Size class bioaccumulation in NES</u>

The existence of significantly higher levels of tDDTs and tCHLs in adult than in juvenile NES indicates a clear pattern of bioaccumulation. The reduced diving capacity of juveniles relative to adults (Le Boeuf et al. 1996), as well as the differences in foraging strategies (Le Boeuf et al. 1993), have been observed during the first 2 years of life of NES (Le Boeuf et al. 1996). This leads to differential use of resources between juveniles of different ages and sexes (Riofrío-Lazo et al., 2012). Despite no significant differences in the mean concentrations of OCs were found between males and females, partially due to the small sample size, there is a remarkable difference in the coefficients of variation calculated for both sexes, showing a greater variability in the concentrations of tDDTs and tPCBs in females than in males. Additionally, the variety of PCB congeners is bigger in females than in males. This could be explained by the fact that NES make two foraging migrations per year, during which adult males and females go to different regions of the Northeastern Pacific Ocean with minimal overlap in range (Le Boeuf et al. 2000). Particularly, males usually feed primarily on benthic prey close to the shore, while females feed on patchily distributed, vertically migrating, epipelagic and mesopelagic prey distributed in mid latitude waters off the coast (Riofrío-Lazo et al., 2012). Velázquez-Castillo and Elorriaga-Verplancken (2017) confirmed this dietary variation via stable isotopes, in terms of more variable (especially offshore) foraging grounds by females relative to males. This higher spatial and resource variability by adult females, which is also related to higher number of foraging strategies (Simmons et al., 2007) should lead to a higher exposure to a greater diversity of OCs in a more variable range of concentrations.

Although adult NES spend long periods in Guadalupe Island every year, they only use the area for reproduction, molt and resting, while the foraging occurs in the North Pacific, in the Gulf of Alaska, and near the Aleutian Islands during their feeding migrations (Stewart et al., 1995; Robinson et al., 2012). Therefore, adult NES could be potentially acquiring the OC loads in this areas.

Also, the milk of most marine mammals contains more fat and less water than that of terrestrial mammals. Female elephant seals produce a highly concentrated milk with a high lipid content, in order to promote rapid weight gain in pups (Le Boeuf et al., 1977). Therefore, the juvenile NES born in Guadalupe Island acquire the contaminant loads through the ingest of milk from the mother.

Prey-predator relationship

Movements of WS around Guadalupe Island are potentially associated with foraging and the seasonal cycles of pinnipeds (Domeier et al., 2012), specifically the NES (Hoyos-Padilla et al., 2016) which has a dietary contribution of 60-70% to WS tissues (Jaime-Rivera et al., 2013). All the target OCs we detected in WS were also detected in NES. In addition, the mean abundance patterns of tDDTs, tCHLs and PCBs were very similar for both prey and predator species (**Fig. 5**). Because of their high lipid content, NES represent a very important source of energy for white sharks. According to Klimley et al. (2001), a juvenile elephant seal contains more energy than required for short-term sustenance of a single WS. For example, a 1.4-year-old, 1.6-m-long elephant seal would have 62.2 kg of fatty tissue, which would provide twice the energy necessary to sustain a WS for 1.5 months. Therefore, WS could be potentially getting an important part of their OCs load from their prey, the NES.

Exposure to OCs compared to other places worldwide

The OCP values found for WS in the present study were lower than those in WS from South Africa by Marsili et al. (2016), using the same sampling method and the same tissues for analysis as the present study (**Table 4**). This could be explained by the different degrees of urbanization and pollution between both locations. While Guadalupe Island is a Biosphere Reserve in an oceanic (far from shore) location and with no urban pollution, South Africa is a coastal developing country showing many agricultural and industrial activities (Marsili et al., 2016) and the WS population inhabiting there is particularly susceptible to accumulate high levels of toxic compounds coming from these activities (Serrano et al., 1997). The results obtained by Mull et al. (2012) showed the same abundance pattern as the present study (tDDTs> tPCBs > tCHLs). This is consistent considering that WS of the Northeastern Pacific migrate between the coasts of California and Baja California. According to Mull et al. (2012) and Schlenk *et al.* (2005), the concentrations of OCs in WS liver are significantly higher than the concentrations in muscle, as the liver has a higher lipid content than the muscle. Due to the sampling method used in the present study, lipid tissue could not be obtained. However, higher concentrations would be expected in livers of the present sampled WS. The values of OCPs and PCBs found for NES in the present work are diffferent than those found in the literature (**Table 4**). This may take place since some studies have found geographical differences in the feeding areas in the North Pacific between colonies of NES from Mexico and from California, located at different latitudes (for example, Año Nuevo, CA vs. San Benito, Baja, Aurioles et al., 2006). In the coast adjacent to the Southern California Bight, where the NES sampled by Blasius et al. (2008) were found, large amounts

organic contaminants were discharged into the Pacific Ocean off the Palos Verdes Shelf (PVS) from 1949 to 1970s. It was determined that over 11 tons of PCBs and 110 tons of DDT remained in the area by 1993 (Lee et al., 2002) and these high levels of contaminants have been shown to have deleterious effects on local marine life (Anderson et al., 1975; Gilmartin et al., 1976). Blasias et al. (2008) reported higher concentrations of tPCB then tDDT in blubber of NES. The greater concentrations of tDDT than tPCB found in NES from Guadalupe Island suggest that their exposure sources are similar to those in NES from the SCB. In the study of Beckman et al. (1997) in California, the concentrations were lower than those found in the present study, probably because the samples of that study were taken in 1992, and the levels of bioavailable contaminants change
constantly over time.

Species	**Sampling location**	**n**	**Tissue**	**tDDTs**	**tPCBs**	**Unitis**	**References**
C. carcharias	Guadalupe Island	32	muscle	97.71 (13.39 - 290.83)	31.07 (0 - 219.72)	ng/g dw	Present study
C. carcharias	Southafrica	15	muscle	475.73 86.72 – 1416.97	2554.45 (379.76-11284.31)	ng/g dw	Marsili *et al.* (2016)
C. carcharias	Southafrica	3	liver	1499.9	-	ng/g dw	Schlenk *et al.* (2005)
M. angustirostris	Guadalupe Island	35	blubber	33994 (5874-193763)	3207 (0-12749)	ng/g lipid	Present study
M. angustirostris	California	24	blubber	5400 (1900-11800)	2000 (720-4300)	ng/g lipid	Beckman *et al.* (1997)
M. angustirostris	California	4	blubber	35580 (8300-110000)	19230 (6100-58000)	ng/g lipid	Kajiwara *et al.* (2001)
M. angustirostris	California	42	blubber	46820 (0-226300)	14020 (60-76000)	ng/g lipid	Blasius and Goodmanlowe (2008)

Table 4. Comparison of mean and ranges of tDDT and tPCB concentrations in WS muscle and NES blubber samples.

Monsanto Corporation was the major producer of PCBs from 1930 to 1977 in the U.S. and marketed certain mixtures of PCBs under the name Aroclor. Aroclors can be identified by a four digit numbering code in which the first two digits indicate the type of mixture and the last two digits indicate the chlorine content by weight percent. In the commercial mixture Aroclor 1260, hexa and hepta-chlorinated congeners are predominant like in the WS and NES samples of the present work. Particularly, the mixture Aroclor 1260 has high concentrations of PCBs 153, 138, which have the highest concentrations for both WS and NES in the present study. These PCBs are more difficult to metabolize and have a higher biomagnification potential (Sawhney, 1986). This could explain why there was a prevalence of these chlorinated congeners in the analyzed samples.

The DDT:PCB ratios found for WS from Guadalupe Island were much higher than the ratios for WS in South Africa (0.16 for males and 0.20 for females) (Marsili et al., 2016), and very similar to the ratios found by Mull et al. (2013) for WS from Southern California (ratios exceeding 4). High DDT:PCB ratios have been associated with juvenile WS feeding near the area of Palos Verdes, CA (Mull et al., 2013). DDT concentrations are proportionally high in biota from the Palos Verdes shelf due to the historic release of large amounts of contaminants in this area (Lyons et al., 2015). The ratios found in the present work, together with the fact that WS from Guadalupe Island have been detected several times in Southern California (Jorgensen et al., 2010, Hoyos et al., 2016) suggest that WS sampled in Guadalupe island could be acquiring part of their contaminant loads while feeding around Southern California.

Despite of the fact that blubber contaminants can not be directly compared to liver contaminants, Kajiwara et al. (2001) reported higher concentrations of tPCB than tDDT in the livers of the harbor seals , while the present work shows higher levels of tDDT than tPCB in the blubber of NES, like in the blubber of three species of pinnipeds sampled in the SCB by Blasius et al. (2008). The greater concentrations of tDDT than tPCB found in NES from Guadalupe Island suggest that the exposure sources could be like those in pinnipeds from the SCB. However, the ratios were higher in NES from Guadalupe Island. Aurioles et al. (2006) found differences in the feeding habits and locations between NES from Mexico and from California. Particularly, they found that female NES from San Benito, Baja California Sur, had a wider longitudinal feeding area that includes inshore and offshore habitats, so its center of distribution would be more coastal than to NES females in Año Nuevo, California. This differences in the location of feeding areas could explain the differences in the DDT:PCB ratios.

As the sampled individuals of the present work were alive and free-ranging, is difficult to know the physiological effects of the contaminants. However, Gelsleichter et al. (2006) found evidence of immunostimulation, via increased proliferation of blood leukocytes in Atlantic stingrays (*Dasyatis sabina*) with high pesticide concentrations. Also, Sawyna et al (2017) provided evidence of OC-correlated immunostimulation, primary driven by PCBs, in multiple elasmobranch tissues.

CONCLUSIONS

The present study evidences for the first time the exposure of WS and NES to OCs in Guadalupe Island, and determines the levels of OCPs and PCBs, establishing a first baseline contaminant levels for both species in the area.

Our results show the bioaccumulation of DDTs and PCBs occurring among the different age classes of both species. The presence of the metabolite 4,4'-DDE as the most abundant compound in all the sampled WS and NES, and the absence of less degraded DDT metabolites, except for one sample, shows no recent use of DDT. Further studies with a bigger sample size need to be done to have more accurate information about patterns between sexes. Due to the use of different tissue for each species, the existence of biomagnification cannot be confirmed. However, there is an obvious predator-prey relationship that confirms the importance of NES as a prey item for WS and establishes it as a potential source of contamination for the WS through its intake. The OCs concentrations found in WS of the present study are lower than the ones previously described in literature. However, higher concentrations of the target OCs would be expected in other tissues with higher lipid content, such as liver. High levels of contaminants have been shown to have deleterious effects on marine life. Therefore, the results of this study stablish the first quantification of OCs in WS and NES from Guadalupe Island, and suggest the need for continuous monitoring of the contaminant levels and the physiological conditions of the contaminated animals as a tool of conservation and management of these species.

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