Chapter 4

Assessing Biomagnification and Trophic Transport of Persistent Organic Pollutants in the Food Chain of the Galapagos Sea Lion (*Zalophus wollebaeki*): Conservation and Management Implications

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Additional information is available at the end of the chapter

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1. Introduction

Bioaccumulation of persistent organic pollutants (POPs) represents a risk to the marine environment and wildlife, including marine mammals and birds [1-4]. Biomagnification is a special case of bioaccumulation and is defined as the process by which concentrations of contaminants or chemical substances (i.e. thermodynamic activities of chemical substances often measured by the lipid normalized concentration) in consumer and higher trophic level organisms exceed those concentrations in the diet or organism's prey [5-7]. This process can occur at each step in a food chain, potentially producing very high and toxic concentrations in upper-trophic-level species [7].

Bioaccumulation and biomagnification are important considerations in the categorization and risk assessment of chemical compounds under the treaty of the Stockholm Convention for POPs and regulatory and management efforts in several nations such as the Canadian Environmental Protection Act Canada (CEPA [8]), the Toxic Substances Control Act (TSCA [9]) in the United States and the Registration, Evaluation, Authorisation and Restriction of Chemicals program (REACH) in the European countries [10]. Due to the long-range atmospheric transport and global fractioning of POPs northward from low or mid latitudes [11, 12], the Arctic and northern hemisphere have remained as active regions of research to study biomagnification of POPs in trophic chains and food webs [2, 13-15]. However, very little is known about the bioaccumulative behaviour and fate of these substances in tropical zones of the planet.

There are several measures that have been used to express the degree of biomagnification. The simplest measure is the Biomagnification Factor (BMF), which is described as the ratio of the chemical concentrations in the organism (C_B) and the diet of the organism (C_D), i.e., BMF = C_B/C_D, where the chemical are usually expressed in units of mass of chemical per kg of the organism (in wet weight or in a lipid basis) and mass chemical per kg of food (in wet weight or in a lipid basis) [6]. Biomagnification of organic contaminants and foraging preferences in aquatic and marine food webs can also be investigated using stable nitrogen isotope as biomarkers of trophic level [15-20]. Stable isotope analysis (SIA) has emerged as a tool in foraging ecology/habitat use, physiology and ecotoxicology, and is applied widely to study marine mammal ecology [21]. Stable nitrogen isotope analysis is a known well established technique for assessing predator–prey interactions and organism trophic levels (TL) in food webs [22-25]. Specifically,$\delta^{15}N$, the concentration ratio of $^{15}N/^{14}N$, expressed relative to a standard (i.e., atmospheric N_2), has been shown to increase with increasing trophic level due to the preferential excretion of the lighter nitrogen isotope [26]. Likewise, carbon isotope signatures ($\delta^{13}C$) provide information on habitat use and general sources of diet of organisms, i.e., marine/freshwater, coastal/oceanic, pelagic/benthic [27].

Studies of the biomagnification and food web transport of POPs in tropical systems such as remote islands around the equatorial Pacific Ocean are lacking. Due to the remoteness and isolation of the Galapagos Islands relative to other better studied geographical areas, the Galapagos Island food web offers a unique opportunity to undertake research related to the transport, bioaccumulative nature and biomagnification of globally distributed contaminants in tropical environments at the ecosystem level. The low population levels and generally good environmental control and management practices on the islands ensures that local pollutant sources are in most cases insignificant compared to global sources. These conditions provide a unique mesocosm to study the behaviour of global pollutants in marine mammalian food-chains.

The Galapagos sea lion(*Zalophus wollebaeki*) is an endemic marine mammal residing year round in the islands and exhibiting a high degree of dietary plasticity, consuming several groups of fish prey (99% of the diet). The Galapagos sea lion diet includes Cupleidae (thread herrings and sardines), Engraulidae (anchovies), Carangidae (bigeye scads), Serranidae (groupers, whitespotted sand bass or camotillo), Myctophidae (lantern fishes), Mugilidae (mullets) and Chlorophtalmidae fishes, and a low proportion of squid, as reported in the existing literature [28-31]. Although the information about diet and trophic level is limited for sea lions at several rookeries in the Galapagos Islands, it is known that the dietary preferences of Galapagos sea lions are also a function of the local variation in prey availability and regional climate-oceanic variability such as the El Niño events, when sea lions can switch their diet composition to more abundant fish items [30, 32, 33]. The Galapagos sea lion has been recognized as a key species for the functioning and health of the marine ecosystem of the islands under the environmental management action plan of the Galapagos Marine Reserve (GMR) [33]. Because of its high trophic position, relative abundance in the islands and non-migratory behaviour, Galapagos sea lions can serve as

local sentinels of food web contamination [33-35]. Concentrations of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) were recently detected in this species, underlying the health risk due to the toxicity and bioaccumulation potential of these contaminants in the Galapagos food web [34, 35]. Thus, equivalent to the role of killer whales as global sentinels of pollution in the Northeastern Pacific [1], the Galapagos sea lion can be used as an eco-marker of environmental pollution and a key indicator of not only the coastal marine health, but the public health in the region.

With the aim to contribute to the understanding of the behaviour and fate of POPs in marine food webs of tropical regions, this chapter provides an advanced primer on biomagnification assessment of POPs in the Galapagos Islands based on the existing literature on baseline levels of DDT detected in Galapagos sea lions [35] and recent unpublished data on organochlorine pesticides (i.e. mirex, dieldrin, chlordanes, β-HCH) and PCBs in Galapagos sea lions and fish preys. To accomplish this work, we made use of concentration data measured in Galapagos sea lions and their fish prey and determination of predator-prey biomagnification factors to assess biomagnification in this tropical system. Insights on the impact of biomagnification and conservation and management implications at the ecosystem level in the Galapagos are discussed.

2. Materials and methods

2.1. Tissue collection from Galapagos sea lion pups

In a recent study [35], blubber biopsy and hair samples of 20 Galapagos sea lion pups of 2–12 months of age were obtained from four rookeries in the Galapagos Archipelago (3°N−4°S, 87°−94°W) between 24-29March 2008. Briefly, pups were sampled at Isabela (Loberia Chica, n = 5), Floreana, (Loberia, n =6) and Santa Cristobal (Puerto Baquerizo, n = 4; Isla Lobos, n = 5) islands. Pups were captured with hoop nets and manually restrained. Age was estimated by visual observation of both the size and weight of the animal. In all circumstances, capture stress and holding time were minimized (< 10-15 min). Hair samples were obtained using a sterile scissor to trim or a scalpel to shave the region to be used prior to the biopsy collection and deposited into labelled zipper bags. Biopsies (100 mg; 6mm−Miltex biopsy punch) were collected from an area 10-20 cm lateral to the spinal column and anterior to the pelvis. The biopsy site was pre-cleaned with alcohol and betadine. Biopsies were wrapped in hexane-rinsed aluminum foil and placed in a cooler with wet ice and transferred into cryovals placed in a cryoship (-20°C) during the field sampling, and, afterwards stored at -80°C in the laboratory until chemical analysis.

Pups were chosen because (a) the animals are readily accessible and relatively easy to capture in most of the rookeries of the Galapagos Islands year round; (b) the animals are of similar age (3-10 months), minimizing the influence of life history parameters on contaminant concentrations; (c) because they are nursed by adult reproductive females they have a high trophic position because they are feeding on mother's milk, ingesting energy and pollutants and analogous to a predator–prey relationship [35]. The rationale of the

study design to justify the use of pups as ecosystem based sentinels of biomagnification is also explained in Figure 1.

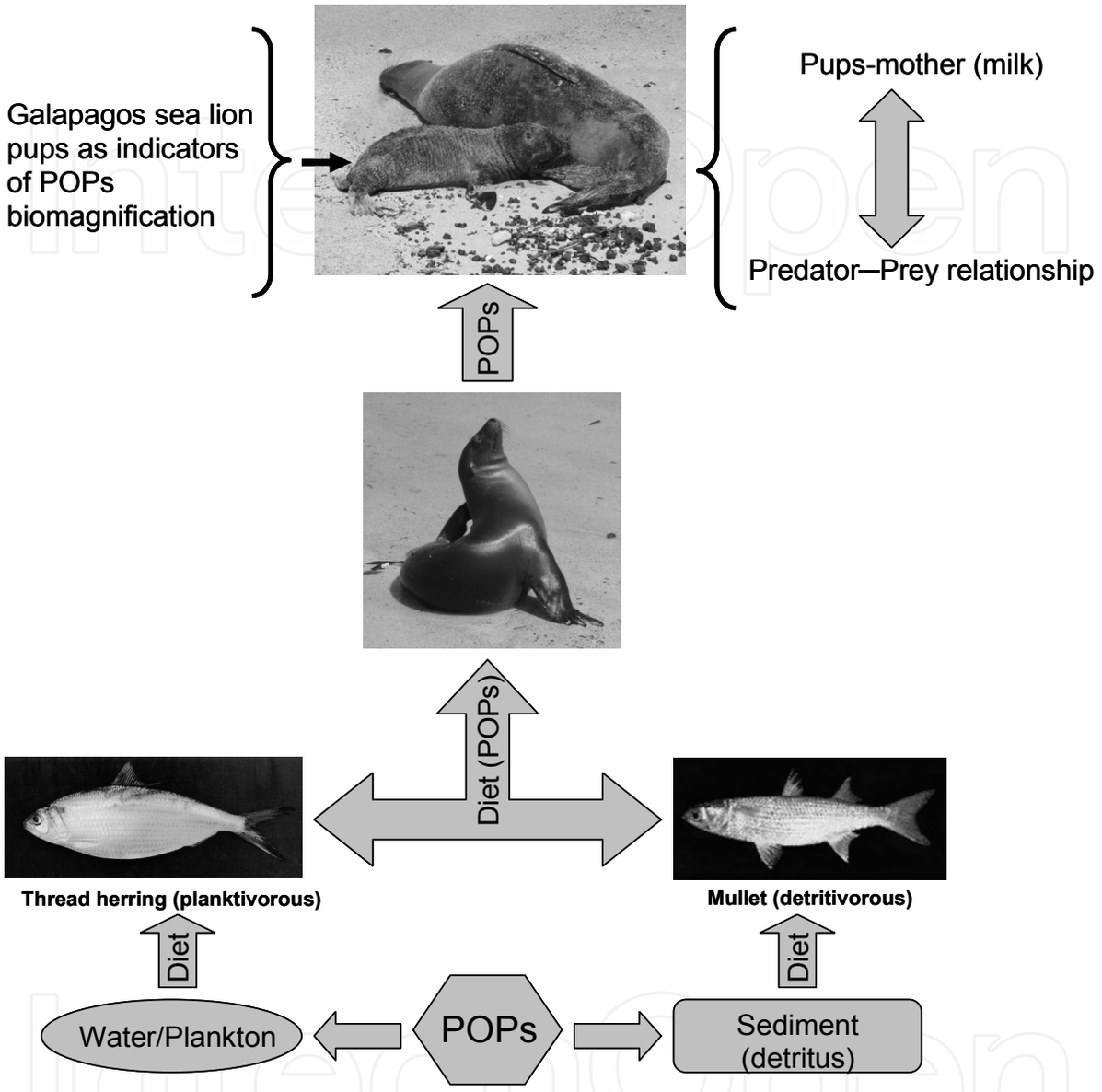

Figure 1. Conceptual model illustrating the bioaccumulation process in a representative, food chain of the Galapagos sea lion. Piscivorous Galapagos sea lions can be exposed to persistent organic pollutants (POPs), mainly through dietary ingestion. Low trophic level prey fish can absorb POPs from water and plankton (planktivorous fish), as well as from sediments (detritivorous fish). Nursing pups can bioaccumulate POPs from adult females by nursing and thus occupy a higher tropic level relative to their mothers because$\delta^{15}N$ isotopic enrichment.

2.2. Fish collection and homogenization

Two species of fish (mullets, *Mugil curema*; n = 11; and, Galapagos thread herrings, *Ophistonema berlangai*; n = 4), which for the purpose of this study were assumed to be major prey items of Galapagos sea lions, were collected from Galapagos waters by fishers during

March-April 2008. Mullets are coastal fish, inhabiting nearshore habitats, and demersal-benthic feeders (detritivorous), grazing on detritus and bottom sediments and digesting the nutritive matter (iliophagous foraging), while Galapagos thread herrings are endemic, pelagic and schooling fishes that filter-feed (planktivorous) mainly on tiny planktonic organisms (e.g., phytoplankton) in open waters [36].

After field collection, fish specimens were frozen until further transportation to the lab, where they were stored at -80ºC. Each fish was measured, weighed and sexed. Muscle biopsies were extracted from the dorsal, lateral muscle of each fish, using a 6mm–biopsy punch (Accuderm, USA), and saved in vials for stable isotope analysis.

Each individual fish was homogenized using a clean, hexane-acetone rinsed meat grinder (Omcam Inc., Italy). The ground fish was then further homogenized in a homogenizer (Omni, USA and/or Polytron, Kinematica, GmbH, Switzerland) at dial position 5-6 for ≈1 min until material was well mixed and homogenous in appearance. Homogenized samples and subsamples were transferred to clean glass jars and stored at -80 ºC until further chemical analysis.

2.3. Sample preparation for Stable Isotopes Analysis (SIA)

Each set of hair samples collected from Galapagos sea lion pups was cleaned for lipid and particle removal by washing the hair three times with a chloroform:methanol 2:1 v/v solution using a clean Pasteur glass pipette. Samples were transferred into labelled scintillation vials and desiccated overnight, and, then, lyophilized using a freeze drier (Free Zone ® Plus 4.5 Liter Cascade; Labconco, Kansas City, MO) for 24 hr (Vacuum pressure set point: 0.01 mBar).

Fish biopsy samples were freeze dried overnight (Vacuum pressure set point: 0.01 mBar). Biopsy samples were weighed and freeze dried again to determine if there were differences in weights after the second freeze drying. Once the sample weight was constant (i.e., no remaining moisture), one set of freeze dried samples was stored in the desiccator until further analysis for $\delta^{15}N$. The set of freeze dried replicates underwent an extraction protocol to remove lipids to be used for $\delta^{13}C$ analysis. First, freeze dried samples were pulverized using a mortar and transferred into a glass tube for lipid extraction by adding 5ml of chloroform:methanol 2:1 v/v; and, then vortex mixed for 30 seconds. Solids were dispersed with sonification in bath sonicator for 10 min. Samples were allowed to settle for 30 min at room temperature, followed by an additional 30 second vortex and sonification. Samples were centrifuged for 5 minutes at 1000 rpm (model GS6R, Beckman, USA) to enhance pellet formation. The solvent was carefully removed with glass Pasteur pipette (pipette was changed for each sample), without transferring any particulate matter, and the solvent was disposed in the waste bottle. A second extraction was repeated. The supernatant was carefully removed with pipette and the residue was left at -20ºC overnight. Samples were dried under Nitrogen and transferred to a clean, amber vial for analysis of stable isotopes of carbon and nitrogen.

2.4. Stable Isotopes Analysis (SIA)

Carbon and nitrogen isotopic analyses on fish biopsies and Galapagos sea lion hair were accomplished by continuous flow, isotopic ratio mass spectrometry (CF-IRMS) using a GV-Instruments® IsoPrime attached to a peripheral, temperature-controlled, EuroVector® elemental analyzer (EA) (University of Winnipeg Isotope Laboratory, UWIL). One-mg samples were loaded into tin capsules and placed in the EA auto-sampler along with internally calibrated carbon/nitrogen standards. Nitrogen and carbon isotope results are expressed using standard delta (δ) notation in units of per mil (‰).The delta values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) represent deviations from a standard. $\delta^{15}N$ isotope ratios (‰) were determined using the following equation [21,26]:

$$\delta^{15}N = [(^{15}N/^{14}N_{SAMPLE}/^{15}N/^{14}N_{STANDARD}) - 1] \times 1000$$

where $^{15}N/^{14}N_{SAMPLE}$ is the isotope ratio of the tissue sample analyzed; and, $^{15}N/^{14}N_{STANDARD}$ represents the ratio of the international standard of atmospheric N_2 (air), IAEA-N-1 (IAEA, Vienna), for $\delta^{15}N$. The equivalent equation for $\delta^{13}C$ isotope ratios (‰) is:

$$\delta^{13}C = [(^{13}C/^{12}C_{SAMPLE}/^{13}C/^{12}C_{STANDARD}) - 1] \times 1000$$

The standard used for carbon isotopic analyses was the Vienna PeeDee Belemnite (VPDB). Analytical precision, determined from the analysis of duplicate samples, was ±0.13‰ for $\delta^{13}C$ and ±0.6‰ for $\delta^{15}N$. The analytical precision based on standards, which are more isotopically homogeneous than samples, was ± 0.19‰ for $\delta^{13}C$ and ±0.24 for $\delta^{15}N$.

2.5. Trophic level estimations

The trophic positions ($TP_{CONSUMER}$) of the prey species (i.e. fish) and the predator (Galapagos sea lion) were determined relative to the baseline $\delta^{15}N$ (assumed to occupy a trophic level 2), using the following algorithm [37, 38]:

$$TP_{CONSUMER} = \frac{\left(\delta^{15}N_{CONSUMER} - \delta^{15}N_{BASELINE}\right)}{3.4} + 2$$

Where $\delta^{15}N_{CONSUMER}$ is the average $\delta^{15}N$ signature value of the predator; $\delta^{15}N_{BASELINE}$ is the $\delta^{15}N$ signature at the base of the food web; and 3.4‰ is the isotopic, trophic level enrichment factor ($\Delta^{15}N$), recommended to be used for constructing food webs when a priori knowledge of $\Delta^{15}N$ is unavailable [39]. The $\delta^{15}N_{BASELINE}$ was established as the $\delta^{15}N$ signature of the particulate organic matter (POM) of bottom sediments in the eastern equatorial Pacific Ocean (250 km south of the islands) with a value of 5.5‰ [31, 40], which is relatively close to the $\delta^{15}N$ value of 7.3‰, reported recently for phytoplankton in the Galapagos [30]. The rationale for using this signature is supported by the fact that the assimilation of nitrogen (i.e., NO_3^-) up taken from near surface marine waters by phytoplankton is reflected by $\delta^{15}N$ values of POM, which is also a major component of the carbon flux and sediments [40].

Although pups instead of adult individual sea lions were sampled in this study, the $\delta^{15}N$ signature in the pup is expected to reflect the isotopic nitrogen signature of the mother, as pups feed only on mothers' tissue (i.e., milk proteins) analogous to a predator-prey relationship, resulting in a $\delta^{15}N$ isototipc enrichment of 2.1‰ and 0.9‰ $\delta^{13}C$ enrichment in relation to adult females [41, 42]. Because of lactation, pups can be at a higher trophic level than their mothers (Figure 1). However, the $\delta^{15}N$ signature in the pups can provide useful information about the foraging habits (i.e., diet) of adult female animals [43].

2.6. Sample preparation for chemical analysis

Contaminant analyses were conducted in the Regional Dioxin Laboratory (RDL) at the Institute of Ocean Sciences (IOS), Fisheries and Ocean Canada (DFO), based on analytical methodologies described elsewhere [44]. In brief, the muscle-blubber biopsy samples of Galapagos sea lion pups (0.053 to 0.212 g wet weight) and subsamples of fish homogenate (9.23 to 10.5 g) were spiked with a mixture of surrogate internal standards which contained all fifteen $^{13}C_{12}$-labeled PCBs, and a mixture of labelled organochlorine pesticides (OCPs): D_3 1,2,4-Trichlorobenzene, $^{13}C_6$ 1,2,3,4 Tetrachlorobenzene, $^{13}C_6$ Hexachlorobenzene, $^{13}C_6$-α-HCH, $^{13}C_6$-γ-HCH, $^{13}C_{10}$ trans Nonachlor, $^{13}C_{12}$ TeCB-47, $^{13}C_{12}$*p,p'*-DDE, $^{13}C_{12}$ Dieldrin, $^{13}C_{12}$*o,p*-DDD, $^{13}C_{12}$*p,p'*-DDD, $^{13}C_{12}$*o,p*-DDT, $^{13}C_{12}$*p,p'*-DDT, $^{13}C_{10}$ Mirex. All surrogate internal standards were purchased from Cambridge Isotope Laboratories (Andover, MA). The spiked samples were homogenized with Na_2SO_4 in a mortar, transferred quantitatively into an extraction column, and extracted with DCM/hexane (1:1 v/v). For some of the samples the extract formed two layers/phases, a waxy-precipitate layer and the solvent layer. The solvent layer was transferred to a clean flask and the waxy precipitate was treated with several aliquots of hexane and DCM. Each of these was transferred to the flask that contained the solvent layer of the extract. Despite the treatment with additional volumes of hexane and DCM, vortexing and pulverization, the waxy precipitate (for sea lions) did not dissolved in the solvents used and as a result it was not included in the corresponding sample extract that was used for lipid and contaminants determinations.The DCM:Hexane sample extracts were evaporated to dryness and the residue was weighted in order to determine the total lipid in the samples. Subsequently the residue was re-suspended in 1:1 DCM/Hexane and divided quantitatively into two aliquots. The larger aliquot (75% of the extract) was subjected to sample-cleanup for PCBs determinations. The remaining (25% of the extract) was used for OCP determinations.

2.7. PCB and OC pesticides analyses

Sample extracts were analyzed for PCB congeners and target OCPs by gas chromatography/high-resolution mass spectrometry (GC/HRMS). The specific methodology and protocols for the quantification and analytical methods to determine PCB congeners and OCPs have previously been reported in prior published papers (34, 35).

2.8. Quality assurance/quality control measures

The mass spectrometry conditions used for all the analyses, the composition of the linearity calibration solutions, the criteria used for congener identification and quantification and the quality assurance – quality control procedures used for the quantification of PCBs and OCPs followed those described in detail elsewhere [34, 35, 44].

2.9. Bioaccumulation parameter

In general, the biomagnification of contaminants is basically quantified as the biomagnification factor in terms of the concentration of a given chemical in the consumer or predator relative to the concentration in the diet or prey (i.e. BMF= C_B/C_D, where C_B is the chemical concentration in the organism and C_D is the chemical concentration of the diet). To quantify biomagnification in the Galapagos sea lions relative to prey items (i.e., thread herring and mullet) and to explore the effect of the magnitude of trophic level differences on the BMF measures, the predator-prey biomagnification factor (BMF $_{TL}$) was used for data interpretation in this study.The criterion applied to indicate the capability of the chemical to biomagnify was a BMF > 1. A BMF statistically greater than 1 indicates that the chemical is a probable bioaccumulative substance [7].

2.9.1. Predator-prey Biomagnification Factor (BMF $_{TL}$)

Following this approach, the mean lipid normalized concentration of each contaminant measured in Galapagos sea lion pups was divided by the mean lipid adjusted concentration in the prey. Then, the biomagnification factor can be adjusted to represent exactly one trophic level in difference using the trophic level estimated from $\delta^{15}N$. Therefore, the field based predator-prey biomagnification factor normalized to trophic position or BMF$_{TROPHIC\ LEVEL}$ (BMF$_{TL}$) is calculated using the following equation [15]:

$$BMF_{TL} = \frac{(C_{PREDATOR}/C_{PREY})}{TL_{PREDATOR} - TL_{PREY}}$$

Where $C_{predator}$ and C_{prey} are chemical concentrations in the predator and prey, expressed in units of mass of chemical (μg) per kg of the predator and mass chemical (μg) per kg of prey in a lipid normalized basis (i.e. BMF$_{LIPID\ WEIGHT}$),and TL $_{predator}$ and TL$_{prey}$ are the trophic levels of the predator and prey. The BMF$_{TL}$ values were used to measure biomagnification in the tropical food chain between two adjacent trophic levels (i.e., the difference in TL between predator and prey is small), assuming steady state in contaminant concentrations between predator and prey. Since BMF$_{TL}$ can be related to the trophic magnification factor (TMF), which describes the increase of contaminants from one trophic level to the other (derived from the slope, b, of the relationship between an organism's log lipid normalized chemical concentration), it can also be expressed as BMF$_{TL}$* as proposed by Conder *et al*. [45]:

$$BMF_{TL}* = 10^{\left\lfloor \frac{\log_{10}([C_{PREDATOR}]/[C_{PREY}])}{TL_{PREDATOR}-TL_{PREY}} \right\rfloor}$$

Where $C_{predator}$ and C_{prey} are appropriately normalized (e.g., lipid normalized) chemical concentrations in the predator and prey, and $TL_{predator}$ and TL_{prey} are the trophic levels of the predator and prey. In essence, the BMF_{TL} is the biomagnification factor normalized to a single trophic level increase in the food-web [45].The use of trophic magnification factors (TMFs) is currently an emerging approach to better assess the biomagnification of POPs in marine food webs [16]. An important number of studies in the northern hemisphere have relied on the use of the TMF for this purpose [15, 16, 18]. Thus, the use of TMF coupled with stable isotope analysis (SIA) to track the amplification and transport of POPs in food webs is a recommended methodology in eco-toxicology to study the biomagnification of POPs. The lack of prey samples and minimal trophic levels required (≥ 3) precluded to undertaking a trophic magnification factor (TMF) assessment in this study.

2.10. Data treatment and supporting statistical analysis

Concentrations of all detected POPs were blank corrected using the method detection limit (MDL), defined as the mean response of the levels measured in three procedural blanks used plus three times the standard deviation (SD) of the blanks ($MDL = Mean_{blanks} + 3*SD_{blanks}$). Following this methodology, the concentration of each PCB congener and OC pesticide was determined based on concentrations above the MDL only. Only PCBs detected in 100% of samples and above the MDL were used for data analysis and calculations of BMFs. Contaminant concentration data were log-transformed to fit the assumption of normality criterion before statistical analysis. ∑PCB concentrations were calculated as the sum of PCB-52, PCB 74, PCB 95, PCB-99, PCB-101, PCB-105, PCB-118, PCB 128, PCB -138/163/164**,** PCB-146, PCB 153, PCB 156, PCB 174, PCB 180, PCB 183, PCB 187, PCB 201 and PCB 202. ∑DDTs were defined as the sum of *o, p'*-DDE, *p, p'*-DDE, *o, p'*-DDD, *p, p'*-DDD, *o, p'*-DDT and *p, p'*-DDT, and ∑chlordanes as the sum of *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor.

To further support the analysis of biomagnification of POPs in the tropical food chain of the Galapagos, statistical comparisons between the concentrations of selected PCBs (e.g., PCBs 153, 180), ∑DDTs, *p,p'*-DDE and other organochlorine pesticides measured in the Galapagos sea lion and those detected in diet items (i.e., mullet and thread herring) were conducted. These comparisons were conducted using analyses of variance (ANOVA) if variances were homoscedastic (i.e., equal variances) or Welch's analyses of variance if variances or standard deviations were heteroscedastic (i.e., unequal variances as tested by Levene's test or Bartlett test, $p< 0.05$), and a Tukey-Kramer honestly significant difference (HSD) test, which is a post-hoc method recommended to test differences between pairs of means among groups that contain unequal sample sizes [46]. Inter-site comparisons among rookeries samples

followed the same statistical methods. Statistical comparison tests were conducted at a level of significance of $p< 0.05$ ($\alpha = 0.05$).

Principal Components Analyses (PCAs) were conducted on the fractions of PCBs and organochlorine pesticides relative to total concentrations by contaminant group (i.e., contaminants expressed as a fraction of total) for each sample to visualize spatial differences in patterns in sea lion pups from different sites within the Galapagos Archipelago and elucidate potential sources (i.e., local versus global-atmospheric). First, samples with undetectable values were replaced by a random number between the lowest and the highest concentration that were detectable (> MDL) to account for uncertainty before PCA (i.e., *trans*-chlordane and PCB 110 showed zero values in blanks in three and two samples out of 20, respectively; therefore; there was not possible to calculate MDLs), or otherwise removed from the PCAs. Secondly, samples were normalized to the concentration total before PCA to remove artefacts related to concentrations differences between samples. Finally, the centered log ratio transformation (division by the geometric mean of the concentration-normalized sample followed by log transformation) was then applied to this compositional data set to produce a data set that was unaffected by negative bias or closure [47]. Regressions, statistical comparisons and PCAs were run using JMP 7.0 (SAS Institute Inc.; Cary, NC, USA).

3. Results and discussion

3.1. Stable isotope profiles and trophic levels

The values of $\delta^{15}N$ and $\delta^{13}C$ (mean ± standard deviation) found here are consistent to those reported in Galapagos sea lion pups (i.e., 13.1‰ ± 0.5‰ for $\delta^{15}N$, and -14.5‰ ± 0.5‰ for $\delta^{13}C$) in a recent study [31].No significant relationship was observed between isotopic values and length of the pups ($\delta^{15}N$: $r = 0.005$, $p = 0.7594$; $\delta^{13}C$:$r = 0.18$, $p = 0.0626$) or weight ($\delta^{15}N$:$r = 0.0001$, $p = 0.9645$; $\delta^{13}C$: $r = 0.18$, $p = 0.0752$). Although female pups appeared to exhibit higher values of $\delta^{15}N$ compared to male pups (t-test = 2.3767, $p = 0.0288$), $\delta^{13}C$ values between males and females were similar (t-test = -0.3326, $p = 0.7433$). In addition, no significant inter-site differences in $\delta^{15}N$ (ANOVA, $p = 0.4235$) and $\delta^{13}C$ (ANOVA, $p = 0.8378$) values were found among rookeries. This indicates that site or foraging location had minimal influence on the isotope ratios. The lack of differences was further minimized by sampling similar ontogenetic stages (i.e., pups of similar age, development and size), and a metabolically inactive tissue (i.e., fur hair), which is corroborated by the fact that hair is an inert tissue containing physiological and dietary information (isotopic signals) [48].

Based on the $\delta^{15}N$values, the trophic level (TL) measured here for the Galapagos sea lion ($\delta^{15}N = 13.0$; TL = 4.2) fall within the range of those recently reported (i.e., $\delta^{15}N$ =12.6−13.4; TL = 4.1−4.4) elsewhere [30, 31, 43]. The $\delta^{15}N$ values for thread herrings and mullets were 9.4‰ ± 1.77‰ (TL = 3.1), and 12.7‰ ± 1.10‰ (TL = 4.1), respectively, while the $\delta^{13}C$ values for thread herrings and mullets were -17.0 ±0.70 and -9.34 ±0.80.

3.2. POP concentrations in animals and inter-site comparisons

3.2.1. Galapagos sea lions

Observed concentrations of selected POPs in Galapagos sea lion and two of its main prey items are summarized in Table 1. Galapagos sea lions represented the largest number of organisms sampled in this study (n = 41) and exhibited the highest concentrations of PCBs and OC pesticides. The multi-comparison post hoc analysis, including sea lions and prey fish, showed that no significant differences in OC pesticides and PCB congener concentrations were observed between male and female pups. Fish prey commonly exhibited significantly lower concentrations than Galapagos sea lion pups (ANOVA and multi-comparisons Tukey-Kramer (HSD) post-hoc test, $p< 0.05$) (Table 1, Figure 2).

Concentrations of ∑DDTs in Galapagos sea lions ranged from 16.0 to 1700 μg/kg lipid and ∑DDTs were the predominant OC pesticide in Galapagos sea lion pups, as previously reported [35]. ∑Chlordanes were the second most abundant group of contaminants present. *Trans*-nonachlor represented 68% of ∑chlordanes, followed by *cis*-chlordane, *cis*-nonachlor and *trans*-chlordane (Table 1), a pattern comparable to that reported in pups of southern elephant seals (*Mirounga leonina*) [49] and Weddell seals (*Leptonychotes weddellii*) [50]. This indicates that *trans*-nonachlor is a predominant chlordane compound in pinnipeds.

Within the hexachlorocyclohexanes (HCHs), β-HCH was the only isomer detectable in all pups (>MDL). β-HCH was the dominant HCH isomer in blubber samples of California sea lions (*Zalophus californianus*) from Baja California [51] and in toothed cetaceans from tropical and temperate waters of the Indian and North Pacific oceans [52] due to the greater biomagnification of the most bioaccumulative β-HCH versus γ-HCH [3, 20]. Interestingly, the mean β-HCH concentration in Galapagos sea lions was higher than the mean ∑HCH concentrations measured in spinner dolphins (*Stenella longirostris*) (21.3 μg/kg lipid) captured in a marine area of the Eastern Tropical Pacific [52] in offshore waters north of the Galapagos.

Both dieldrin and mirex were detected in all pups with concentrations ranging from 0.85 to 24 μg/kg lipid for mirex and from 9.00 to 83.0 μg/kg lipid for dieldrin. Concentrations of ∑PCBs (i.e., sum of 20 PCB congeners) ranged between 16.0 and 380 (μg/kg lipid) in pups and from 1.0 to 140 (μg/kg lipid) in fish preys (Table 1).

3.2.2. Fish prey

OC pesticides, including ∑DDTs, chlordanes, β-HCH, dieldrin and mirex, and individual PCB congeners detected in Galapagos sea lion pups were also detected (> MDL) in all sampled thread herring and mullet prey samples. Significantly lower concentrations of OC pesticides and PCBs were found in thread herrings and mullets than in Galapagos sea lion pups (ANOVA and multi-comparisons Tukey-Kramer (HSD) post-hoc test, $p< 0.05$; Table 1). PCB 202 was the only congener exhibiting similar concentrations in sea lions and

fish (ANOVA, $p > 0.05$), suggesting a lack of its bioaccumulation in the food chain. Although thread herring and mullet showed differences in $\delta^{15}N$ values or trophic levels and foraging strategies, concentrations of POPs in these two species were similar (Figure 2) with the exception of mirex and *cis*-nonachlor, which were higher in planktivorous thread herrings than in mullets. Endosulfan sulphate was detected in all mullet samples ranging from 0.07 to 0.22 μg/kg lipid, with an arithmetic mean of 0.16 μg/kg lipid. Only two thread herring samples exhibited detectable concentration of this pesticide (0.002–0.05 μg/kg lipid). Endosulfan sulphate was not detected in any of the biopsy samples of pups.

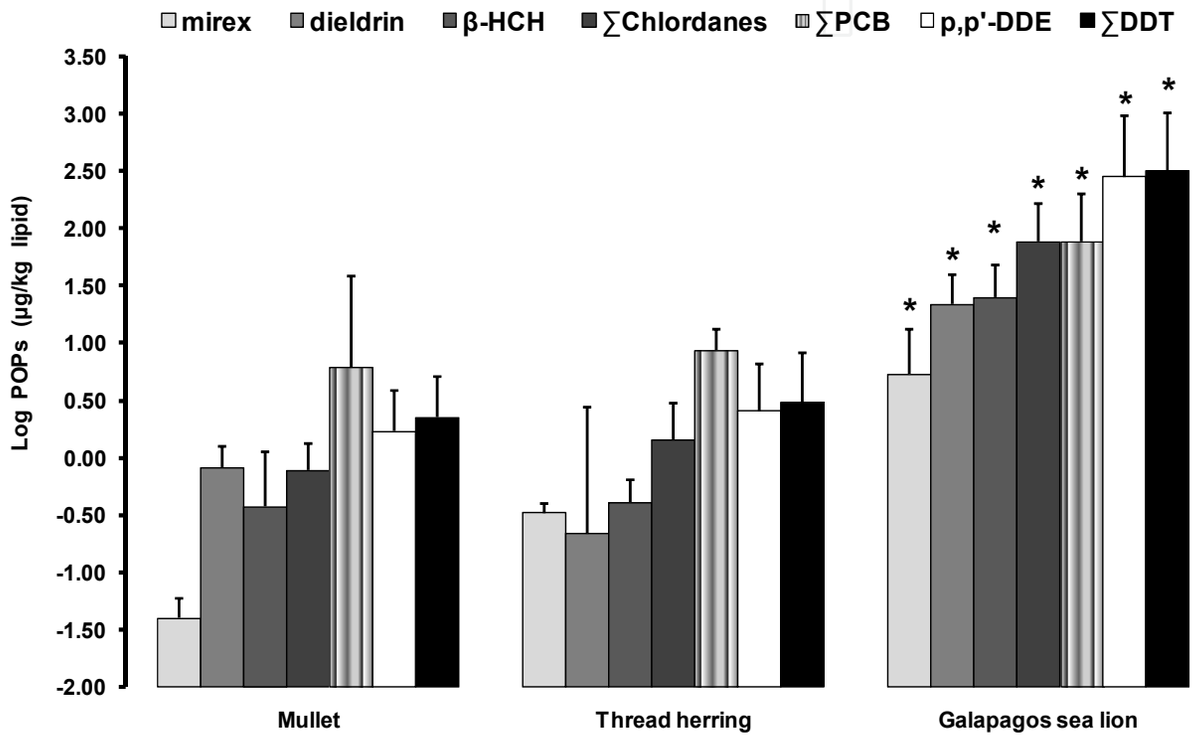

Figure 2. Inter-species comparisons of ∑PCB and organochlorine pesticide (mirex, dieldrin, β-HCH, ∑Chlordanes, *p,p*-DDE, ∑DDT) concentrations. Asterisks indicate that concentration in the Galapagos sea lion were significantly higher ($p < 0.05$) than those found in mullets and thread herrings. Error bars are standard deviations.

The PCB composition in prey showed a different composition of PCB congeners compared to that of sea lions pups (Figure 3). Higher chlorinated PCBs, i.e., Hepta, Octa and Nona-chlorinated biphenyls (PCBs 180–201) were more abundant in thread herrings and mullets than in Galapagos sea lion pups. This indicates the possible role of biotransformation, reduced uptake of PCBs, or a natural placental barrier for heavier PCBs in sea lions. Lower chlorinated PCB congeners, ranging from PCB 43/44 to PCB 118 (Tetra to Penta- chlorinated biphenyls), make up an important contribution (≈ 37% ± 7.25%) to the total PCB concentrations suggesting a lighter PCB signature ("equatorial fingerprint") in the Galapagos sea lion, mullet and thread herring compared to that observed in many arctic biota.

	Galapagos sea lion (predator)		Fish (prey)		*p*-value
	Female pups	Male pups	Thread herring	Mullet	
	(*n* = 10)	(*n* = 10)	(*n* = 4)	(*n* = 6)	
Lipid (%)	75.9 ± 3.50	77.8 ± 2.45	1.22 ± 0.86	2.86 ± 2.00	
***p,p′*-DDE**	480 ± 120 **A**	505 ± 180 **A**	3.30 ± 1.00 **B**	2.22 ± 0.700 **B**	<0.05*
	(65.4–1183)	(13.6–1650)	(0.669–5.00)	(0.620–5.20)	
***p,p'*-DDT**	13.0 ± 2.85 **A**	8.60 ± 1.08 **A**	0.070 ± 0.046 **B**	0.130 ± 0.051 **B**	<0.05*
	(1.70–29.0)	(0.974–12.0)	(ND–0.195)	ND–0.300	
***p,p'*-DDD**	20.0 ± 4.73 **A**	17.0 ± 4.60 **A**	0.440 ± 0.140 **B**	0.550 ± 0.170 **B**	<0.05*
	(1.88–44.0)	(0.965–54.0)	(0.036–0.70)	(0.155–1.30)	
∑DDT	516 ± 125 **A**	533 ± 183 **A**	4.00 ± 1.26 **B**	3.00 ± 0.910 **B**	<0.05*
	(71.2–1230)	(16.3–1666)	(0.705–6.05)	(0.820–6.80)	
Mirex	8.60 ± 1.76 **A**	6.40 ± 2.20 **A**	0.330 ± 0.030 **B**	0.040 ± 0.008 **C**	<0.05**
	(2.50–21.0)	(0.850–24.0)	(0.250–0.400)	(0.028–0.080)	
Dieldrin	31.0 ± 7.26 **A**	22.0 ± 4.80 **A**	0.600 ± 0.204 **B**	0.880 ± 0.128 **B**	<0.05**
	(9.00–83.0)	(9.00–63.0)	(0.005–0.90)	(0.400–1.30)	
***β*-HCH**	34.2 ± 4.00 **A**	26.0 ±7.05 **A**	0.440 ± 0.090 **B**	0.495 ± 0.095 **B**	<0.05**
	(18.3–52.0)	(7.75–78.0)	(0.229–0.620)	(0.041–0.650)	
***trans*-chlordane**	0.410 ± 0.100 **A**	0.65 ± 0.10 **A**	0.070 ± 0.027 **B**	0.040 ± 0.015 **B**	<0.05**
	(ND–0.840)	(0.273–1.03)	(ND–0.130)	(ND–0.110)	
***cis*-chlordane**	17.2 ± 2.67 **A**	15.0 ± 2.75 **A**	0.455 ± 0.140 **B**	0.250 ± 0.053 **B**	<0.05*
	(6.800–34.0)	(3.60–31.0)	(0.049–0.670)	(0.120–0.482)	
***trans*-nonachlor**	73.0 ± 12.0 **A**	65.0 ± 22.0 **A**	0.860 ± 0.191 **B**	0.40 ± 0.072 **B**	<0.05**
	(37.0–146)	(11.0–214)	(0.430–1.30)	(0.160–0.570)	
***cis*-nonachlor**	16.0 ± 3.20 **A**	10.0 ± 2.10 **A**	0.300 ± 0.109 **B**	0.195 ± 0.050 **C**	<0.05*
	(3.7–31.8)	(3.56–25.8)	(ND–0.510)	(0.075–0.380)	
∑Chlordanes	107 ±15.0 **A**	90.5 ± 25.2 **A**	1.70 ± 0.445 **B**	0.870 ± 0.175 **B**	<0.05*
	(48.1–180)	(18.8–255)	(0.481–2.50)	(0.372–1.50)	
PCB 52	3.20 ± 0.530 **A**	2.10 ± 0.610 **A**	0.210 ± 0.030 **B**	2.20 ± 1.85 **B**	<0.05**
	(1.13–5.60)	(0.332–7.05)	(0.136–0.270)	(0.055–11.0)	
PCB 74	2.60 ± 0.410 **A**	2.00 ± 0.510 **A**	0.100 ± 0.009 **B**	0.280 ± 0.220 **B**	<0.05**
	(1.40–5.10)	(0.340–4.40)	(0.050–0.085)	(0.012–1.40)	
PCB-95	2.80 ± 0.303 **A**	2.20 ± 0.320 **A**	0.300 ± 0.090 **B**	2.02 ± 1.70 **B**	0.05**
	(1.63–4.83)	(0.873–3.75)	(0.018–0.413)	(0.026–10.4)	

PCB-99	11.0 ± 2.07 **A** (4.99–27.0)	8.30 ± 2.70 **A** (1.30–23.0)	0.570 ± 0.073 **B** (0.390–0.740)	2.62 ± 2.14 **B** (0.090–13.0)	<0.05**
PCB-101	8.70 ± 1.38 **A** (4.36–18.3)	4.30 ±1.38 **A** (1.79– 16.4)	0.630 ± 0.186 **B** (0.115–0.980)	3.35 ± 2.70 **B** (0.090–17.0)	<0.05**
PCB-105	2.05 ± 0.630 **A** (0.715–7.40)	1.30 ± 0.445 **A** (0.140–4.10)	0.205 ± 0.070 **B** (0.062–0.374)	0.760 ± 0.600 **B** (0.020–3.70)	<0.05**
PCB-118	14.0 ± 3.50 **A** (5.70–43.0)	9.70 ± 3.40 **A** (1.26–32.0)	1.00 ± 0.170 **B** (0.710–1.46)	3.80 ± 3.00 **B** (0.118–19.0)	<0.05**
PCB 128	2.50 ± 0.750 **A** (0.740–8.76)	1.60 ± 0.570 **A** (0.201–5.25)	0.180 ±0.060 **B** (0.071–0.350)	0.560 ± 0.450 **B** (0.015–2.80)	<0.05**
PCB 138/163/164	24.0 ± 6.70 **A** (7.80–80.0)	15.50 ± 5.60 **A** (2.080–50.0)	1.30 ± 0.360 **B** (0.690–2.20)	3.30 ± 2.60 **B** (0.150–16.0)	<0.05*
PCB 146	6.00 ± 1.40 **A** (2.10–16.0)	2.80 ± 1.10 **A,B** (0.620–11.5)	0.40 ± 0.078**B,C** (0.210–0.570)	0.600 ± 0.460 **C** (0.030–3.00)	<0.05**
PCB 153	35.0 ± 8.90 **A** (11.3–99.3)	25.0 ± 9.80 **A** (2.60–95.4)	1.60 ± 0.580 **B** (0.601–3.10)	3.80 ± 3.00 **B** (0.180–19.0)	<0.05*
PCB-156	0.610 ± 0.137 **A** (0.170–1.60)	0.40 ± 0.110 **A** (0.090–1.07)	0.17 ± 0.035**A,B** (0.075–0.240)	0.400 ± 0.320 **B** (0.012–1.96)	<0.05**
PCB-174	0.680 ± 0.110 **A** (0.140–1.30)	0.420 ± 0.096 **A** (0.100–0.860)	0.090 ± 0.050 **B** (0.025–0.230)	0.370 ± 0.300 **B** (0.014–1.80)	<0.05**
PCB 180	16.0 ± 4.24 **A** (3.90–44.0)	12.0 ± 4.40 **A** (1.00–44.0)	1.66 ± 0.420 **B** (0.600–2.60)	1.90 ± 1.50 **B** (0.130–9.10)	<0.05*
PCB-183	2.20 ± 0.669 **A** (0.516–7.45)	1.40 ± 0.536 **A** (0.170–5.26)	0.215 ± 0.072 **B** 0.008–0.330	0.440 ± 0.350 **B** 0.030–2.20	<0.05*
PCB 187	3.40 ± 0.812 **A** (0.965–9.50)	1.45 ± 0.43 **A,B** (0.470–4.55)	0.620 ± 0.130 **B** (0.230–0.840)	0.930 ± 0.680 **B** (0.080–4.32)	<0.05*
PCB 201	1.20 ± 0.515 **A** (0.140–5.60)	0.60 ± 0.20 **A,B** (0.050–2.00)	0.140 ± 0.04**A,B** (0.060–0.240)	0.370 ± 0.280 **B** (0.030–1.80)	<0.05*
PCB 202	0.355 ± 0.180 **A** (0.022–1.90)	0.160 ± 0.050 **A** (0.008–0.470)	0.070 ±0.020 **A** 0.033–0.126	0.120 ± 0.090 **A** 0.010–0.600	>0.05*
∑PCBs	136 ± 32 **A** (50.2–384)	91.0 ± 30.0 **A** (16.0–282)	9.35 ± 1.90 **B** (5.40–14.0)	28.0 ± 22.0 **B** (1.20–138)	<0.05**

*Homocedastic: Welch's analysis of variances not used; **Heteroscedastic: Welch's analysis of variances used; ND = non-detectable concentration

Table 1. POP concentrations (μg/kg lipid) in Galapagos sea lion, thread herring and mullet sampled in 2008. Lipid contents are arithmetic mean ± standard deviations (SD). Concentrations are mean ± standard error (SE), and the range is indicated between brackets. Different letters (i.e. A, B, and C) indicate significant differences among sea lion pups and fish species (ANOVA and multi-comparisons Tukey-Kramer (HSD) post-hoc test)

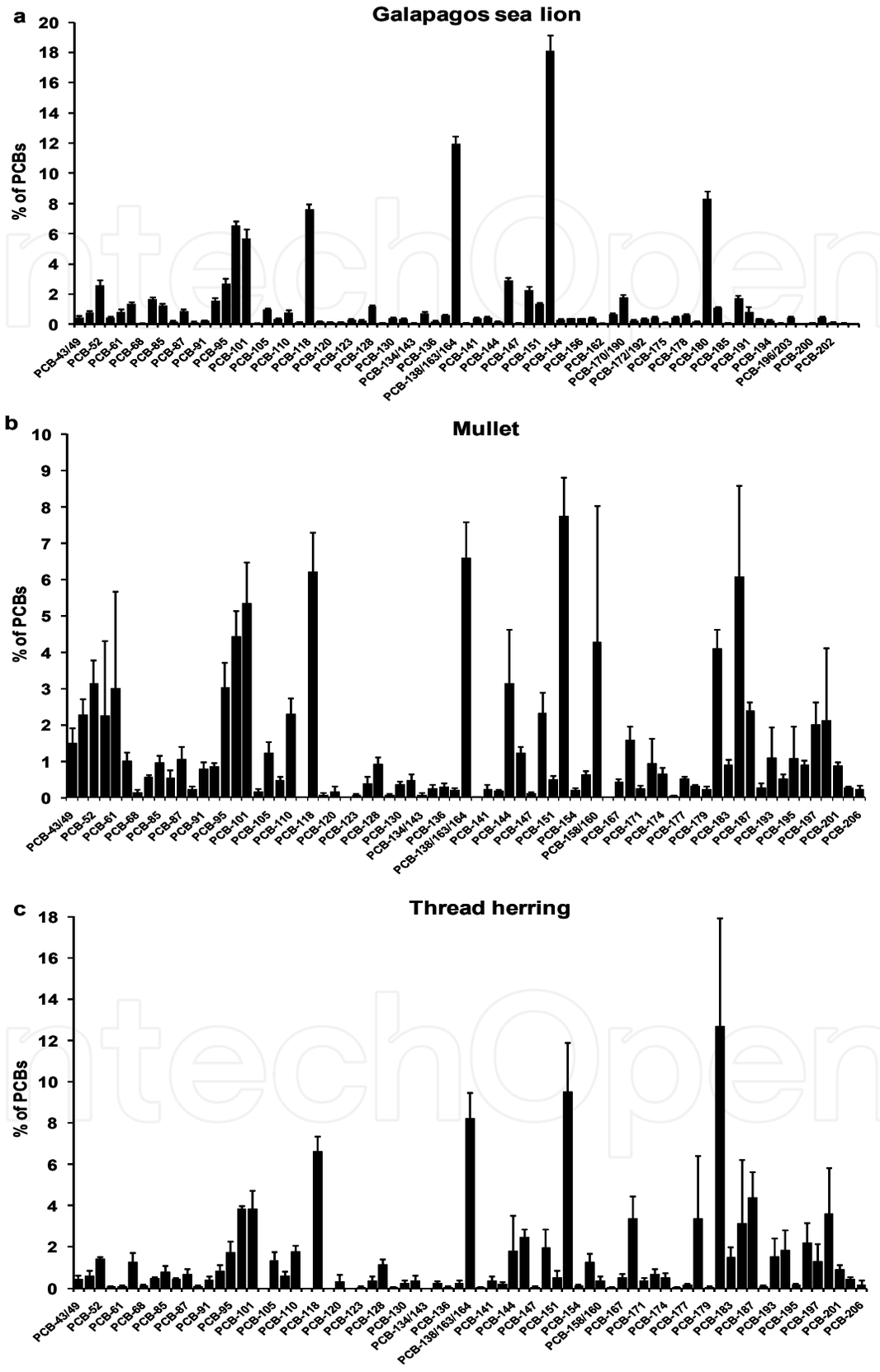

Figure 3. Composition of PCB congeners in Galapagos sea lion pups (a), mullet (b) and thread herring (c). Error bars are standard errors.

3.2.3. Intersite comparisons

The relative concentrations of contaminants observed in all sites exhibited a general common pattern, ∑DDT > ∑Chlordane > ∑PCBs >*β*-HCH> dieldrin > mirex, which was dominated by ∑DDTs, followed by chlordanes and PCBs, and secondly by *β*-HCH, dieldrin and mirex. Concentrations of ∑PCBs and OC pesticides detected in Galapagos sea lion pups showed no significant differences among rookeries (ANOVA for all comparisons, $p > 0.05$). This might suggest a common, global source of contamination delivering POPs to the animals, and that localized sources play a little role in contributions of POPs.

3.3. Biomagnification factors

The interpretation of the data resulting from the use of biomagnification factors are focused on BMF_{TL} as the BMF and BMF_{TL}* was used in this study as an optional approach for evaluation of BMF methods. When the BMF is calculated for the Galapagos sea lion/thread herring case, the BMF values were consistent among the methodologies used (Table 2). In contrast, the three methods differed markedly from 9 to 9.5×10^{18} orders of magnitude higher for OC pesticides and from 4.8 to 1.9×10^{7} orders of magnitude higher for PCBs when the predator-prey BMF_{TL} approaches versus the conventional $C_{PREDATOR}/C_{PREY}$ ratio in the Galapagos sea lion/mullet relationship are compared. These fluctuations appear to be driven by the effect of the magnitude resulting from the differences in trophic levels. While the trophic level difference ($TL_{predator} - TL_{prey} = 1.1$) between the Galapagos sea lion and the thread herring is large, the trophic level difference ($TL_{predator} - TL_{prey} = 0.11$) between the Galapagos sea lion and the mullet is statistically insignificant ($p > 0.05$) and cannot be used in the calculation of the predator-prey BMF_{TL} .Thus, the predator-prey biomagnification factor methodologies (BMF_{TL}) are sensitive to small differences in trophic levels (i.e., Galapagos sea lion-mullet). Based on this observation, the best way of expressing the BMF is the calculation of the BMF calculated as the $C_{PREDATOR}/C_{PREY}$ ratio, which was similar between the Galapagos sea lion/herring and Galapagos sea lion/mullet cases.The use of different biomagnification factor measures showed that BMF_{TL} and BMF_{TL}* are more appropriate to assess biomagnification if differences in trophic levels of predator/prey relationships are large (i.e. >1), as depicted in Table 2.

Calculated biomagnification factors of OC pesticides and PCB congeners, including octanol-water (K_{OW}) and octanol-air partition coefficients (K_{OA}), are shown in Table 2. The BMF_{TL} of OC pesticides ranged from 7.3 (*trans*-chlordane) to 140 (*p*,*p*′-DDT) kg/kg lipid in Galapagos sea lion/thread herring and from 130 (*trans*-chlordane) to as high as 2000 (*p*,*p*′-DDE) kg/kg lipid in Galapagos sea lion/mullet, while BMF_{TL} for PCB congeners ranged from 2.7 (PCB 156) to 30 (PCB 74) kg/kg lipid in Galapagos sea lion/thread herring, and from 11 (PCB 52) to 72 (PCB 153) kg/kg lipid in Galapagos sea lion/mullet (Table 3). No significant correlations were found between the BMF_{TL} of OC pesticides and K_{OW} (Figure 4b,d). Yet, BMF_{TL} values decrease for some pesticides (e. g., mirex; trans-chlordane) when a K_{OW} of $10^{5.5}$ or $10^{6.0}$ is exceeded. As a function of the octanol-air partition coefficient (K_{OA}), the BMF_{TL} for OC pesticides increased markedly as the K_{OA} increased from $10^{7.5}$ to 10^{9}, and then dropped for the rest of pesticides as K_{OA} exceeds $10^{9.5}$ (Figure 4a,c).

Compound	Log K_{OW} 25-26 ºC	Log K_{OA} 37 ºC	BMF sea lion/ thread herring	BMF sea lion/ mullet	BMF_{TL} sea a lion/ thread herring	BMF_{TL} sea lion/ mullet	BMF_{TL} * sea lion/ thread herring	BMF_{TL} * sea lion/ mullet
p,p'-DDE	6.93	9.44	150	220	140	2000	100	2.10×10^{21}
p,p'-DDT	6.39	10.7	150	84.0	140	760	106	3.00×10^{17}
p,p'-DDD	6.30	10.3	41.0	33.0	38.0	300	31.0	6.60×10^{13}
∑DDT	6.41	10.7	132	180	122	1630	92.0	3.10×10^{20}
***β*-HCH**	3.81	10.5	68.5	60.7	60.0	550	50.0	1.60×10^{16}
***trans*-chlordane**	6.27	10.1	7.90	14.0	7.00	130	6.80	2.67×10^{10}
***cis*-chlordane**	6.20	10.1	35.0	65.0	33.0	590	27.0	2.86×10^{16}
***trans*-nonachlor**	6.35	10.0	80.0	177	74.0	1610	57.5	2.70×10^{20}
***cis*-nonachlor**	6.08	8.38	44.0	68.0	40.0	615	33.0	4.30×10^{16}
∑Chlordanes			58.0	113	54.0	1030	43.0	4.70×10^{18}
Mirex	7.50	7.96	22.0	176	21.0	1600	18.0	2.50×10^{20}
Dieldrin	5.48	8.73	45.0	30.0	41.0	270	34.0	2.70×10^{13}
PCB-52	5.90	8.39	12.5	1.21	12.0	11.0	10.0	5.80×10^{0}
PCB 74	7.70	8.41	32.0	7.87	30.0	72.0	25.0	1.40×10^{8}
PCB 95	7.30	8.98	8.78	1.25	8.10	11.0	7.50	7.50×10^{0}
PCB-99	6.60	9.36	16.7	3.64	15.5	33.1	13.5	1.30×10^{5}
PCB-101	6.30	9.11	10.3	1.90	9.53	18.0	8.66	4.20×10^{2}
PCB-105	6.80	9.56	8.10	2.20	7.50	20.0	6.95	1.28×10^{3}
PCB-118	6.70	8.24	12.0	3.17	11.0	29.0	10.0	3.60×10^{4}
PCB 128	7.00	9.16	11.4	3.60	10.5	33.0	9.50	1.10×10^{5}
PCB - 138/163/164	7.20	10.0	15.0	5.90	14.0	54.0	12.0	1.10×10^{7}
PCB-146	7.30	9.22	11.8	7.33	11.0	67.0	9.80	7.30×10^{7}
PCB 153	6.90	9.79	19.0	7.90	18.0	72.0	15.0	1.50×10^{8}
PCB 156	7.40	9.74	2.95	1.28	2.70	12.0	2.72	9.15×10^{0}
PCB 174	7.00	9.62	6.05	1.50	5.60	14.0	5.30	3.90×10^{1}
PCB 180	7.20	9.83	8.30	7.40	7.70	67.0	7.10	7.60×10^{7}
PCB 183	7.00	9.88	8.50	4.10	7.90	38.0	7.30	4.00×10^{5}
PCB 187	7.25	9.71	3.95	2.60	3.70	24.0	3.60	6.90×10^{3}
PCB 201	7.10	10.3	6.26	2.35	5.80	21.0	5.50	2.40×10^{3}
PCB 202	7.10	NR	3.80	2.10	3.55	19.0	3.50	9.40×10^{2}
∑PCBs			12.0	4.10	11.0	37.0	10.0	3.65×10^{5}

NR= non reported;
Values for log K_{OW} and log K_{OA} were obtained from Kelly *et al.* [2] and Mackay *et al*. [56].

Table 2. Biomagnification factors (BMF), Predator-prey Biomagnification factors (BMF_{TL}) and Log Predator-prey Biomagnification factors (BMF_{TL}*) in units of kg/kg lipid for organochlorine pesticides (OCP) and PCB congeners in the Galapagos sea lion. The logarithmic values of the octanol-water (K_{OW}) and octanol-air (K_{OA}) partition coefficients for each contaminant are also reported as supporting indicators of bioaccumulation.

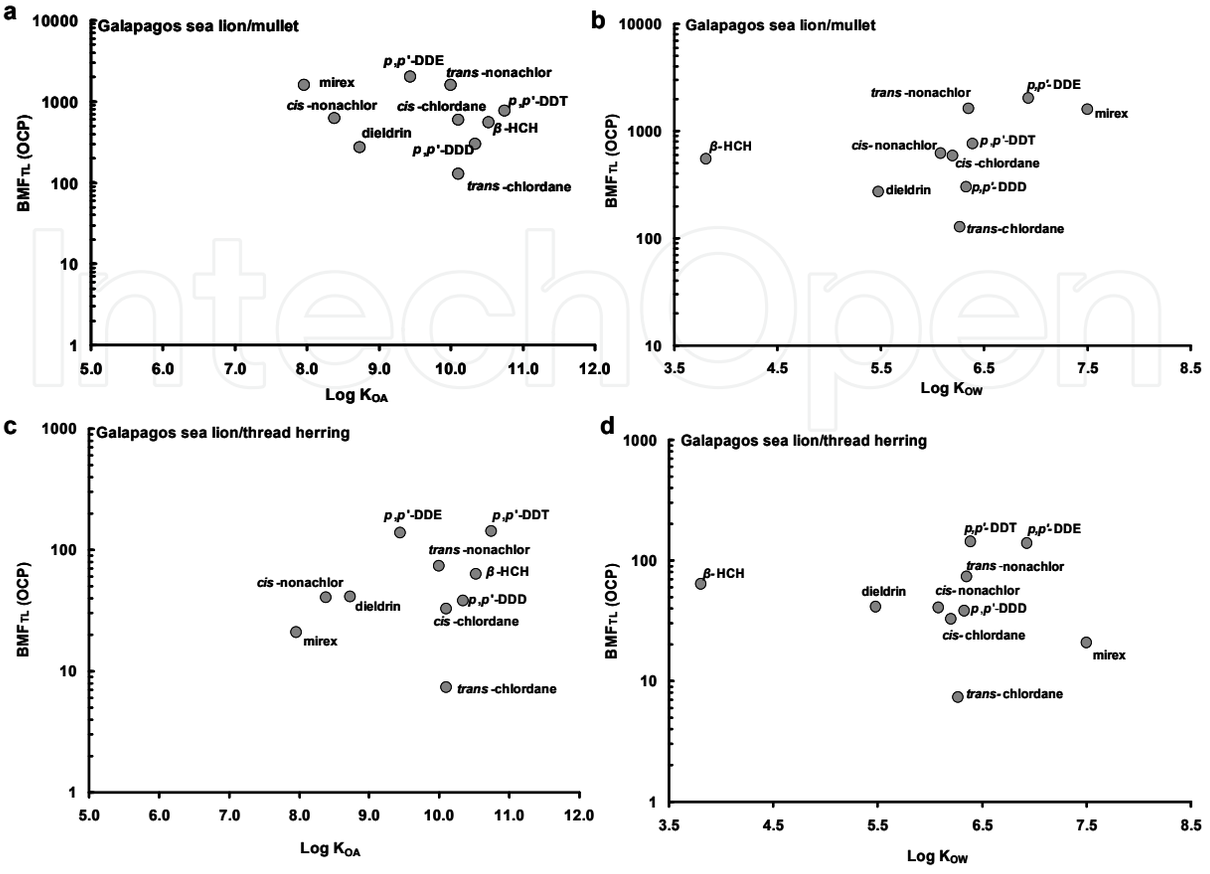

Figure 4. Predator-prey biomagnification factors (BMF_{TL}) in the Galapagos sea lion as expressed by the OC pesticide concentration ratios sea lion/ mullet (a, b) and sea lion/ thread herring (c, d) as a function of log K_{OA} (a, c) and log K_{OW} (b, d).The figure illustrates that while the Stockholm Convention for POPs uses a log K_{OW}> 5 as a criterion to identify bioaccumulative substances, substances including β-HCH with a log K_{OW}< 5 can biomagnify in marine mammals. Log K_{OA} appears to be a better predictor of substances that have the potential to biomagnify in marine mammals. Values for log K_{OW} and log K_{OA} were obtained from Kelly *et al.* [2] and Mackay *et al*. [56].

The BMF_{TL} of PCBs showed different trends when looking a different prey items in terms of K_{OA}. While no correlation was found between the BMF_{TL} of PCBs and log K_{OA} in the Galapagos sea lion/ mullet relationship (Figure 5a), BMF_{TL} for PCBs increased as the K_{OA} increased from $10^{7.6}$ to $10^{8.4}$ and then appeared to decrease gradually with increasing log K_{OA} in the Galapagos sea lion/thread herring relationship (Figures 5c). No correlation was found between the BMF_{TL} of PCBs and log K_{OW} for the Galapagos sea lion/thread herring or Galapagos sea lion/mullet feeding relationship (Figure 5b, d).

These observations demonstrate that these halogenated substances biomagnify and achieve concentrations in Galapagos sea lions that exceed those in their prey, although physiological processes and biotransformation may limit the biomagnification of some contaminants. When comparing the plots of BMF_{TL} of PCBs versus log K_{OW} or versus log K_{OA} similar patterns were observed for both Galapagos sea lion/thread herring and Galapagos sea lion/mullet feeding relationships (Figure 5a,d and Figure 5b,d, respectively). This is explained by the strong correlation usually observed between log K_{OA} and log K_{OW} of PCBs [53].

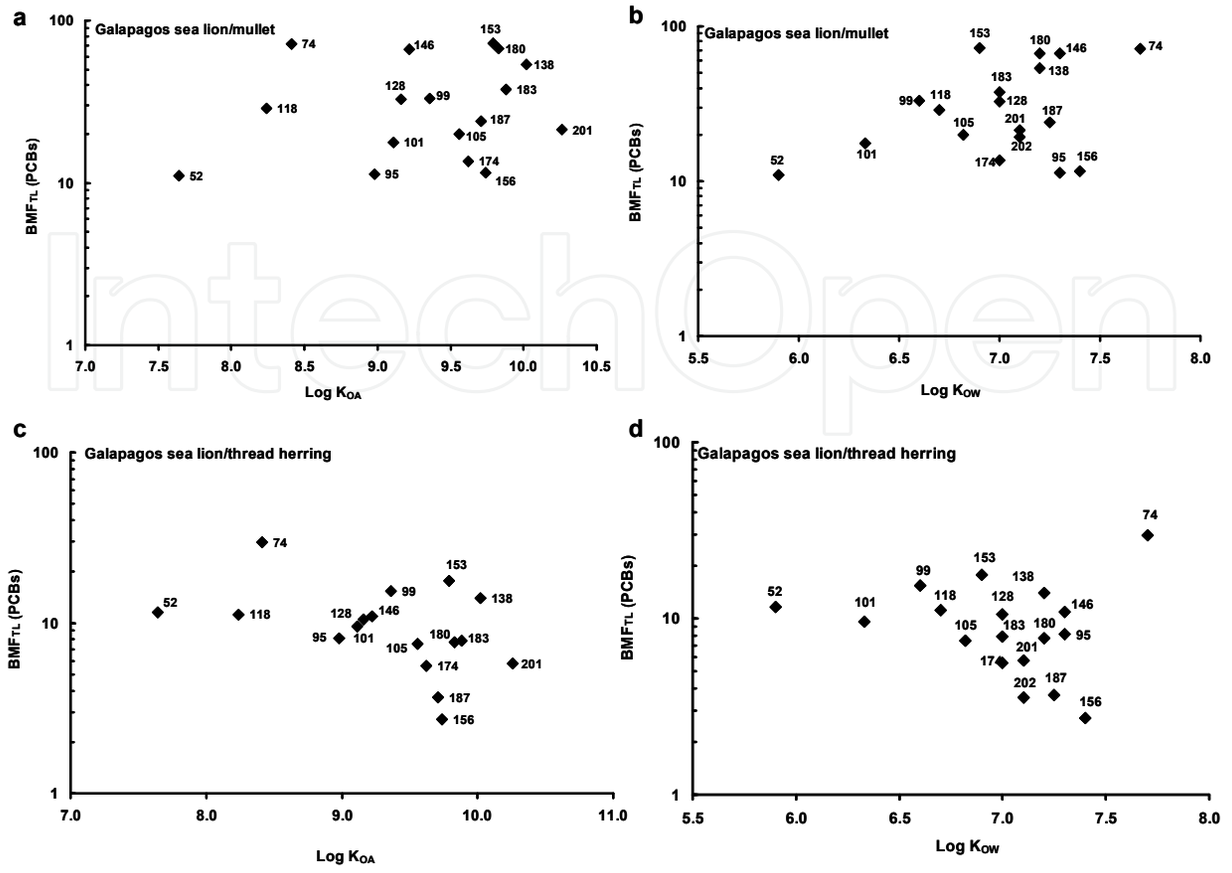

Figure 5. Predator-prey biomagnification factors (BMF_{TL}) in the Galapagos sea lion as expressed by the PCB congeners' concentration ratios sea lion/mullet (a, b) and sea lion/thread herring (c, d) as a function of log K_{OA} (a, c) and log K_{OW} (b, d). For PCBs, log K_{OW} appears to be an adequate predictor of the bioaccumulative potential of PCBs in marine mammals because all PCBs tested have a high log K_{OA}> 6. Values for log K_{OW} and log K_{OA} were obtained from Kelly *et al.* [2] and Mackay *et al*. [56].

The BMF_{TL} for organochlorine pesticides expressed by the concentration ratios sea lion/thread herring and sea lion/mullet of the Galapagos sea lion are higher than those reported for harp seals (*Pagophilus groenlandicus*) from the contaminated Barents Sea [15], (Table 3). However, the BMF_{TL} for PCBs of the Galapagos sea lion are lower than those reported for harp seals. This indicates the biomagnification predominance of organochlorine pesticides in tropical-equatorial regions versus the predominant biomagnification of PCBs in Arctic regions. To further explore these comparisons, the ratio of the BMF_{TL} for *p*,*p*'-DDE (the DDT dominant metabolite) to the BMF_{TL} for PCB 153 (used here as the most recalcitrant PCB congener) was calculated for both species of pinnipeds and then compared. As shown in Table 3, the ratio *p*,*p*'-DDE BMF_{TL}/PCB 153 BMF_{TL} was much higher in the Galapagos compared to that of the Barents Sea, which is driven by the predominance of *p*,*p*'-DDE biomagnification in the Galapagos. Vapor pressures of organic contaminants are expected to be higher in tropical systems due to warmer/higher temperature in comparisons to cold/lower temperature in the Arctic; and, therefore, higher thermodynamic gradients and increase in concentrations are likely to occur during the trophic transfer of contaminant mass from prey to predator, resulting in a high biomagnification factor.

	Galápagos Islands (Ecuador) Galapagos sea lion	Barents Sea Harp seal[a]
	BMF_{TL}	BMF_{TL}
p,p'-DDE	139–2014	319
p,p'-DDT	142–760	NR
∑DDT	122–1631	NR
β-HCH	63.0–552	4.1
cis-chlordane	32.7–587	NR
trans-chlordane	7.34–128	NR
trans-nonachlor	73.7–1609	141.7
∑Chlordanes	54.1–1029	NR
PCB 52	11.0–11.6	NR
PCB 99	15.5–33.1	147.0
PCB 101	9.53–17.7	NR
PCB 105	7.51–20.0	18.1
PCB 118	11.2–28.8	41.6
PCB 138	13.9–53.9	327.7
PCB 153	17.7–72.2	416
PCB 180	7.72–66.9	NR
∑PCBs	11.2–37.2	NR
Ratio BMF_{TL} *p,p'*-DDE to BMF_{TL} PCB 153	7.85–27.9	0.77

NR= non reported
[a] Borga *et al*. [15].

Table 3. Comparison of BMF_{TL} for remote marine food chains between the Galapagos Islands and an Arctic reion for selected organochlorine pesticides and PCBs. The BMF_{TL} for Galapagos sea lions are expressed as the range of concentration ratios of both sea lion/thread herring and sea lion/mullet feeding relationships.

3.4. Biomagnification behaviour of POPs in the Galapagos food-chain

It is well recognized that the increase in organic chemical concentrations in lipids of organisms with increasing trophic level in food-webs originates from the magnification of the chemical concentration in the gastro-intestinal tract caused by food digestion and absorption [5,14]. In this study, the biomagnification capacity of organochlorine contaminants in the tropical food chain of the Galapagos sea lion is established (i.e. $C_{PREDATOR} > C_{PREY}$, BMF > 1).

However, a range of various factors directly or indirectly affect magnification process in predators, including animal ecologies and physiologies, feeding preferences, life history parameters (sex, age, body size and corporal condition), reproduction, geographic locations and stochastic-climatic events. Furthermore, the composition of contaminants can be shaped through toxicokinetics processes (i.e., uptake, metabolism, respiration and excretion), influencing the persistence and food-web biomagnification of POPs. Due to these factors, it is complex to elucidate whether a wild predator is at a steady state with its diet; therefore,

calculated BMFs may not always reflect actual biomagnification [54]. As shown in this study, predator-prey BMFs revealed the biomagnification capacity of POPs in the food chain of the Galapagos sea lions, which is an apex predator possessing flexible feeding preferences (dietary plasticity).

Efficient uptake and dietary assimilation and slow depuration/excretion rates of these compounds (PCBs with K_{OW} ranging 10^5–10^7, and OC pesticides K_{OW} ranging $10^{3.8}$–$10^{7.0}$) explain the high degree of biomagnification in the Galapagos marine food chain. Dietary absorption efficiencies of Penta and Hexachlorobiphenyls are typically between 50-80% in fish and 90-100% in mammals [55] and chemical half-lives ($t_{1/2}$) for recalcitrant PCBs such as PCB 153 in organisms exceed 1000 days [56]. The analysis of BMF_{TL} estimates of PCBs and OC pesticides (Figures 4-5) indicates that OC pesticides and PCBs are accumulated by fish and sea lions and also biomagnify in the food chain. Based on contaminants' predator-prey BMFs, the DDT metabolites, *p*,*p*'-DDT and *p*,*p*'-DDE, followed by *trans*-nonachlor (Figure 4), are the most bioaccumulative pesticides, while PCB 74 and 153 are the most bioaccumulative PCB congeners in the Galapagos sea lion (Figures 5). The less bioaccumulative compounds are *trans*-chlordane and PCB 156.

Of particular importance is the biomagnification behaviour of *β*-HCH with a $K_{OW} < 10^4$ ($K_{OW} = 10^{3.8}$; Figure 4b,d), but with a K_{OA} of $10^{8.9}$–$10^{10.5}$ (Figure 4a,c), contrasting with the regulatory criteria and current management policies (i.e. Stockholm Convention; CEPA) for POPs that consider only chemicals with K_{OW} values $>10^5$ as bioaccumulative substances [7]. The predator-prey biomagnification factors (BMF_{TL} = 63–552) of *β*-HCH in Galapagos sea lions exceed equivalent biomagnification factors of PCB 153 (BMF_{TL} =18.0–72.2) and PCB 74 (BMF_{TL} =30.0–72.0), as shown in Table 2. This portrays that *β*-HCH, a relatively hydrophilic and nonmetabolizable chemical, biomagnifies in the tropical marine mammalian food chain of an air breathing organism (the Galapagos sea lions), which is explained by the relatively high $K_{OA}$of *β*-HCH ($K_{OA} > 10^{7.0}$) and its negligible respiratory elimination. Biomagnification of *β*-HCH was evident in the lichen-caribou-wolf terrestrial food chain, in the maritime and interior grizzly bears' food chains, and in a marine mammalian food web (including water-respiring and air-breathing organisms) from temperate regions of Canada and the Canadian Arctic [2,14,19].

3.5. Environmental transport of contaminants

Lack of significant differences and consistent uniformity of PCBs and OC pesticides, particularly for PCBs, among sites might indicate common sources of contamination. Concentrations of PCBs were also similar among rookeries in an earlier baseline study [34], although DDT concentrations were found to be significantly different [35]. Furthermore, principal components analysis represented a more comprehensive approach for exploring spatial differences and behaviour of POPs. The two first principal components (i.e., PC 1 and PC2) accounted for 55.2% of the total variation in Galapagos sea lion pups. PCA score plot results for the 2008 data further revealed that contaminants follow a similar trend, aggregated near to the centre of the axes, among sites, showing lack of discrimination and differentiation in contaminant patterns (Figure 6a). The first principal component (i.e.,

loading plots, PC1: 40.1% of the total variance) segregated in a significant degree the heavier PCB congeners (upper and lower left quadrants) from the lighter PCBs (upper and lower right quadrants; as seen in Figure 6b). A high positive PC1 score was correlated with higher percentages of low chlorinated PCBs (e.g., PCBs 43/49, 47/48/49, 52, 60, 61, 66, 74, 85, 86/97,87, 92, 95, 101, 110, 123, 132, 135, 136, 141, 144, 149) and *p,p'*-DDD, *p,p'*-DDT, dieldrin, *cis*-nonachlor, *trans*-chlordane, *cis*-chlordane and *β*-HCH, while a high negative score in PC 1 (upper and lower left quadrant) was correlated with a lower proportion of heavily and several, more persistent chlorinated PCBs (e. g. PCBs 118, 138/163/164, 137, 153, 158/160, 171, 177, 180, 183, 170/190, 172/192, 193, 194, 195, 196/203, 201, 202), as well as the semi-volatile and more bioaccumulative *p,p'*-DDE. These patterns show that PC1 appeared to be related to vapour pressure (Henry's Law constant or H) due to a high contribution of more volatile halogenated contaminants (pesticides) and less chlorinated (lighter) PCB congeners. A significant correlation was also observed between the log of the Henry's law constant (Log H) for the PCBs and PC1 (the variable loadings of the first principal component;$p < 0.05$, $r = 0.27$; Figure 7), suggesting that log H represented an important factor influencing the transport pathways and partitioning of PCB mixtures in remote environments; and, therefore, affecting the ultimate composition pattern observed in Galapagos sea lions. The Henry's law constant for each PCB is a fundamental parameter that represents the air-water equilibrium partitioning between surface waters and the atmosphere [57]. This indicates that local sources of exposure for high chlorinated PCBs are minimal in the Galapagos and that most of the contamination by POPs is coming from common atmospheric or continental sources.

Dieldrin is a metabolite of aldrin, which was used for agriculture and public health purposes at beginning of the 1950s until its production was cancelled in 1989 in North America, but as with other pesticides, it continues to enter the environment via erosion of soils contaminated in the past and atmospheric deposition [58]. Mirex is a very unreactive and hydrophobic insecticide that was used in North America to control fire ants and as a fire retardant, persisting in the environment because of chronic small inputs from the atmosphere [59]. The presence of this compound in these blubber samples might be related to the past use of mirex in continental Ecuador [60] because of the possible use as insecticide (bait) to control invasive ants in the Galapagos and continental Ecuador. *β*-HCH is a major constituent of technical HCHs, which is likely one of the sources of this residue. Another potential source of *β*-HCH can be lindane (i.e., γ-HCH) since this pesticide is currently being used in several countries in the southern hemisphere as evidenced by its detection in blubber samples of southern elephant seals and minke whales (*Balaenoptera acutorostrata*) from the Antarctic Ocean [49, 61]. At the continental coast of Ecuador, lindane has recently been detected in sediments and aquatic organisms from the Taura River in the Gulf of Guayaquil [62]. The atmospheric influx of HCHs source formulations used in the Asian and South American tropics (i.e., lindane) and North America (i.e. technical HCH) might explain the incidence of *β*-HCH in these samples. Uncertain records of use of legacy OC pesticides exist for the Galapagos, although anecdotic suggested the use of CUP for agriculture (Dr. Alan Tye, former Head Scientist, Department of Plant and Invertebrate Science, Charles Darwin Foundation, Galapagos Islands), and the widespread use of DDT to eliminate introduced rats in the Galapagos by the US Armed Forces during the 1940s and 1950s [35].

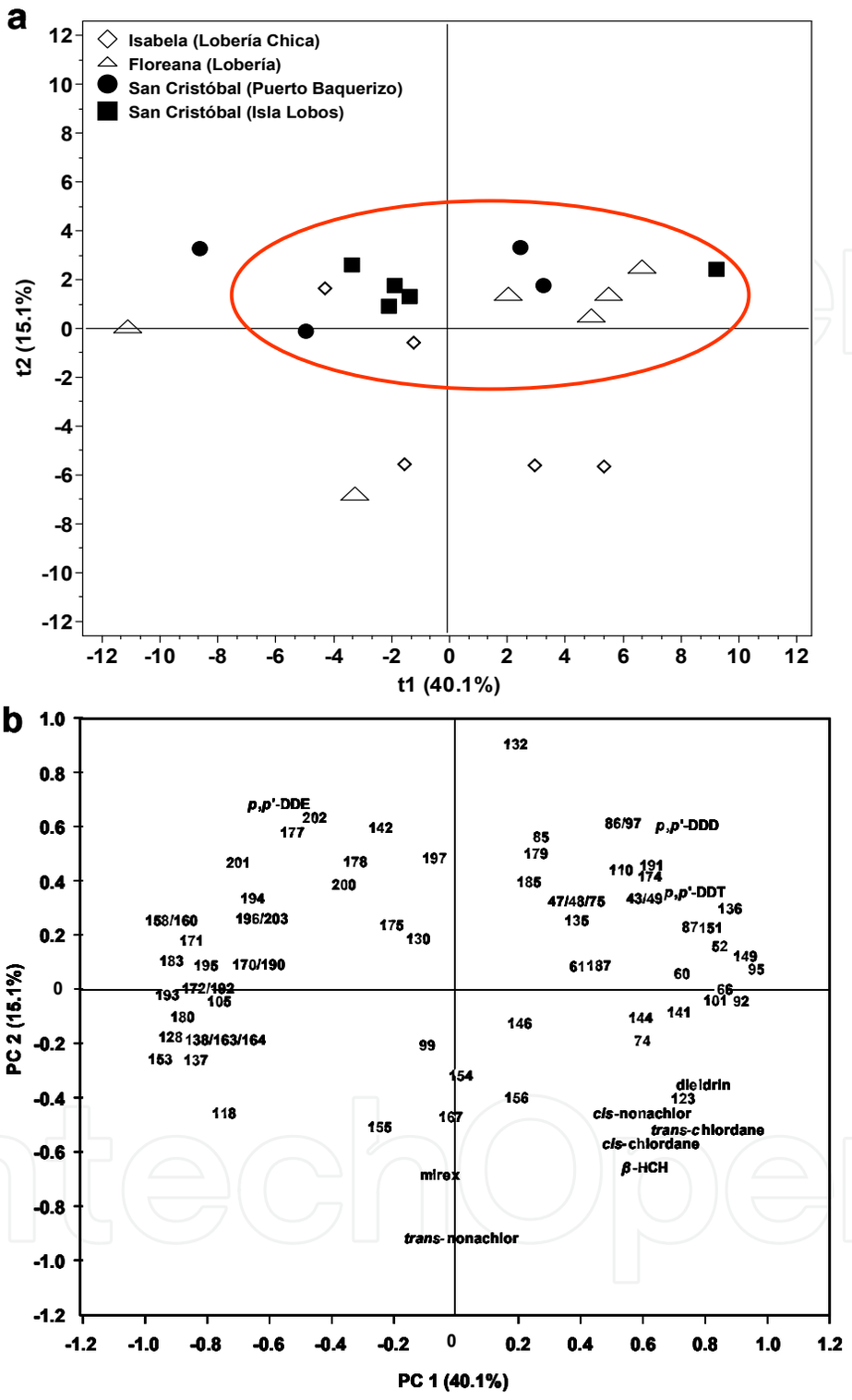

Figure 6. Principal components analysis where the variance accounted for by each principal component is shown in parentheses after the axis label: (a) score plots for patterns of POPs for the first two principal components shows that most of the pups from different rookeries have a similar contaminant pattern, as demonstrated here by the sample scores plot (t1 and t2) of 20 individuals; (b) loadings plots (PC1 and PC2) showing values of individual PCB congeners and pesticides in Galapagos sea lion pups, where numbers are PCB congeners based on the IUPAC system.

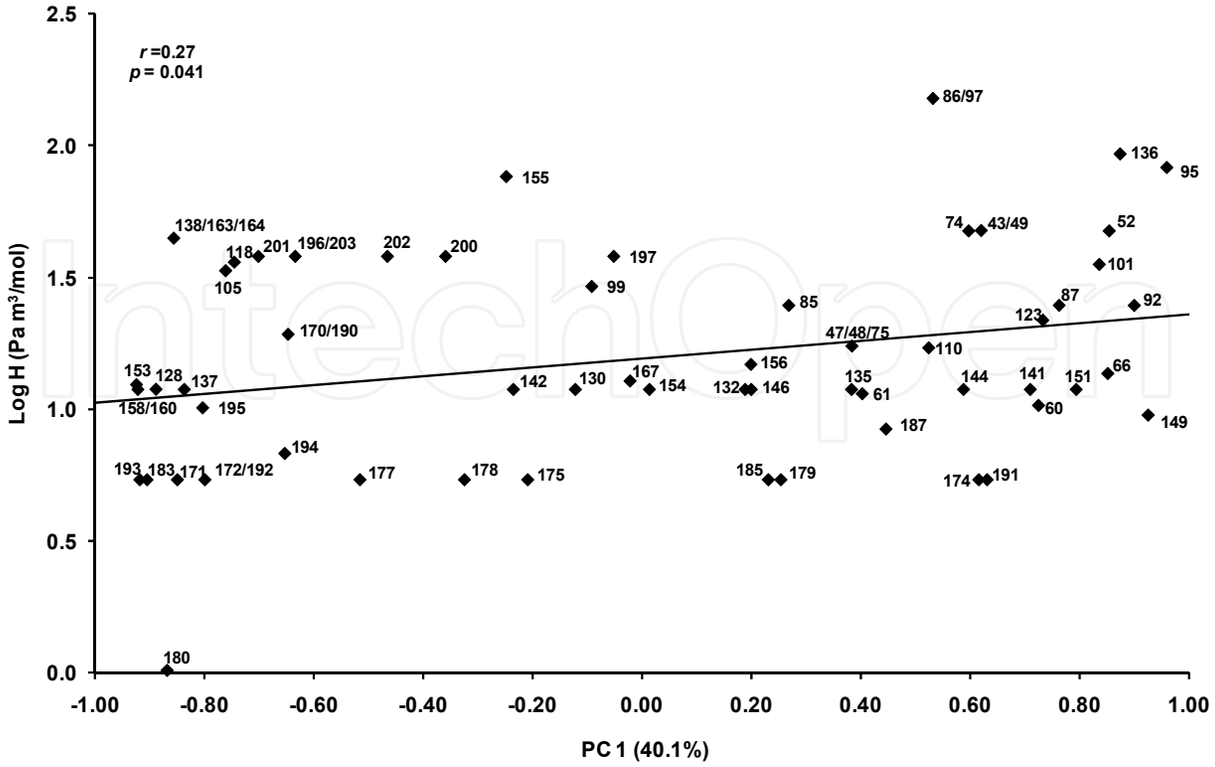

Figure 7. Relationship between the Henry's law constant (Log H) for polychlorinated biphenyl (PCB) congeners and the first principal component (PC1). PC1 is significantly correlated with Log H for PCB congeners, suggesting that Galapagos sea lions from the remote Galapagos Islands are more exposed to light PCB mixtures, consistent with atmospheric signals. Numbers are PCB congeners based on the IUPAC system.

The long range atmospheric transport coupled with global fractionation have usually been described as the major mechanism delivering POPs from lower or mid latitudes to the polar regions [11, 63, 64], but it is likely that a similar mechanism or redistribution from mid latitudes may be also expanding or delivering volatile or semi-volatile pesticides such as HCHs and DDTs to isolated islands around the equator (i.e., the Galapagos Archipelago). These observations suggest that the contamination by organochlorine pesticides might be coming from both local and continental sources because pesticides were used in the recent past in countries in the southern hemisphere [49, 65]. Trans-Pacific air pollution of contaminants from tropical Asia to the eastern Pacific [63, 66] cannot be ruled out as a global and common pathway of POPs of atmospheric origin.

3.6. Health risk assessment

The health risk of POP biomagnification in Galapagos sea lions is of serious concern in the long term, as we have previously reported that 1% of the male pups exceeded the *p*,*p*′-DDE toxic effect concentration associated with potent anti-androgenic effects [35]. DDT concentrations in Galapagos sea lion pups are near levels expected to be associated with impacts on the immune systems, and in minor degree on the endocrine systems in males. Adult male Galapagos sea lions can be expected to exhibit DDT concentrations that are

higher than those in pups as DDTs accumulate throughout the animal's life because they are unable to offload contaminants during reproduction [35].While concentrations of DDTs pose protracted health risk because of lifetime exposure, the ∑PCB concentrations in Galapagos sea lion pups were lower than the new toxicity reference value of1,300 μg/kg lipid for risk of immunotoxicity and endocrine disruption in harbor seals [67]. Other POPs with a similar mode of toxicity such as polybrominated diphenyl ether (PBDEs) flame retardants, which were also detected recently in these animals [34], can further exacerbate the immune and endocrine response. A compromised immune and endocrine system impairs the ability of animals to combat disease and to successfully reproduce.

4. Conservation implications and future research

The Galapagos is one of the last evolutionary biology labs to preserve biodiversity. Yet, it has already been declared a UNESCO-Heritage site at risk because of invasive species, escalating human population growth and burgeoning tourism [68]. This study corroborated that POPs biomagnify to a significant degree in the tropical marine food chain of the Galapagos' marine ecosystem. This has important implications for management and control of organochlorine pesticides and conservation of marine ecosystems in tropical regions since pollution in the Galapagos has been categorized as an aesthetic issue rather than a chronic problem.

Recently, the World Health Organization (WHO) has reactivated the use of the malaria mosquito-fighting pesticide DDT in tropical countries because of increasing malaria cases [69]. While the concentrations of DDT and associated health risks in wildlife are generally believed to be declining, this may no longer be the case in tropical countries where DDT is increasingly used and can biomagnify in food chains. A renewed use of DDT to combat malaria is likely to increase DDT concentrations in the Southern Hemisphere and in particular put bird and marine mammal populations at greater risk because of the biomagnification of these substances in their food webs.

Since the ratification of the UN Stockholm Convention on POPs by Ecuador in 2004, the National Plan for the Inventory and Management of POPs was undertaken [70, 71]. DDT is included on Schedule 2 of the Stockholm Convention because of its damaging health effects in human and wildlife populations. Continuation of this initiative will help to control DDT contamination in the Galapagos. While DDT can save human lives, it can also adversely affect wildlife, local food production and opportunities for ecotourism. DDT use requires that tradeoffs need to be made between the conservation of valued, sensitive wildlife (e.g. Galapagos sea lions), fragile ecosystems and public health programs to control malaria.

Additional research and field sampling efforts may include other organisms integrating the trophic guilds of the Galapagos sea lion food web by measuring legacy and emerging POPs, stable isotopes and subsequent estimations of trophic levels. This will allow assessing in a higher degree the food web amplification of pollutants through the use of TMFs and food web bioaccumulation models in marine ecosystem of the remote Galapagos Islands.

Our findings provide sound scientific information on food chain contamination and potential ecological impacts in the Galapagos that can be used for conservation plans at the ecosystem level, and portrays the implications for environmental management and control of bioaccumulative, persistent and toxic contaminants (e. g. DDT). Finally, this study serves as a reference point against which possible future impact of DDT use in tropical marine ecosystems can be measured, underlying the use of more environmental friendly substances to control pests and vectors in developing countries.

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