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DDT in endangered Galapagos sea lions (*Zalophus wollebaeki*)

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ABSTRACT

We characterize for the first time the presence of DDT and its metabolites in tropical Galapagos sea lions (*Zalophus wolleabeki*). ∑DDT concentrations in Galapagos sea lion pups sampled in 2005 and 2008 ranged from 16 to 3070 μg/kg lipid. Concentrations of ∑DDT in pups in 2008 averaged 525 μg/kg lipid and were 1.9 times higher than that (281 μg/kg lipid) detected in pups in 2005. These concentrations are lower than those reported in many pinnipeds elsewhere, comparable to those in Hawaiian monk seals, and higher than those in southern elephant seals. The health risk characterization showed that 1% of the male pups exceeded the *p,p′*-DDE toxic effect concentration associated with anti-androgenic effects reported in rats. The findings provide preliminary guidance on the relationship between DDT use and ecological impacts, serving as a reference point against which possible future impact of tropical DDT use can be assessed.

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1. Introduction

Global contamination by dichlorodiphenyltrichloroethane (DDT) and other persistent organic pollutants (POPs) remains a serious health concern for protecting biodiversity on the planet. The Stockholm Convention on POPs was established as an international treaty on 17 May 2004 to eliminate the world's most persistent, bioaccumulative and toxic substances, including DDT (UNEP, 2001).

Because of the long range transport characteristics of these substances (Wania and Mackay, 1993; Iwata et al., 1993), the impact of DDT and other POPs on wildlife and human populations inhabiting remote Arctic regions has remained an active area of research (Muir et al., 2000; Kelly et al., 2007; Guglielmo et al., 2009). However, limited information is available on the status and impacts of DDT on remote tropical regions. This is unfortunate, since DDT is still used in tropical regions for malaria control (Roberts et al., 2000; Schenker et al., 2008; Van den Berg, 2008). Recently, The World Health Organization recommended a renewed indoor use of DDT in human habitations of developing countries (WHO, 2006). In addition, an increase in the use of DDT to combat malaria was endorsed by the 34th G8 summit in July 2008. Since its first use in the 1940s, DDT has caused serious impacts to many wildlife populations. For instance, DDT was associated with catastrophic impacts on birds and fish-eating wildlife populations (Hickey and Anderson, 1968; Blus, 2003). Therefore, the renewed use of DDT renews concerns about the impacts of DDT on human and ecosystem health, especially in tropical regions where DDT may be increasingly used (Blus, 2003; Van den Berg, 2008).

Several studies have reported high concentrations of DDTs in abiotic media (i.e., soil, sediment, river, water and air), and subsequent volatilization, with pronounced meridional transport (multi-grass hopping) northward, from tropical developing regions in southern Asia and Oceania, including oceanic surface water samples, and western boundaries of Africa and the Americas (Iwata et al., 1993, 1994; Guglielmo et al., 2009). High concentrations of DDTs resulting from biomagnification of DDT have also been detected in fish of African tropical lakes (Kidd et al., 2001; Manirakiza et al., 2002), and Amazonian river dolphins (*Inia geoffrensis*) from the Brazilian Amazon (Torres et al., 2009). Accumulation of DDT in ospreys (*Pandion haliaetus*) suggests that breeding grounds in North America are still a substantial source for higher DDT exposure (Elliott et al., 2007). A recent study in migratory White faced

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Ibis (*Plegadis chihi*) found higher exposure of DDT on wintering grounds further down in tropical areas (Yates et al., 2010). Likewise, DDT levels in White faced Ibis from Mexico and Adélie penguins (*Pygoscelis adeliae*) from the western Antarctic Peninsula have not decreased between 1985 and 2006 (Geisz et al., 2008; Yates et al., 2010).

Since the early 1970s, reproductive impairment and a high rate of abortions and stillbirths in California sea lions (*Zalophus californianus*) were associated with DDT (Le Boeuf and Bonell, 1971; Delong et al., 1973). More recently, high levels of DDTs were linked to a high prevalence of neoplasms and carcinoma, and associated mortality, in California sea lions (Ylitalo et al., 2005). In addition, POPs have been linked to effects on the immune system (e.g., impairment of T-lymphocyte function, phagocytosis, and respiratory burst) and the endocrine system (e.g., disruption of Vitamin A and thyroid hormones) of several pinnipeds, including harbor seals (*Phoca vitulina*) and California sea lions, as well as small cetaceans (Ross et al., 1995; Lahvis et al., 1995; Debier et al., 2005; Tabuchi et al., 2006). Reduced immune function increases susceptibility to infectious diseases and poses population level risks (Ross, 2002).

Of the two endemic pinnipeds inhabiting the Galapagos Archipelago, the Galapagos sea lion (*Zalophus wollebaeki*) population has decreased by 50–60% since the late 1970s (Alava and Salazar, 2006), and is listed as "endangered" by the International Union for the Conservation of Nature (IUCN) (Aurioles and Trillmich, 2008). Notable stressors have included the El Niño events of 1982–1983 and 1997–1998, fisheries interactions, illegal hunting, oil spills, enzootic diseases, as well as infectious diseases transmitted by rats and dogs (e.g., *Leptospira* and Morbilliviruses, including Canine Distemper Virus) (Alava and Salazar, 2006; Aurioles and Trillmich, 2008).

The possible role of DDT and related contaminants in the Galapagos sea lion decline is unclear. There is no historical report on the use DDT in the Galapagos Islands. However, relatively low concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polybrominated diphenyl ethers (PBDEs) have been reported in Galapagos sea lion pups (*Z. wollebaeki*) (Alava et al., 2009). In adjacent areas (≈3350 km to the north), high concentrations of DDTs are still detected in California sea lions, harbor seals and elephant seals (*Mirounga angustirostris*) from California, USA (Blasius and Goodmanlowe, 2008). The extent that the Galapagos are affected by local and atmospherically-transported DDT from such 'hotspots' is unknown.

The objective of this study was to investigate the concentrations, patterns, temporal trends and possible health risks of DDT in Galapagos sea lions, with a goal of providing input to the changing international regulations.

2. Materials and methods

2.1. Collection of samples

Muscle-blubber biopsy samples were collected from 41 free-ranging, live captured Galapagos sea lion pups (*Z. wollebaeki*) of 2–12 months of age from eight rookeries of the Galapagos Islands Archipelago during two expeditions carried out on March 13–21 in 2005 and March 26–29 in 2008. Pups were sampled at Santa Cruz (Caamaño, $n = 11$; and Plaza Sur, $n = 4$), Fernandina (Punta Espinoza, $n = 3$) and Pinta (Puerto Posada, $n = 3$) islands in 2005; and, from Isabela (Loberia Chica, $n = 5$), Floreana (Loberia, $n = 6$) and Santa Cristobal (Puerto Baquerizo, $n = 4$; Isla Lobos, $n = 5$) islands in 2008 (Fig. 1).

Reproduction in Galapagos sea lions follows a yearly reproductive cycle, principally during the cold season, with peak pupping taking place between August and November (Villegas-Amtmann et al., 2009). The young are weaned after approximately 12–24 months (e.g., Trillmich, 1986; Trillmich and Wolf, 2008). Nursing 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, minimizing the influence of life history parameters on contaminant concentrations; (c) as they are nursed by adult reproductive females they have a high trophic position as they are feeding on mother's milk, analogous to a predator–prey relationship.

Pups sampled in 2005 were captured with hoop nets and immobilized following the field isoflurane gas (0.5–2.5%) anesthesia methodology developed by Páras et al. (2002) (Supporting Information), while those sampled in 2008 were captured with hoop nets and manually restrained without involving anesthesia. In all circumstances, capture stress and holding time were minimized (<10–15 min). Biopsies (100 mg; 6 mm−Miltex biopsy punch) were collected from the supraspinatus muscle, located just above of the pectoral flipper (Villegas-Amtmann and Costa, 2010), or 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 an placed in a cooler with wet ice and transferred into cryovals placed in a cryoship (−20) during the field sampling, and, afterwards stored at −80 °C in the laboratory until chemical analysis. Standard length, weight, girth, and sex for each pup were recorded. The body condition of the pups was measured using the Fulton's condition factor ($FCF = weight \times 10^5/\text{standard length}^3$) to compare body weight of sea lion pups of different standard length within a given reproductive season and eliminate the effect of size on weight (Luque and Aurioles-Gamboa, 2001; Castro-Gonzalez et al., 2001). Age was estimated by visual observation of both the size and weight of the animal. Details of morphometric and field data of the pups can be found in the Supporting Information (see Table S1 in Supplementary material).

2.2. Contaminant analysis

Muscle-blubber biopsy samples (0.004–0.212 g) were analyzed for DDTs by gas chromatography and high resolution mass spectrometry (GC/HRMS) as discussed elsewhere (Ikonomou et al., 2001). The DDT analytes quantified included *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT, and *p,p′*-DDT. The intact biopsy samples were spiked with a mixture of surrogate internal standards which contained $^{13}C_{12}$ *p,p*-DDE, $^{13}C_{12}$ o,*p*-DDD, $^{13}C_{12}$ *p,p*-DDD, $^{13}C_{12}$ *o,p*-DDT, and $^{13}C_{12}$ *p,p*-DDT. 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). The solvent layer was transferred to a clean flask and the waxy precipitate was treated with several aliquots of hexane and DCM, and 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 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 lipid content of the samples. Subsequently the residue was re-suspended in 1:1 DCM/hexane and divided quantitatively into two aliquots. The lager aliquot (75% of the extract) was subjected to sample-cleanup for PCBs, PCDDs, PCDFs, and PBDEs determinations and the results have been reported elsewhere (Alava et al., 2009). The remaining (25% of the extract) was used for DDT determinations. The lower

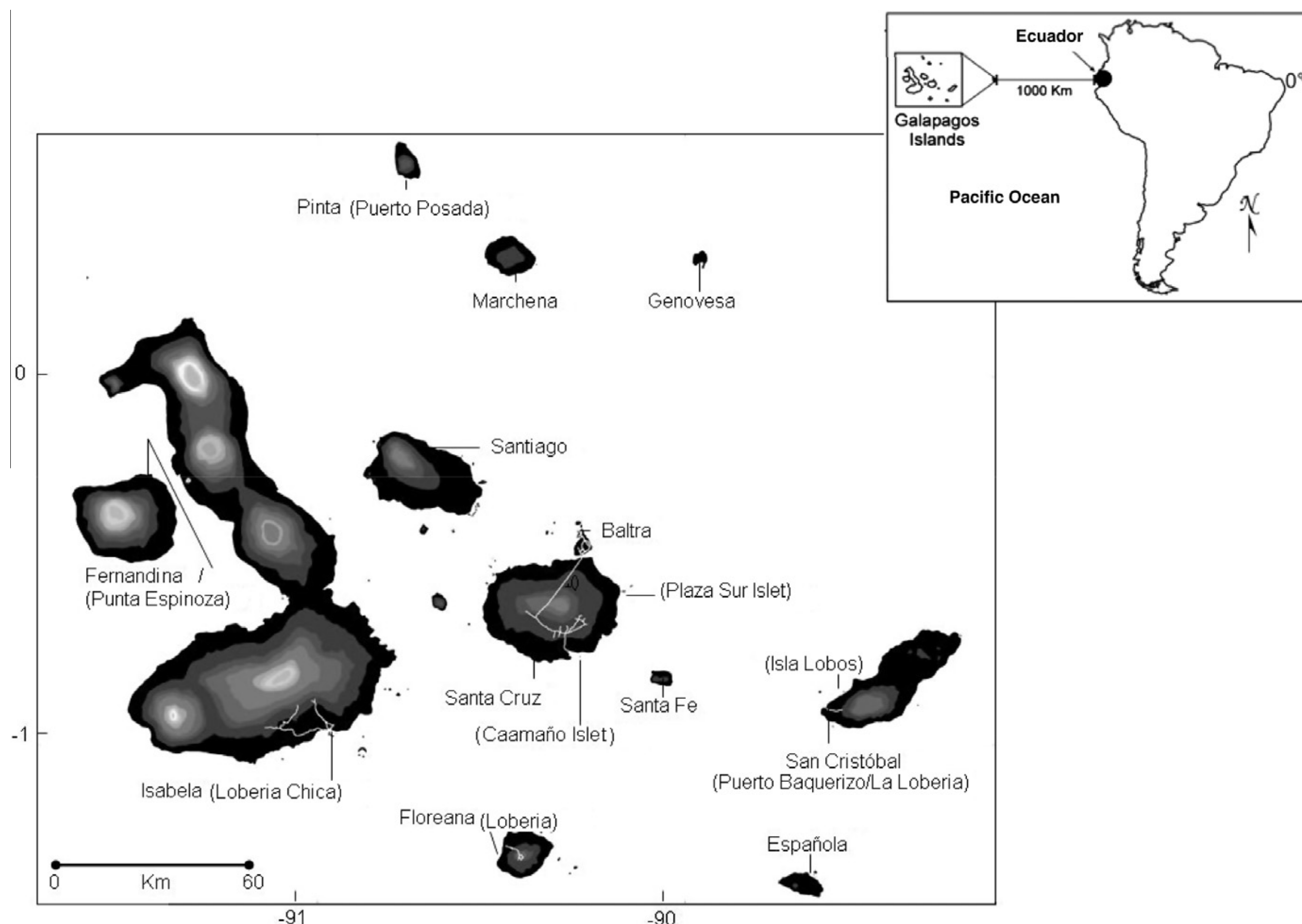

Fig. 1. Map of Galapagos Archipelago at 1000 km off the Ecuadorian continental coast (01°40′N–01°25′S and 89°15′W–92°00′W), showing the islands' names and sites harboring the rookeries (in brackets) of Galapagos sea lions pup (*Zalophus wollebaeki*) sampled during the expeditions carried out in 2005 and 2008.

volume fraction of the sample extract was loaded onto a Florisil column (8 g of 1.2% water deactivated Florisil slurry packed with hexane into a fritted column) and eluted with 60 mL 1:1 DCM:hexane. Cleaned extracts were concentrated to less than 10 μL and spiked with the ^{13}C-labeled method performance standard ($^{13}C_{12}$-PeCB-111) prior to instrumental analysis. Details on the conditions used for sample clean-up and the quality assurance quality control protocols followed are reported in detail elsewhere (Ikonomou et al., 2001).

The corresponding extracts were analyzed for target organochlorine pesticides by GC/HRMS. The high resolution mass spectrometer was a Micromass Ultima (Micromass, UK) instrument equipped with an HP-6890 gas chromatograph and a CTC autosampler. For the OCPs analyses a DB-5 column was used (45 m × 0.25 mm, 0.1 μm film, J&W Scientific, Folsom CA), initial temperature 80 °C for 3 min, increased at 15 °C/min–160 °C, then at 5 °C/min–270 °C and held for 1 min, and lastly at 15 °C/min–300 °C. The injector temperature was held at 200 °C. Splitless injection of 1 μL sample and 1 μL air were performed and the purge was activated 2 min after injection. For all analyses the HRMS was operated at 10,000 resolution under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). The source temperature was maintained at 280 °C and the GC/HRMS interface at 260 °C.

2.3. Quality assurance/quality control measures

Samples were processed in batches of 12 samples each containing one or two procedural blanks, an in-house performance evaluation sample containing known concentrations of the analytes of interest and a certified reference material (CRM), i.e., NIST Standard Reference Material (SRM) 1945 (whale blubber homogenate), and nine or 10 real samples. Method blanks, consisting of Na2SO4, were processed according to the same procedure as the samples and analyzed with every batch of 12 samples to check for potential background contamination. Analytes were identified only when the GC/HRMS data satisfied the following criteria: (i) two isotopes of the analyte were detected by their exact masses with the HRMS operating at 10,000 resolution during the entire chromatographic run; (ii) the retention time of the analyte peak was within 3 s of the predicted time obtained from analysis of authentic compounds in the calibration standards (where available); (iii) the maxima for both characteristic isotopic peaks of an analyte coincided within 2 s; (iv) the observed isotope ratio of the two ions monitored per analyte were within 15% of the theoretical isotopic ratio; and (v) the signal-to-noise ratio resulting from the peak response of the two corresponding ions was $\geqslant 3$ for proper quantification of the analyte. Analyte concentrations were calculated by the internal standard isotope-dilution method using mean relative response factors (RRFs) determined from calibration standard runs made before and after each batch of samples was analyzed. Concentrations of analytes were corrected for the recoveries of the surrogate internal standards. The validity of data correction was confirmed from the tight accuracy and precision data obtained from the analyses of CRM and in-house reference samples. The recoveries of all pesticide surrogate internal standards were between 65% and 110% and the accuracy of determining the target DDT analytes in spiked samples was between 15% and 20%.

2.4. Data and statistical analyses

Concentrations of pesticides measured were blank-corrected using the method detection limit (i.e., MDL on a pg/sample basis), defined here as the mean response of the levels measured in three procedural blanks used plus three times the standard deviation (SD) of the blanks ($MDL = Mean_{blanks} + 3 \times SD_{blanks}$) (Alava et al., 2009). Concentrations below the MDL were substituted using half of the MDL. Concentrations were lipid normalized to account for differences in the lipid content of the samples (μg/kg lipid) and were log-transformed before conducting statistical analyses. ∑DDT concentrations were calculated as the sum of *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT, and *p,p′*-DDT. Because lipid normalized DDT concentrations were not normally distributed in Galapagos sea lion pups (with the exception of female pups sampled in 2008) as tested by the Shapiro–Wilk *W* test ($p < 0.05$), the contaminant data were log transformed to meet the normality criterion before statistical analyses.

To examine whether morphometric factors or sex affected contaminant concentrations, life history parameters (i.e., length, weight, corporal condition or FCF) and lipid content of both sexes were compared through the Welch ANOVA assuming unequal variances (Zar, 1999). Linear regression (Pearson correlation) was used to determine whether life history parameters are correlated with contaminant concentrations.

To determine differences in contaminant concentrations between females and males, a Welch two-tailed *t*-test for unequal variances was used. Differences in contaminant concentrations and percent lipid among sea lion rookeries were evaluated using analysis of variance (ANOVA), where variances among sites were equal (i.e., homoscedastic as tested by the Levene's test and Bartlett test, $p > 0.05$), or Welch ANOVA, where variances were unequal (i.e., heteroscedastic; Levene's test or Bartlett test, $p < 0.05$). This was followed by a Tukey–Kramer honestly significant difference (HSD) multiple comparison test, which is a post hoc method recommended to test differences between pairs of means among groups that contain unequal sample sizes (Zar, 1999). Concentrations were expressed in term of the geometric mean with an upper and lower standard deviation (±SD) unless otherwise specified (i.e., arithmetic mean ± SD). Statistical analyses were conducted using JMP 7.0 (SAS Institute Inc.; Cary, NC, USA, 2007) at a level of significance of $p < 0.05$ ($\alpha = 0.05$).

2.5. Health risk assessment

In absence of toxicological and health studies of DDT on Galapagos sea lions, we attempted to interpret observed DDT concentrations in terms of potential DDT related health effects by comparing *p,p′*-DDE concentration distributions to *p,p′*-DDE circulatory levels related to immunotoxicity in bottlenose dolphins (*Tursiops truncatus*) (Lahvis et al., 1995) and anti-androgenic effect in mammalian (i.e., rat) cell cultures (Kelce et al., 1995). To make comparable these reference values, we normalized them to lipid and protein content of blood reported for bottlenose dolphins (e.g., Bossart et al., 2001; Woshner et al., 2006; Houde et al., 2006; Yordi et al., 2010) and rats (Poulin and Krishnan, 1996; DeBruyn and Gobas, 2007) to express the concentrations in equal units and in similar media, using the following equation:

$$TEC_{\text{BLOOD-LIPID NORMALIZED}} = TEC_{\text{BLOOD-WET WEIGHT}}/(f_{\text{L,BLOOD}}) + (f_{\text{P,BLOOD}})0.05$$

where $TEC_{\text{BLOOD-LIPID NORMALIZED}}$, and $TEC_{\text{BLOOD-WET WEIGHT}}$ are the circulatory toxic effect concentrations of *p,p′*-DDE in a lipid and wet weight basis, respectively; $f_{\text{L,BLOOD}}$ is the fraction of lipid in blood, and $f_{\text{P,BLOOD}}$ is the fraction of protein in the blood. The coefficient 0.05 is the sorptive capacity of proteins in relation to that of lipids (DeBruyn and Gobas, 2007). Lipid, protein fractions and lipid normalized effect concentrations for bottlenose dolphin and rats are available in Table S2 (supplementary material). In an effort to conduct the health risk characterization, the relative frequency of the population sampled (i.e., pups), here expressed as the normal probability density distribution function of the log *p,p′*-DDE concentrations measured in a lipid weight basis in pups, were plotted (Gaussian distribution) against the lipid normalized log values of *p,p′*-DDE toxic effect concentrations above documented to assess what proportion of the pups (i.e., frequency) exceed target threshold *p,p′*-DDE concentrations.

3. Results and discussion

3.1. Morphometrics and lipid content

The mean ± SD of the standard length, body weight, corporal condition and lipid content of the 41 pups is shown in Table 1. When compared to males sampled in 2005 and pups (male and females) sampled in 2008, female pups sampled in 2005 were significantly longer (Barlett test, $p = 0.0007$; Welch ANOVA, $p < 0.0001$; Tukey–Kramer test, $p < 0.05$) and heavier (Barlett test, $p = 0.0009$; Welch ANOVA, $p < 0.0001$; Tukey–Kramer test, $p < 0.05$). Because of differences in body size (i.e., length and weight), the corporal condition of 2005-females was significantly different from the body condition of 2005-males (Welch *t*-test = 3.343, $p = 0.0036$, df = 18); 2008-males (*t*-test = 2.580, $p = 0.0179$, $df = 20$); and, 2008-females (*t*-test = 2.942, $p = 0.0081$, $df = 20$). This likely reflects the more rapid growth and the higher body density of male otariid pups, as they allocate a larger fraction of milk energy to muscular and skeletal growth than females (Luque and Aurioles-Gamboa, 2001).

Pups appeared nutritionally healthy (i.e., lipid measurements > 50%). No significant differences were observed in lipid content among any group of pups (Welch ANOVA, $p = 0.7358$;

Table 1
Sample size, lipid content, length, weight, corporal condition and Pearson correlation coefficients (*r*) with *p* values resulting from the linear regression analyses of the log transformed lipid concentrations of ∑DDTs vs morphometric parameters by sex categories in Galapagos sea lion pups, *Zalophus wollebaeki*.

Sex	Year	Sample size (*n*)	Lipid (%)	Weight (kg)	Standard length (cm)	Body condition (FCF)[a]	Standard length vs ∑DDTs	Weight vs ∑DDTs	FCF vs ∑DDTs
Males	2005	8	70.2 ± 9.34	20.6 ± 0.95	102 ± 1.85	1.94 ± 0.03	$r = -0.373$ $p = 0.3622$	$r = -0.409$ $p = 0.3144$	$r = 0.124$ $p = 0.7696$
Females	2005	13	73.3 ± 3.92	66.9 ± 7.01*	155 ± 7.67*	1.71 ± 0.06*	$r = -0.894$ $p < 0.0001$*	$r = -0.777$ $p = 0.0018$*	$r = 0.686$ $p = 0.0096$*
Males	2008	10	77.8 ± 2.45	22.3 ± 2.34	105 ± 3.03	1.94 ± 0.06	$r = 0.2041$ $p = 0.5716$	$r = 0.1910$ $p = 0.6225$	$r = -0.280$ $p = 0.4667$
Females	2008	10	75.9 ± 3.50	21.1 ± 2.21	102 ± 3.28	2.01 ± 0.08	$r = 0.1698$ $p = 0.6390$	$r = -0.1769$ $p = 0.6488$	$r = -0.413$ $p = 0.2693$

[a] FCF is the Fulton's Condition Factor ($FCF = weight \times 10^5/standard\ length^3$).
* Asterisk indicates a statistically significant comparison or correlation between morphometric parameters and ∑DDT concentrations in female pups sampled in 2005.

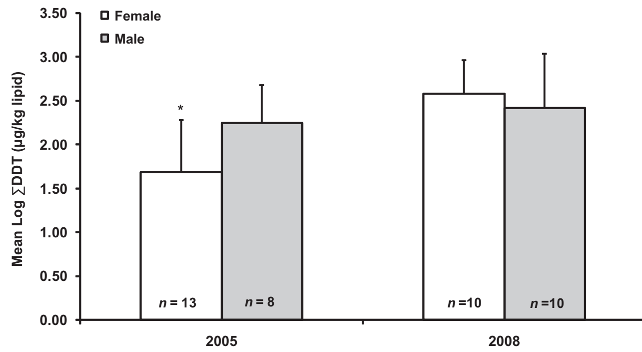

Fig. 2. Temporal comparisons of mean log ∑DDT concentration by sex categories. The asterisk indicates that the concentration was significantly different from the other concentrations. Error bars are standard errors.

Tukey–Kramer test, $p > 0.05$). The mean ± SD lipid content of the pup samples ranged from 70.2 ± 9.34% to 77.8 ± 2.45% (Table 1).

3.2. Biological factors as determinants of ∑DDT concentrations in pups

To reduce the possible influence of age and body condition on DDT concentrations, only biopsy samples from nursing animals of similar age (i.e., <2 year) were collected. DDT concentrations in Galapagos sea lion pup females captured in 2005 were significantly lower than the DDT concentration found in the 2005 males (t-test = 2.320, $p = 0.0316$, df = 19) and in pups, both males (t-test = 2.873, $p = 0.0091$, $df = 21$) and females (t-test = 4.126, $p = 0.0005$, $df = 21$), sampled in 2008 (Fig. 2, Table 2). Since the study animals were immature, differences in concentrations between male and female pups due to reproductive losses (i.e., milk secretion and parturition) (e.g., Addison and Smith, 1974; Addison and Brodie, 1987) can be ruled out as a cause.

Table 2
Overall and arithmetic mean ± standard error (SE) concentrations of ∑DDTs (µg/kg lipid) and metabolites (µg/kg lipid) in muscle-blubber samples of Galapagos sea lion pups.

Sample	Sex	*o,p*-DDE	*p,p*-DDE[a]	*o,p*-DDD	*p,p*-DDD	*o,p*-DDT	*p,p*-DDT	∑DDTs[b]
2005 samples								
PIP-02	M	0.06	1140	2.10	33.5	1.60	25.60	1200
PIP-08	F	3.10	2900	8.30	106	9.15	39.15	3070
PIP-10	M	2.70	134	1.70	11.0	1.15	10.30	161
PEP-01	F	2.13	115	0.450	6.50	0.100	2.50	130
PEP-03	M	2.70	390	0.850	14.2	1.10	3.05	412
PEP-07	M	2.70	67.5	0.500	7.20	0.700	7.00	85.5
PSP-01	M	1.80	107	1.300	11.2	0.800	7.35	130
PSP-02	M	1.10	90.0	0.300	6.35	0.200	8.70	107
PSP-03	M	2.50	181	0.900	10.1	1.10	5.20	200
PSJ-06	M	13.2	20.1	2.300	2.20	5.60	7.60	50.0
CAAF-01	F	3.20	23.0	0.600	1.05	1.40	1.80	30.8
CAAF-02	F	2.72	28.6	0.500	3.20	1.20	1.60	38.0
CAAF-03	F	2.20	32.0	0.100	2.70	0.900	1.30	40.0
CAAF-04	F	2.00	11.2	0.300	0.700	0.800	1.10	16.0
CAAF-05	F	2.72	65.3	0.500	0.600	1.20	1.60	72.0
CAAF-06	F	2.21	32.0	0.400	1.90	0.950	1.50	38.5
CAAF-07	F	2.22	26.5	0.400	2.50	0.950	1.30	33.8
CAAF-08	F	2.14	14.3	0.400	0.450	0.900	1.20	19.4
CAAF-09	F	2.20	21.0	0.400	1.10	0.900	1.80	27.0
CAAF-10	F	4.00	7.00	0.700	0.800	1.70	2.30	16.3
CAAF-11	F	2.20	0.150	0.400	0.450	0.900	32.6	37.0
2005 females		2.50 ± 0.16	252 ± 221*	1.02 ± 0.60	9.80 ± 8.00*	1.60 ± 0.60	6.90 ± 3.60*	274 ± 233*
2005 males		3.40 ± 1.40	266 ± 130	1.20 ± 0.30	12.1 ± 3.30	1.50 ± 0.60	9.30 ± 2.45	293 ± 135
2008 samples								
IZS-01	F	1.11	1058	1.18	44.1	1.06	16.8	1122
IZS-02	M	0.00	193	0.34	9.31	0.24	8.80	212
IZP-04	F	0.20	65.4	0.16	2.97	0.76	1.69	71.2
IZP-05	M	0.13	13.6	0.17	0.96	0.37	0.97	16.3
IZP-06	F	0.00	143	0.11	1.88	0.40	2.29	148
FPZ-01	F	0.00	293	0.16	9.9	0.27	17.4	320
FPZ-02	F	0.16	231	0.22	4.05	0.16	5.04	241
FSZ-03	M	0.00	1647	0.00	9.44	0.00	9.44	1666
FPZ-04	M	0.28	81.9	0.35	7.25	0.33	5.91	96.0
FPZ-05	M	0.69	147	1.98	20.5	1.56	9.66	181
FPZ-06	M	1.06	132	1.97	16.7	1.79	9.10	163
SCPZ-01	F	0.62	1183	0.00	26.2	0.11	21.6	1231
SCPZ-02	F	2.08	637	3.03	38.0	1.92	16.6	699
SCSP-03	F	2.02	273	2.19	25.1	3.46	13.6	320
SCPZ-04	M	0.52	947	0.74	16.3	0.56	12.4	977
ILPZ-01	M	1.74	1172	2.32	53.6	2.13	11.6	1243
ILPZ-02	F	0.63	542	1.61	17.7	0.85	7.03	570
ILSP-03	M	1.53	89.6	1.89	11.9	0.99	6.45	112
ILPZ-04	F	0.00	377	1.97	27.8	2.31	29.4	438
ILPZ-05	M	1.78	625	2.56	22.2	1.18	11.5	664
2008 females		0.680 ± 0.255	480 ± 120	1.06 ± 0.340	20.0 ± 4.70	1.10 ± 0.35	13.0 ± 2.85	516 ± 125
2008 males		0.770 ± 0.220	505 ± 180	1.20 ± 0.315	17.0 ± 4.60	0.920 ± 0.20	8.60 ± 1.10	533 ± 183

[a] The mean log ± standard deviation of ∑*p,p*′-DDE concentrations for males and females were 2.14 ± 0.52 and 1.39 ± 0.93 µg/kg lipid in 2005, and 2.36 ± 0.65 and 2.55 ± 0.39 µg/kg lipid in 2008, respectively.

[b] The mean log ± standard deviation of ∑DDT concentrations for males and females were 2.25 ± 0.43 and 1.69 ± 0.60 µg/kg lipid in 2005, and 2.42 ± 0.62 and 2.58 ± 0.38 µg/kg lipid in 2008, respectively.

Regression analyses showed that there were no significant correlations between measured life history parameters and ∑DDT concentrations in male pups captured in 2005 and pups sampled in 2008 (regression analysis for all pup groups, Table 1; $p > 0.05$). In contrast, concentrations of ∑DDTs in female pups sampled in 2005 were negatively correlated with increasing length and weight ($p < 0.005$; Table 1; Fig. S1 in supplementary material). The low concentrations of DDT in the 2005 females can be explained due to the growth dilution effect since negative, significant correlation were observed between DDT concentration and body size in this particular group of pups (Table 1). Under the assumption of growth dilution, an apparent dilution on contaminant concentrations occurs in the body mass as a result of growth and possible shift to diet items containing lower levels of contaminants (Alava et al., 2009; Gobas and Arnot, 2010).

3.3. DDT contamination and patterns

Mean concentrations of ∑DDT and ∑*p,p*′-DDE ranged from 274 ± 233 to 533 ± 183 μg/kg lipid, and from 252 ± 221 to 505 ± 180 μg/kg lipid, respectively (Table 2). The range of concentrations for ∑DDT and ∑*p,p*′-DDE in sea lion pups were 16.0–3070, and 0.15–2900 μg/kg lipid, respectively. ∑DDT concentrations detected in pups sampled in 2005 were lower than the concentrations of ∑DDT measured in 2008, suggesting a possible temporal increase in DDT concentrations, as illustrated in Fig. 2. Male pups showed significantly higher concentrations of major DDT metabolites, *p,p*′-DDD (*t*-test, 2.92 $p = 0.0087$), *p,p*′-DDT (*t*-test, 2.45; $p = 0.0239$) and, *p,p*′-DDE (Welch *t*-test = 2.37, $p = 0.0286$), compared to females in 2005. The metabolite *p,p*′-DDE contributed the highest proportion (>90%) of ∑DDT compounds (Fig. 3). The second most dominant metabolite was *p,p*′-DDD, followed by *p,p*′-DDT.

The composition pattern of each DDT metabolite did not differ between males and females in 2005 (Welch two-tailed *t*-test for all comparisons, $p > 0.05$), or between males and females in 2008 (Welch two-tailed *t*-test for all comparisons, $p > 0.05$). However, significant differences were observed when comparing the temporal (2005 and 2008) composition of DDT metabolites among all groups of pups (Fig. 3). While the contribution of *p,p*′-DDE to the total of DDT compounds in the 2005 females was significantly lower to that observed in females sampled in 2008 (Barlett test, $p < 0.0001$; Welch ANOVA, $p = 0.0271$; Tukey–Kramer test, $p < 0.05$), the contributions of *o,p*-DDE and *o,p*-DDT were significantly higher in females sampled in 2005 compared to male and female pups sampled in 2008 (Barlett test, $p < 0.0001$; Welch ANOVA, $p = 0.0019$; Tukey–Kramer test, $p < 0.05$ for *o,p*-DDE; and, Barlett test, $p < 0.0001$; Welch ANOVA, $p = 0.0085$; Tukey–Kramer test, $p < 0.05$ for *o,p*-DDT). No significant differences in the composition pattern of *p,p*-DDD (ANOVA, $p = 0.2528$; Tukey–Kramer test, $p > 0.05$) and *p,p*-DDT (Barlett test, $p < 0.0001$, Welch ANOVA, $p = 0.2224$; Tukey–Kramer test, $p > 0.05$) were observed among pups. This indicates that male and female pups were exposed to DDT mixtures of similar composition in either 2005 or 2008, although temporal differences in composition pattern (e.g., *p,p*′-DDE) were detected possibly due to the historical or former use of DDT in the past or recent times.

3.4. Site differences of DDT concentrations

Inter-site comparisons showed that concentrations of ∑DDT detected in pups from Caamaño (Santa Cruz) exhibited the lowest levels and were significantly lower than ∑DDT concentrations measured in pups from Puerto Posada (Pinta), Punta Espinoza (Fernandina) and Plaza Sur (Santa Cruz) (Levene's test, $p = 0.0310$; Welch ANOVA, $p = 0.0238$; Tukey–Kramer test, $p < 0.05$), sampled in 2005; and, also significantly lower than those measured in pups from rookeries of San Cristobal Island (Isla Lobos and Puerto Baquerizo) and La Loberia (Floreana), when all sites, sampled in both 2005 and 2008, were compared (ANOVA, $p < 0.0001$; Tukey–Kramer test, $p < 0.05$) (Fig. 4). Concentrations of ∑DDTs in pups from Plaza Sur were also significantly lower than DDT concentrations in pups of Pinta Island in 2005 (Tukey–Kramer test, $p < 0.05$). ∑DDT concentrations in the four sites sampled in 2008 were not significantly different from each other (ANOVA, $p = 0.1357$; Tukey–Kramer test, $p > 0.05$). Pups with low age estimates from Pinta Island (pups PIP-02, male of 2 months and PIP-08, female of 3 months; Table 2), one of the most remote and uninhabited islands (Fig. 1), exhibited the highest concentrations of ∑DDTs compared to the rest of the samples. Although it cannot be ruled out that newborns and youngest pups of marine mammals can have low contaminant concentrations, concentrations of contaminants increase as newborns and pups nurse and absorb contaminant from lipid rich milk during lactation. This contaminant load is especially high for first born calves (Ylitalo et al., 2001; Hickie et al., 2007), which might be the case in the two pups from Pinta Island.

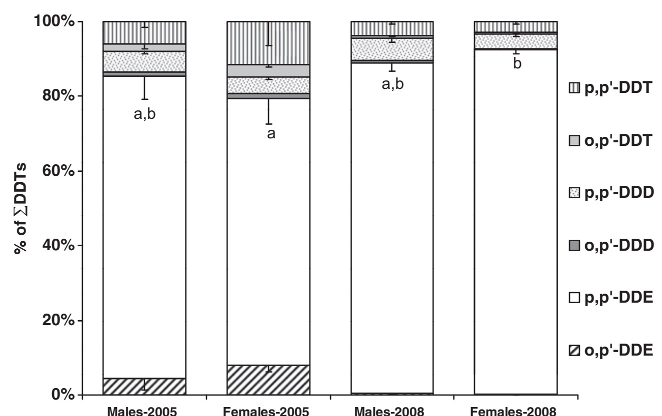

Fig. 3. Composition pattern of DDT metabolites (i.e., *o,p*-DDE, *p,p*-DDE, *o,p*-DDD, *p,p*-DDD, *o,p*-DDT, and *p,p*-DDT) in males and females of Galapagos sea lion pups (*Zalophus wollebeaki*). Error bars are standard errors.

Gender and size of pups (e.g., females from Caamaño sampled in 2005), and sample size as well as inter-island sea lion movements (i.e., home range) and foraging trips (feeding areas) of Galapagos sea lion adult females might partly explain the spatial differences in DDT contamination of Galapagos sea lions. A recent study confirmed that adult females undertake trips to the sea to forage and spend a significant proportion of time on islands (i.e., multiple haul-out sites) other than their breeding colonies (Villegas-Amtmann et al., 2008; Villegas-Amtmann and Costa, 2010). Proximity to populated urban areas in some islands (e.g., Santa Cruz, San Cristobal and Floreana) seems not to influence or elevate the concentration of DDT as the pups sampled from rookeries close to human centers exhibited either lower or similar levels compared to those existing on more remote islands (e.g., Pinta and Fernandina; Figs. 1 and 4).

3.5. Global comparison

∑DDT concentrations in Galapagos sea lion pups are lower than those detected in pinnipeds from the Northern Hemisphere (Kajiwara et al., 2001; Kannan et al., 2004; Debier et al., 2005; Del Toro et al., 2006; Blasius and Goodmanlowe, 2008; Mos et al., 2010), but greater than those detected recently in adult subdominant males, adult females, juveniles and pups of southern elephant seals (*Mirounga leonina*) from Elephant Island, Antarctica (Miranda-Filho

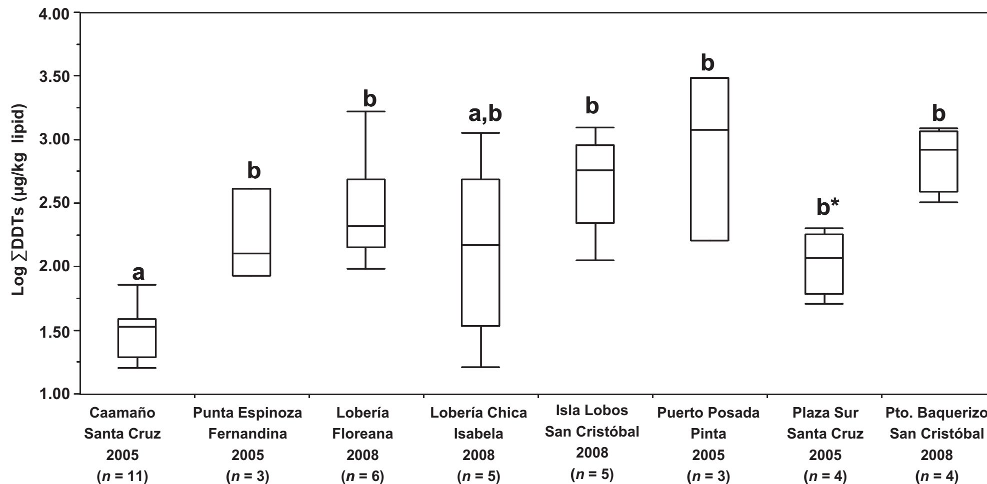

Fig. 4. Inter-site comparisons showing box plots of log DDT concentrations among rookeries of Galapagos sea lion pups and sampling year**.** The internal line across the middle of the box identifies the median sample values; the ends of the box are the 25% and 75% quartiles; and the whisker bars are the minimum and maximum values. Concentrations in rookeries not connected by the same letter are significant different. An asterisk right after the letter indicates that the concentration was also significantly different from the preceding box plot. When congeners were undetectable, half of the method detection limit was assigned in samples.

et al., 2007) (Table 3; Fig 5). Interestingly, Galapagos sea lion pups exhibited ∑DDT concentrations similar to those detected in juveniles of Hawaiian monk seals (*Monachus schauinslandi*) from several subpopulations in the Northwestern Hawaiian Islands (Ylitalo et al., 2008). The maximum concentrations (i.e., 1000–3000 µg/kg lipid) observed in our study pups are similar to DDT concentrations observed in adult individuals of California sea lions (*Z. californianus*) from Baja California, Mexico, (Del Toro et al., 2006), but lower than those found in California sea lions from the coast of California, USA (Fig. 5).

The DDT concentrations measured in some of the animals (for example, pups from Pinta Island; mean = 1490 µg/kg lipid, ranging 177–3097 µg/kg lipid) are comparable or higher to the DDT levels detected in adult male spinner dolphins (*Stenella longirostris*; 2553 µg/kg lipid) from the Eastern Tropical Pacific (Prudente et al., 1997), captured northwest of the Galapagos Archipelago, and in Amazonian River dolphins (*Inia geoffrensis*; 1624 µg/kg lipid) from the Brazilian Amazon, where DDT has been sprayed (Torres et al., 2009). These observations might indicate a resident "background" DDT contamination of the Eastern Tropical Pacific Ocean and the Americas region.

The apparent increase of DDT levels from 2005 to 2008 in remote Galapagos sea lions is not an isolated event since concentrations of DDT in Adélie penguins (*Pygoscelis adeliae*) from remote areas of the western Antarctic Peninsula have not decreased between 2004 and 2006 (Geisz et al., 2008). Likewise, concentrations of DDT in human breast milk from Japan have not decreased since 1998 (Kunisue et al., 2006).

3.6. DDT health effects assessment

Marine mammals are at a particular risk of endocrine disruption and reduced immune function due to their high trophic position in the food-chain and long lifespan (e.g., Ross et al., 2000; Ross, 2006; Mos et al., 2010). Experimental studies using in vitro tests and laboratory animals have demonstrated estrogenic and anti-androgenic effects of DDT metabolites (Kelce et al., 1995; Andersen et al., 1999; Freyberger and Ahr, 2004). For example, transcriptional activity of androgen receptors in mammalian cell cultures is inhibited at *p,p′*-DDE concentrations of 64 µg/kg wet weight (Kelce et al., 1995). Also, *p,p′*-DDE concentrations ranging between 13 and 536 µg/kg wet weight have been associated with decreased proliferative responses of lymphocytes in free ranging bottlenose dolphins (Lahvis et al., 1995) and splenocytes in beluga whales (De Guise et al., 1998). The risk characterization showed that while >99% of the concentrations were below the *p,p′*-DDE anti-androgenic effect reference value in pup sampled in 2005, the *p,p′*-DDE concentrations in 2% of females and 3% of males were above the minimum *p,p′*-DDE immunotoxic effect concentration in bottlenose dolphins (Fig. 6A). In 2008, 8% of males and 9% of females exceeded the minimum *p,p′*-DDE immunotoxic effect threshold, while close to 100% of females are below the *p,p′*-DDE anti-androgenic reference value; however, 1% of the males surpass the *p,p′*-DDE anti-androgenic effect (Fig. 6B). This indicates that 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. Other pollutants with a similar mode of toxicity such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ether (PBDEs) flame retardants, which were also detected in these animals (Alava et al., 2009), can further elevate the immune and endocrine response. A compromised immune and endocrine system affects the ability of animals to combat disease and to successfully reproduce.

Since our study animals comprised only pups aged 2–12 months, our risk categorization here may be considered as a conservative estimate at the population level. Adult male Galapagos sea lions can be expected to have DDT concentrations that are higher than those in pups as DDTs accumulate throughout the animal's life (Addison and Smith, 1974; Addison and Brodie, 1987; Ross et al., 2000).

The 50% decline in the Galapagos sea lion population between the 1970s and 2001 continues to raise questions about underlying causes. While malnutrition and starvation associated with the El Nino events of 1982–1983 and 1997–1998 can cause large-scale populations declines, DDT metabolites can contribute to population level declines through immunotoxicity and developmental impacts of nutritionally stressed animals (Alava and Salazar, 2006). A return to heavy reliance on DDT may represent a significant long-term health risk for Galapagos sea lions.

3.7. Regional vs global transport of DDT

DDT in the Galapagos sea lion pups likely originate from continental sources since there are no historical records indicating the use of DDT in the Galapagos. DDT was never imported to the islands (Dr. H. Jurado, Servicio Nacional de Erradicacion de la Malaria

Table 3
Global comparisons of mean concentrations (mg/kg lipid) of ∑DDT in muscle-blubber of pinniped species.

Species	Stage/sex	∑DDT	Reference
Location and year of collection			
Zalophus californianus	Adult female and male	1450	Le Boeuf and Bonell (1971)
Coastal California, USA, 1970			
Z. californianus	Full term parturient female	120	Delong et al. (1973)
San Miguel Island, California, USA, 1970	Premature term parturient female	980	
Z. californianus	Adult male	830	Kajiwara et al. (2001)
Coastal California, USA, 1991–1997	Adult female	110	
	Subadult male	870	
Mirounga angustirostris	Yearling male	9	Kajiwara et al. (2001)
Coastal California, USA, 1991–1997	Yearling female	62	
Z. californianus	Adult male	140	Kannan et al. (2004)
North, Central and South California Coast, USA, 2000	Adult female	283	
	Subadult male	63	
Z. californianus[a]	Juvenile (e.g., yearlings)	28	Debier et al. (2005)
Año Nuevo, Central California, USA, 2002			
Z. californianus	Stranded adult male	380	Ylitalo et al. (2005)
Central California Coast, USA, 1993–2003	Stranded adult female	250	Ylitalo et al. (2005)
Z. californianus	Stranded adult and subadult male	4	Del Toro et al. (2006)
Baja California, Mexico, 2000–2001			
Z. californianus[b]	Pup	2500	Blasius and Goodmanlowe (2008)
Southern California Bight, USA, 1994–1996			
Phoca vitulina	Pup	1940	Blasius and Goodmanlowe (2008)
Southern California Bight, USA, 1994–1996			
Mirounga angustirostris	Pup	77	Blasius and Goodmanlowe (2008)
Southern California Bight, USA, 1994–1996			
Phoca vitulina	Pup	1.0	Mos et al. (2010)
Norteastern Pacific Ocean: British Columbia, Canada, and Washington State, USA, 1996–1997			
Mirounga leonina	Adult male	0.20	Miranda-Filho et al. (2007)
Shetland Islands, Elephant Island, Antarctica, 1997–2000	Adult female	0.20	
	Juvenile	0.10	
	Pup	0.10	
Monachus schauinslandi[c]	Juvenile	0.56–0.90	Ylitalo et al. (2008)
Hawaiian Islands: French Frigate Shoals, Laysan Island and Midway Atoll, 1997–2002			
Zalophus wollebaeki (this study)	Pup	0.28	Present study
Galapagos Islands, Ecuador, 2005			
Zalophus wollebaeki (this study)	Pup	0.53	Present study
Galapagos Islands, Ecuador, 2008			

[a] Concentrations detected in the serum of juvenile California sea lions.
[b] Mean concentrations for pups of the three pinniped species from the Southern California Bight (CA, USA) were calculated as the sum of the mean concentrations reported for pup males and females and divided by the total number of pups; see Table 2 in Blasius and Goodmanlowe (2008).
[c] Range of means concentrations of *p,p′*-DDE for Hawaiian monk seals; see Table 1 in Ylitalo et al. (2008).

(SNEM)-National Malaria Eradication Service Center of Ecuador, pers. comm.). This is supported by the fact that malaria and its mosquito vector (*Anopheles* sp.) have never been found in the Galapagos, although historical, anecdotic communications suggest that DDT was used in huge amounts by military personnel from the US Navy (former American Air Force and Naval Base in Baltra, Santa Cruz Island, used during the second World War) to eliminate introduced rats as invasive species in human housing from urbanized areas and into the islands between 1940s and 1950s in the last century (M.P. Harris, Centre for Ecology and Hydrology, Banchory Research Station, Banchory, UK, pers. comm.; M. Cruz, GGEPL-Galapagos National Park, pers. comm.). In continental Ecuador, DDT was applied inside homes (intra-domestic applications) and in agriculture between 1957 and 1999 to control malaria and crop pests (Ministerio del Ambiente, 2004). The national inventory of organochlorine pesticide use in continental Ecuador reported that approximately 134,000 kg/year DDT was used in 1993. DDT use then dropped to approximately 1400 kg/year in 1998 (Fig. S2 in supplementary material). Ecuador stopped importing DDT in 1994. At present, a stock of 1636 kg of DDT is available for emergency malaria control (Ministerio del Ambiente, 2004, 2006).

The high ratio *p,p′*-DDE/∑DDT (0.91–0.94) suggests a scenario of past DDT contamination and insignificant contributions from recent or fresh DDT sources. The use of DDE/DDT ratio to estimate the time of exposure is based on the assumption that DDT is being metabolized to DDE over time. A higher ratio implies a longer period of reaction and hence more DDE in relation to the amount of DDT. However, it must be emphasized that biota and in particular marine mammals are able to metabolize DDT to *p,p′*-DDE (Jensen and Jansson, 1976; Letcher et al., 1995), which may also explain the high proportion of *p,p′*-DDE detected in Galapagos sea lion pups. The concentration ratio is similar to that found (0.93) in southern elephant seals of Antarctica (Miranda-Filho et al., 2007). In comparison, *p,p′*-DDE/∑DDT concentration ratios measured in sediment and aquatic organisms of the Taura River in Continental Ecuador are 0.66 in sediments and 0.14 in fish (Montaño and Resabala, 2005), and indicate a more recent DDT contamination and a potential regional source of DDT contamination. Although linking the use of DDT in Ecuador and other Central and South American countries to the concentrations detected in the Galapagos sea lion pups is difficult, it is not unrealistic to assume that DDT use in continental Ecuador contributes to current concentrations of DDT in

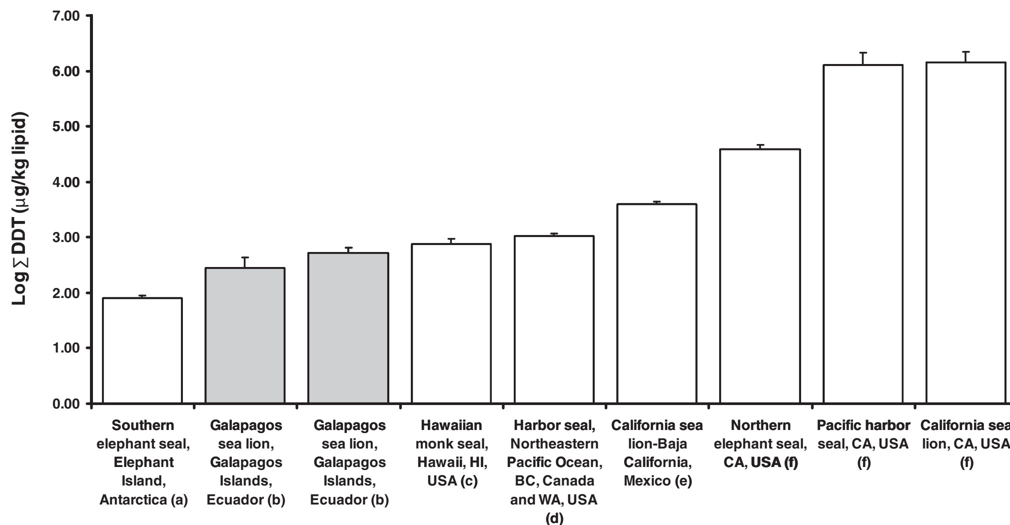

Fig. 5. Global comparisons of log ∑DDT mean concentrations (µg/kg lipid) among pinniped species from the Pacific and Antarctica: (a) Miranda-Filho et al. (2007); (b) Present study (2005 and 2008 samplings, respectively); (c) Ylitalo et al. (2008); (d) Mos et al. (2010); (e) Del Toro et al. (2006); (f) Blasius and Goodmanlowe (2008). Except for California sea lions from Baja California (Mexico), used here as reference, all the individuals are pups. Error bars are standard errors (SE).

Galapagos sea lions. Recent estimates of annual DDT emissions from 1940 to 2005 (Schenker et al., 2008) indicate that the major use of DDT on the latitudinal band between 6°N and 6°S, encompassing part of the tropics and the equator (i.e., latitude 0°), took place from 1945 and 1965, as shown by the steep increase of DDT emissions (Fig. S3 in supplementary material). Annual DDT emissions have since decreased slowly from 1965 to 2005 in this latitudinal zone, with a reduction of approximately 94% (Fig. S3).

In the mid 1970s, Goldberg (1975) described a global fractionation process, commonly known as "the Grasshopper Effect", to illustrate the atmospheric transfer of DDT from continents to oceans (i.e., global distillation), which has been recently confirmed (Guglielmo et al., 2009). While substantial work has been carried out on the fate and behaviour of POPs and their atmospheric transport into the polar regions, very little has been conducted to investigate equatorial deposition of DDT from high-use regions. Despite the fact that the Galapagos are located 1000 km from continental Ecuador or more than 3000 km from legacy DDT hot spots in California, it cannot be ruled out that this mechanism might be playing a role in DDT transport to and contamination in the Galapagos.

The regional atmospheric-oceanic system, including the confluence of the NE and SE trade winds (i.e., the Inter-Tropical Converge Zone-ITCZ), winds from the west and oceanographic currents (i.e., Panama and Humboldt currents, and the Equatorial undercurrent or Cromwell current coming from the west) may contribute to the distribution of these contaminants in this particular region of the Southeastern Pacific Ocean. DDT in Galapagos might also originate from tropical countries in Asia by means of trans-Pacific air pollution (Wilkening et al., 2000). This is supported by the fact that tropical Asia is a significant global emission source of contaminants, including the long-range atmospheric transport of POPs (Iwata et al., 1993).

Recent modeling work reports that residence times and proportions of the total global masses of DDT are 10–15 days and 2% in the atmosphere, and 1.2 years and 26% in the global ocean with 30% of the DDT mass bounded to the organic matter phase in the equatorial Pacific Ocean, where high primary productivity is found due to existence of wind driven upwelling delivering nutrient enriched waters (Guglielmo et al., 2009), as those found in Galapagos waters (Alava, 2009). These observations portray that the physical–chemical properties of DDT, oceanographic conditions and atmospheric inputs are the driven forces explaining the presence of DDT in the islands.

3.8. Management implications

Since the ratification of the Convention by Ecuador in 2004, the National Plan for the Inventory of POPs and Management was undertaken (Ministerio del Ambiente, 2004, 2006). 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 are made between the conservation of valued, sensitive wildlife (i.e., Galapagos sea lions) and public health objectives to control malaria. The toxicological paradigm that the "dose makes the poison" provides a theoretical foundation for an approach that minimizing ecological damage while optimizing human health benefits. However, the application of this approach requires rigorous control of DDT use and emissions while continuously monitoring the concentrations and ecological effects of DDT in wildlife. Programs for monitoring DDT emissions and ecological impacts in tropical areas do not exist at this time, but will be instrumental to achieving human health and environmental objectives.

DDT may become a significant factor shaping the evolutionary processes that are so keenly studied in the Galapagos Islands. While we recognize that our study is limited in scope, due to the highly protective measures in place on the Galapagos Islands and the difficult sampling and analysis protocols, it provides a unique and timely warning signal to the dangers of an increased reliance of DDT for malaria control in tropical countries. The results from this study may help to provide preliminary guidance on the relationship between DDT use and ecological impacts and serve as a reference point against which possible future impact of tropical DDT use can be measured.

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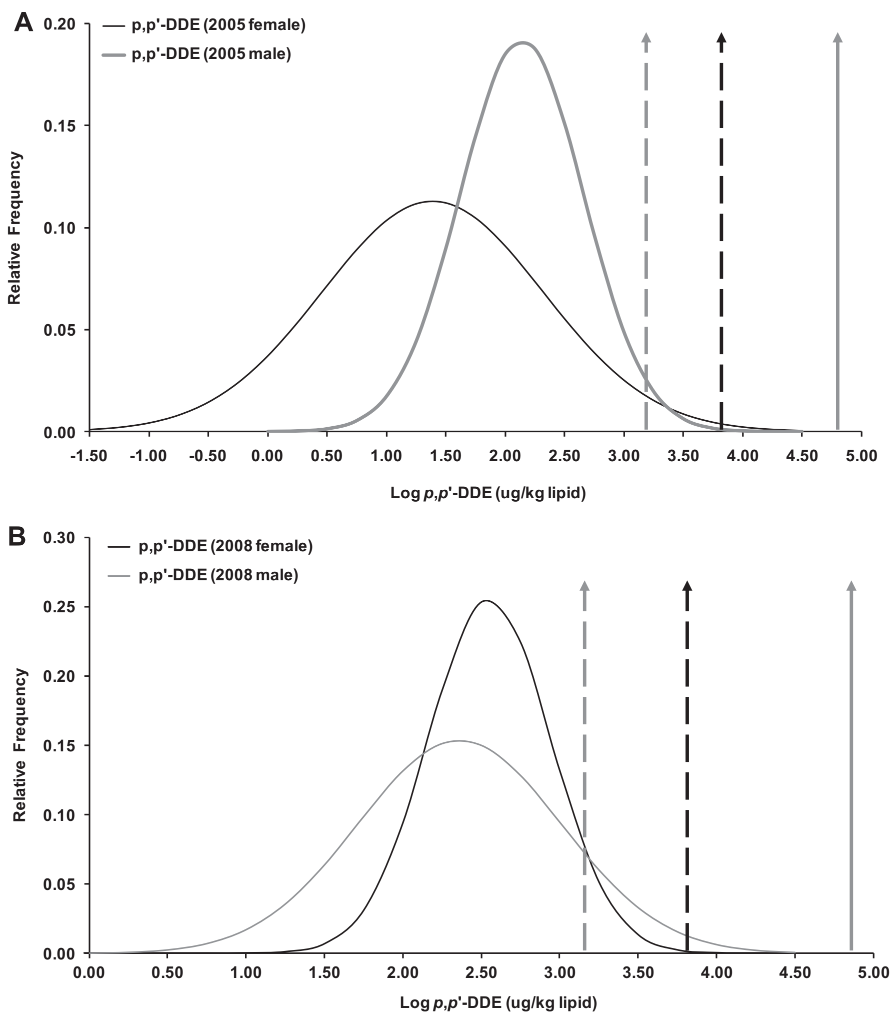

Fig. 6. Normal probability density distributions of *p,p′*-DDE concentrations (i.e., cumulative frequency) of log-transformed *p,p*-DDE concentrations (μg/kg lipid) in biopsy samples of Galapagos sea lion pups sampled in 2005 (A) and 2008 (B) shown in relation to the *p,p*-DDE anti-androgenic effect concentration 64 μg/kg wet weight (Kelce et al., 1995) in mammalian species, equivalent to 6890 μg/kg lipid and represented by the black dashed arrow; and, the range of *p,p*-DDE concentrations (13–536 μg/kg wet weight) associated with a decreased lymphocyte proliferation response in bottlenose dolphins (Lahvis et al., 1995), equivalent to 1430 μg/kg lipid (minimum concentration represented by grey dashed arrow) and 58,900 μg/kg lipid (maximum concentration represented by the solid grey arrow). (A) The cumulative distribution of *p,p′*-DDE concentrations is shown by the grey solid curve in males and by the black solid curve in females in 2005; and, (B) the cumulative distributions of *p,p′*-DDE concentrations is shown by the grey solid curve in males and by the black solid curve in females in 2008.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2011.01.032.

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