PRELIMINARY RESULTS ON ECOTOXICOLOGICAL INVESTIGATION ON GRAY WHALES (*Eschrichtius robustus*) IN THE SAN IGNACIO LAGOON (MEXICO)

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ABSTRACT

The main objective of this pilot project was to investigate for the first time the ecotoxicological status of gray whale (Eschrichtius robustus) in the San Ignacio Lagoon (Mexico). The differences in CYP1A1 and CYP2B expression, and contaminant levels (OCs) were investigated in two different period of the breeding season and between mothers and calfs using skin biopsy as diagnostic tool. The DDTs level were slightly higher in calf blubber in comparison to the mother, despite the PCB concentration are similar, suggesting the maternal transfer of POPs trough lactation. In conclusion these preliminary data point out that there is an accumulation of chlorinated POPs in gray whale calves resulting from the lactational transfer of these compounds from their mothers. Exposure to POPs (such as DDTs) at early life stages may have toxic impacts on their developing endocrine, immune and neural systems. However, the impact of these contaminants on health and development of gray whale calves is currently unknown.

KEYWORDS

Eschrichtius robustus, skin-biopsy, OCs, Biomarkers, CYP1A1, CYP2B, San Ignacio Lagoon (Mexico).

INTRODUCTION

The Eastern North Pacific gray whale population (*Eschrichtius robustus*) has been the focus of ongoing research and population monitoring. It is an example of a population of large whales that has successfully recovered from over-exploitation (Jones and Swartz, 2002). This population may be approaching, or possibly already exceeding its carrying capacity level (Moore, *et al.* 2001). Recent range-wide increases in mortality of all age and sex classes suggest the population may have become food limited (Le Boeuf *et al.* 2000). Research on the breeding biology and phenology of gray whales has contributed and continues to contribute valuable information on the importance of their coastal lagoon habitats to their reproductive success.

In this paper we present the preliminary results on contaminant levels (Organochlorine Compounds - OCs,) and cytochrome P450 1A1 (CYP1A1) and cytochrome P450 2B (CYP2B) expression on 21 specimens of *Eschrichtius robustus* collected in the San Ignacio Lagoon (Mexico) in two different period of the breeding season in the 2012. Six couples of mother and calf were also investigated in order to explore the potential transfer of POPs during the lactation.

The protein level of CYP1A1 and CYP2B, involved in responses to different endogenous and environmental stress, are used in this project as a potential indicator of a broad spectrum of toxicological health status of the species in different phase of the breeding season in the San Ignacio calving-breeding lagoons. Cytochrome P450 1A is a member of the superfamily of enzymes involved in Phase I oxidative metabolism of exogenous compounds, playing a key role in biotransformation of contaminants like dioxins, furans, PCBs and PAHs. Induction of CYP1A is mediated by the aryl hydrocarbon receptor (AHR) pathway which is activated by PAHs and planar halogenated compounds (PHAHs); CYP1A and AHR are therefore widely used as

biomarker of exposure to these compounds, also in marine mammals (Fossi *et al.,* 2006; Hirakawa *et al.*, 2007; Godard *et al.*, 2004; Montie *et al.*, 2008; Niimi *et al.*, 2005; Wilson *et al.*, 2007; Fossi *et al.*, 2010).

METHODS

Study site

Laguna San Ignacio (LSI) is one of the three primary calving-breeding lagoons and winter aggregation areas of the Eastern North Pacific gray whale (*Eschrichtius robustus*) along the Pacific coast of Baja California Sur, Mexico. The lagoon is located in the west coast of the Baja California Peninsula (Fig. 1), and lies within the El Vizcaíno Biosphere Reserve.

Sampling

Integument biopsies (epidermis, dermis and blubber) were collected from 33 free-ranging *Eschrichtius robustus* during the winter 2012 using biopsy darts launched with a crossbow (CITES Nat. IT 025IS, Int. CITES IT 007). 13 specimens (12 female and 1 male) were sampled in January 2012 and 19 specimens 9 calf (6 female and 3 male) and 10 mothers were sampled in March 2012. Sex was determined according to Bérubé and Palsbøll (1996). A total of 21 specimens were analyzed for contaminant levels (OCs,) and cytochrome P450 1A1 and cytochrome P450 2B in this preliminary work. Each gray whale was included or identified in a photo ID catalogue.

Contaminants analysis

Organochlorine Compounds (OCs) - The analytical method used for quantitative and qualitative analysis of HCB, DDTs and PCBs was High Resolution Capillary Gas chromatograph equipped with an electron capture detector (63Ni ECD)(AGILENT 6890/N), according to the U.S. Environmental Protection Agency (EPA) 8081/8082, modified by us (Marsili and Focardi, 1996). The gas chromatograph had a SPB-5 bonded phase in a 30 m long fused silica capillary column.

Biomarkers analysis

CYP1A1 and CYP2B western blot

CYP1A and ***CYP2B*** have been detected in cetacean skin biopsy (Fossi *et al.*, 2006; Fossi *et al.*, 2008). For WB analysis, S9 fractions of tissue homogenates (in duplicate for each sample) were separated by SDS-PAGE (10% polyacrylamide gels – Criterion XT Precast Gel - BioRad) and blotted onto nitrocellulose sheets for 1 hour at the constant voltage of 200 V. The membranes were saturated by incubating them with a blocking solution (3% gelatin dissolved in Tris Buffered Saline containing 0.05% Tween-20, TTBS) for 1 hour at room temperature. Primary polyclonal rabbit antibodies from Oxford Biochemical Research were used (Oxford MI, USA). Goat anti-rabbit CYP1A1 and anti CYP2B4, diluted 1:5000 and 1:1000, respectively, in TTBS-1% gelatin, were incubated overnight at room temperature with cetacean proteins. Incubation with anti-rabbit HRP-labelled secondary antibody (1:3000 final dilution) was performed for 1.5 hours at room temperature and protein detection was done according to the BioRad Immun-Star HRP Chemiluminescent Kit booklet, using standardized times. Semi-quantitative analysis was performed for each WB (in triplicate) with Quantity One software (BioRad, 1-D Analysis Software) using the methods proposed by Fossi *et al.* (2008).

RESULTS AND DISCUSSION

The main objective of this pjlot project was to investigate for the first time the ecotoxicological status of gray whale (*Eschrichtius robustus*) in the San Ignacio Lagoon (Mexico). The differences in CYP1A1 and CYP2B expression, and contaminant levels (OCs) were investigated in two different periods of the breeding season (January 2012 and March 2012) and between mothers and calves using skin biopsy as diagnostic tools.

Seasonl variations of OCs and cytochrome P450 levels during the permanence in calving-breeding lagoons - In Fig 2 we show the results of OCs (PCBs and DDTs), cytochrome P450 1A1 and cytochrome P450 2B of 7 free-ranging *E. robustus* (females) sampled during the January 2012 compared with 7

(females) sampled in March 2012 during the permanence in calving-breeding lagoons. A slight increasing on DDTs level were detected in the blubber of the females analyzed at the end of the breeding season, on the contrary the PCBs concentration are similar. The body mass, overall fat content, girth and blubber thickness of the gray whales are significantly higher during the southbound migration to their breeding grounds than during the permanence in calving-breeding lagoons and the return northbound migration. The use of the blubber reserve during the pregnacy and lactation can generete the concentration of POPs in the analyzed tissues despite the evident excration during the lactation period (see Fig 3C). Gray whale levels of OCs in blubber, also compared the exiting data published by Tilbury et al (2002), are lower than in other mysticete species.
On the opposite, high level of CYP1A were detected in the speciments sampled during January 2012 compared with females sampled after the permanence in calving-breeding lagoons, suggesting the high metabolization of CYP1A inducers (such as POPs and PAHs) during the migratory route.

Transfer of OCs between mother and calf and cytochrome P450 levels - In Fig 3 we show the results of OCs (PCBs and DDTs), cytochrome P450 1A1 and cytochrome P450 2B of 6 calves and mothers of *E. robustus* sampled in March 2012. The DDTs level were slightly higher in calf blubber in comparison to the mother, despite the PCB concentration are similar. The use of the blubber reserve during the lactation can generete the increasing concentration of POPs in the calf despite the evident excration of liphofilic contaminant during the lactation activities. Mothers show higher levels of CYP1A and CYP2B in comparison to the the calves.

CONCLUSIONS

These preliminary data show that:
a) the gray whale levels of OCs in blubber are lower than in other mysticete species;
b) a slight increasing on DDTs level were detected in the blubber of the females analyzed at the end of the breeding season, despite the PCBs concentrations are similar in the two periods;
c) the use of the blubber reserve during the lactation can generete the increasing concentration of POPs in the mother despite the evident excration of liphofilic contaminant during the lactation activities;
d) the DDTs level were slightly higher in calf blubber in comparison to the mother, despite the PCB concentration are similar, suggesting the maternal transfer of POPs trough lactation;
d) the protein level of CYP1A1, involved in responses to different endogenous and environmental stress, is suggested in this project as a potential indicator a broad spectrum of toxicological health status of the species in different phase of the breeding season in the San Ignacio lagoon.
In conclusion these preliminary data point out that there is an accumulation of chlorinated POPs in gray whale calves resulting from the lactational transfer of these compounds from their mothers. Exposure to POPs (such as DDTs) at early life stages may have toxic impacts on their developing endocrine, immune and neural systems. However, the impact of these contaminants on health and development of gray whale calves is currently unknown.

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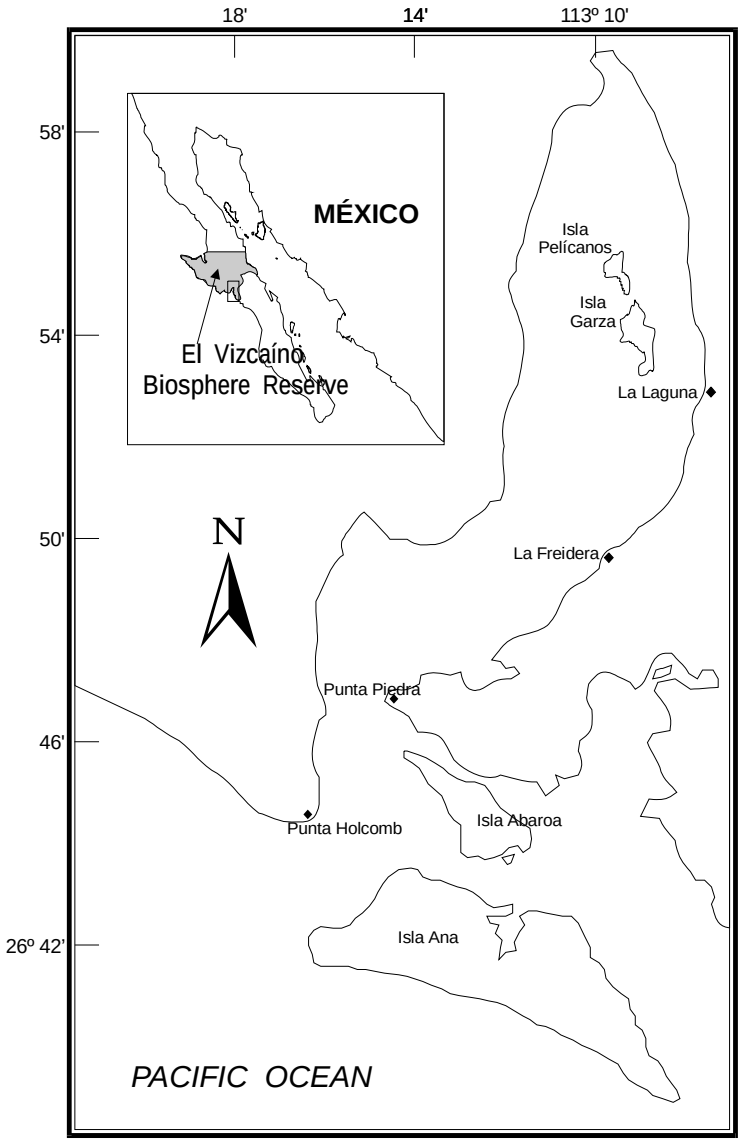

Figure 1 – Sampling area. Laguna San Ignacio (LSI) is located in the west coast of the Baja California Peninsula and lies within the El Vizcaíno Biosphere Reserve (Mexico).

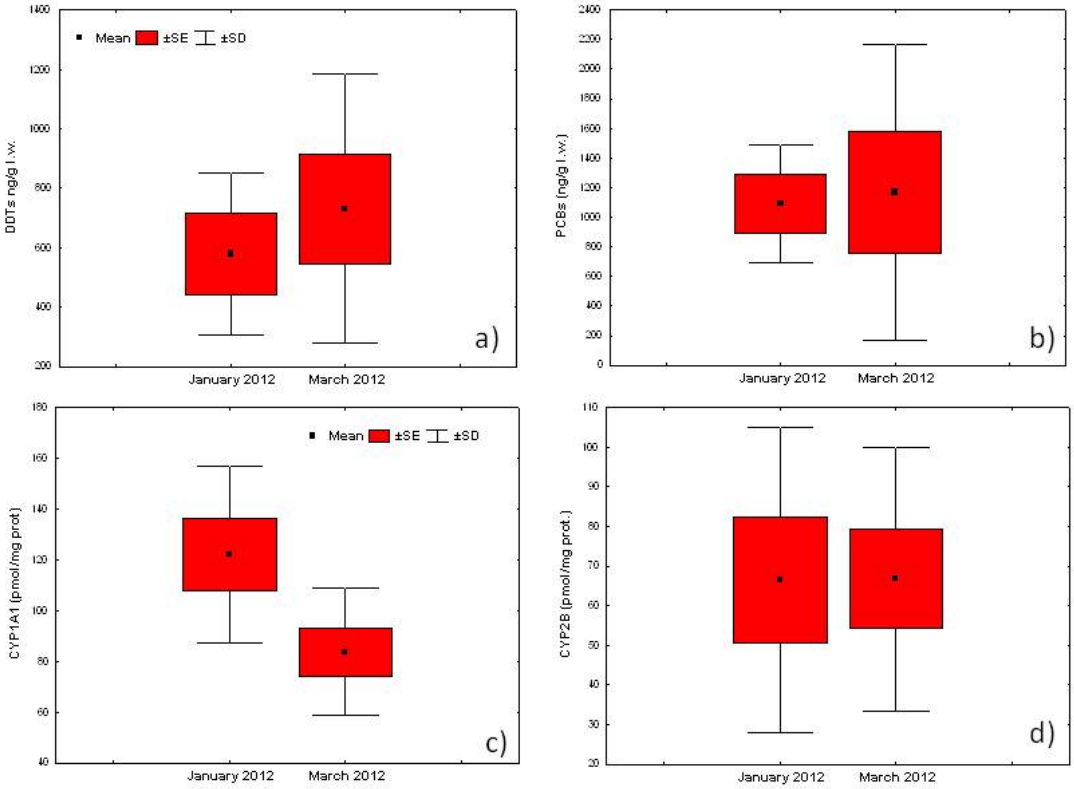

Figure 2 - PCBs levels (a), DDTs levels (b), CYP1A1 (c) and CYP2B (d) in skin biopsy of 7 free-ranging *Eschrichtius robustus* (female) sampled during the January 2012 compared with 7 (female) sampled in March 2012 during the permanence in calving-breeding lagoons (Laguna San Ignacio – Mexico).

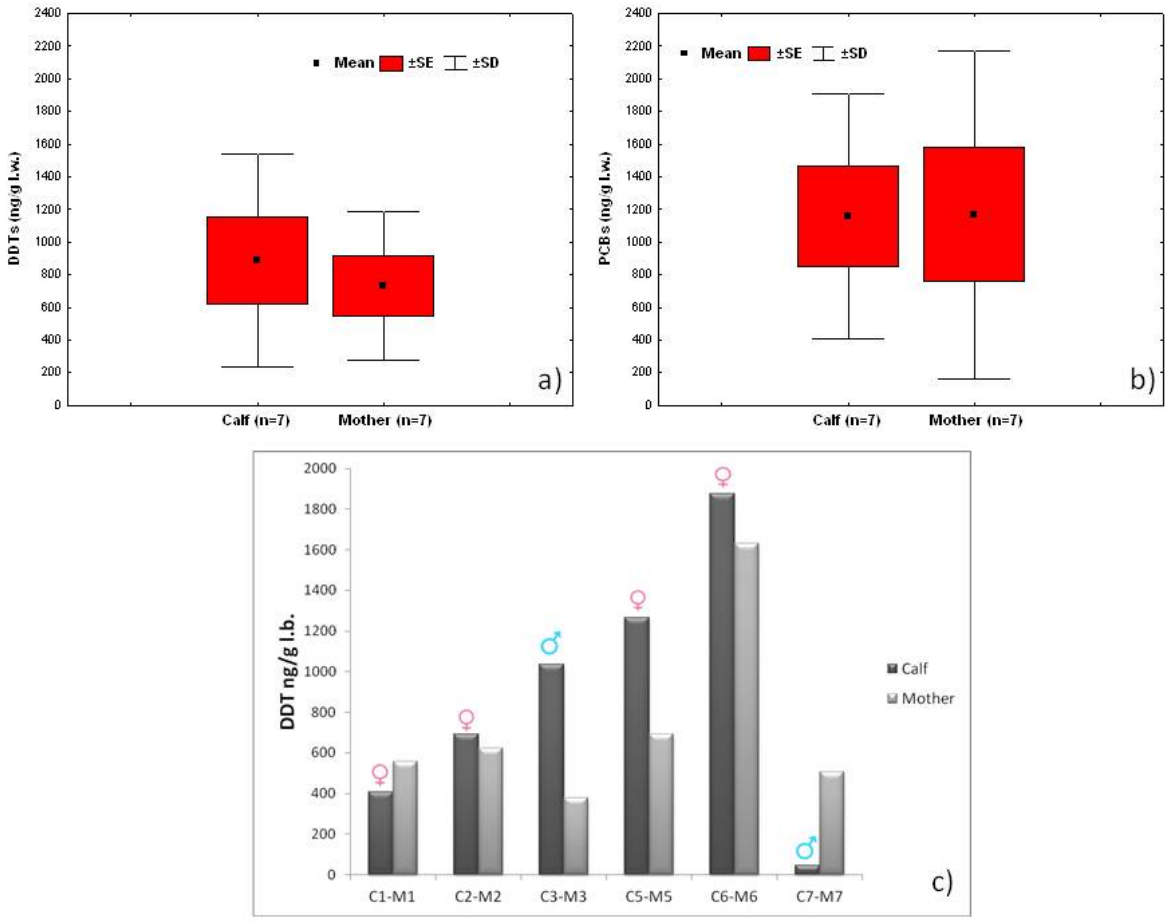

Figure 3 - PCBs levels (a), DDTs levels (b) in skin biopsy of 7 calves and mothers of *Eschrichtius robustus* sampled in March 2012 (Laguna San Ignacio - Mexico). C) details of DDTs differences between calves (c) and mothers (m).