PRELIMINARY RESULTS OF ANTHROPOGENIC CONTAMINANTS IN QUEENSLAND'S COASTAL DOLPHINS: LEVELS AND TOXICOLOGICAL EFFECTS

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ABSTRACT

In this study we used biopsy samples to assess contaminants levels (OCs and PAHs) and biomarker responses (CYP1A1-CYP2B) in three Australian inshore dolphin species: Australian snubfin (*Orcaella heinsohni*), Indo-Pacific humpback (*Sousa chinensis)* and bottlenose dolphins (*Tursiops* spp.*)*. Preliminary results showed a significance geographical difference in the induction of cytochrome CYP1A1 and CYP2B when samples from the *Tursiops truncatus* from Mediterranean seas were compared to samples from Australia regardless the species. Significantly higher levels of PAHs were found in samples from Queensland (QLD) compared to samples from the Mediterranean Sea (MS). On contrary OCs levels were generally smaller, but no significant difference in PCBs was found among QLD and MS samples.

KEYWORDS

HUMPBACK DOLPHINS, AUSTRALIAN SNUBFIN DOLPHINS, BOTLLENOSE DOLPHINS, COASTAL POLLUTIONS.

INTRODUCTION

In Australia inshore dolphins due to their restricted coastal distribution in close proximity with detrimental human activities are particularly subject to coastal pollution. As such, there is increasing scientific concern about threats to cetaceans occasioned by multiple stress factors due to bioaccumulation and effects of anthropogenic contaminants. Inshore dolphins are among the most threatened species from coastal pollution. In this study we will use biopsy samples to assess contaminants (OCs, PAHs and heavy metals) levels and effects in Australian snubfin (*Orcaella heinsohni*), Indo-Pacific humpback (*Sousa chinensis)* and bottlenose dolphins (*Tursiops aduncus.).*

In Australia a study conducted on a limited number of samples detected a range of organohalogenated pollutants in the blubber of four bottlenose dolphins (*Tursiops* spp.), one common dolphin (*Delphinus* spp.), and also from seven dugongs from north-east Queensland (Vetter et al. 2001). In three species of dolphins from South Australia (*T. aduncus*, *T. truncatus* and *D. delphis*), high to moderate Cd, Hg and Se concentrations were recorded in comparison with similar species elsewhere, including the more contaminated regions in the Northern Hemisphere (Lavery 2008). In the same study, differences in the concentration of metals and selenium were recorded between inshore and offshore species, with higher concentrations of Cd, Pb, Hg, Se, Zn recorded in inshore bottlenose dolphins (Lavery 2008).

The recovery in Central Queensland of several carcasses of inshore dolphin species, for which the cause of death could not be determined (Queensland marine wildlife stranding and mortality database 1996-2008), has raised concerns regarding the potential impact that contaminants are having on the health of coastal dolphins. In Central Queensland the input of agricultural and urban-sourced pollutants has been identified as a major threat to the coastal water quality in the region (Haynes & Michalek-Wagner 2000). The coastal environment of Central Queensland receives pollutants into the marine habitat from a variety of sources spread across these regions. These include air and water emissions from several industrial sources, shipping and handling, coal stockpiles, power station corrosion products, leachate from landfill, urban development, sewage treatment, historical copper mining and oil shale exploration. Recent surveys of pollutant concentrations in Great Barrier Reef habitats have indicated that nearshore marine sediments contain a range of organochlorine pollutants including DDT and its breakdown products, as well as dieldrin and lindane and PCDD/Fs (Haynes et al., 2000). In Queensland, elevated concentrations of a number of heavy metals are also present in coastal sediments and fish species (Moss and Costanzo, 1998; Reichelt-Brushett and Harrison 1999, 2004).

Despite concerns on the effects of pollutants on the health of coastal dolphins along the Queensland coast no studies have been done to determine and quantify the impact of coastal pollution on species likely to be affected: Australian snubfin (*Orcaella heinsohni*), humpback dolphins (*Sousa chinensis*) and bottlenose dolphins (*Tursiops* spp.). The aim of this study is to determine the concentrations and potential effects of anthropogenic contaminants in Australian snubfin, Indo-Pacific humpback using new analytical techniques.

MATERIAL AND METHODS

Sampling.

Sampling was planned in three different areas selected based on the different levels of coastal human development and for the presence of resident dolphins populations: Port Curtis (the major industrial port in QLD and second in Australia), Fitzroy River (primarily used for agricultural and grazing activities) and Port Clinton (no human presence) (Fig.1). At present for the purpose of this study biopsy samples have been collected only from the Fitzroy River.

Sampling was done using the PAXARM biopsy system. All surveys were conducted during calm sea conditions (i.e. Beaufort Sea state ≤ 3 and swell ≤ 1m) using a 5.5 m centre console vessel powered with 100 hp engine available full time for the project. Surveys were based on methods successfully employed in previous studies by the research team (e.g. Parra et al. 2006; Cagnazzi et al. in press). We darted dolphins using the PAXARMS when they were travelling at slow to moderate speed (1-5 km/h) parallel to the vessel. Photographs of individuals that were successfully sampled were taken for individual identification and to avoid resampling the same individuals. In the field samples were stored in liquid nitrogen, while once in the lab sample will be stored in the freezer at -80°C.

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. Total PCBs were quantified as the sum of 30 congeners. The congeners constituted 80% of the total peak area of PCBs in all tissues. Total DDTs were calculated as the sum of op'DDT, pp'DDT, op'DDD, pp'DDD, op'DDE and pp'DDE. For DDTs and PCBs analysis, the samples were freeze-dried and extracted with n-hexane in a Soxhlet apparatus followed by sulphuric acid clean-up and Florisil chromatography (Marsili & Focardi, 1997). Results were expressed as ng/g lipid weight (l.w.).

Policiclycic Aromatic Hydrocarbons: PAHs were analyzed by HPLC with fluorescence detection as described in Marsili et al. (2001). Extraction was carried out according to (Marsili et al. 2001) and samples were concentrated to 1 ml in acetonitrile. A reverse-phase column (Supelcosil LC-18, 25 cm X 4.6mm i.d., 0.5µm particle size) and an acetonitrile/water gradient were used. Initial gradient was 60% acetonitrile and increased to 100% over 20min, then remaining stable for 10 min. Flow-rate was 1 ml/min. The external standard consisted of 16PAHs from Supelco (EPA 610 PAH mixture). Results were expressed as the sum of fifteen PAHs (naphtalene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, Benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene) per ng/g on a lipid basis (l.w.).

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 (Fossi *et al.*, 2008). 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).

Statistical analysis

Because samples are available only from one site, tests for geographical differences in contaminant levels are not possible at this stage.

To test interspecific differences in the induction of cytochromes 1A1 and 2B a non parametric Kruskal-Wallis test (KW) for multiple independent samples was used. A post hoc test with Bonferroni correction for multiple comparisons was used to test difference among two species (Oh = *Orcaella heinsohni*, Sc = *Sousa chinensis*, Ta = *Tursiops aduncus*, Tt = *Tursiops truncatus*). Samples of *Tusiops truncatus* (n = 14) collected from Mediterranean Sea were used as out group (MS group).

Due to the small sample size, we did not attempt to test for interspecific differences in PAH and OC levels. All the biopsy samples collected from the Fitzroy River were grouped together (QLD group). Differences in PAH and OC levels between QLD and MS groups were investigated using a two-samples randomization test with Monte-Carlo Method.

RESULTS

Due to negative weather condition we were able to collect samples only from one site, Keppel Bay, where a total of 34 biopsy samples from all the species (Sc = 13, Oh = 12, Ta = 9) were collected. Data are available only from a subset of these samples. The following results are based on a very small samples size need to be evaluated consciously.

- The cytochromes 1A1 and 2B were detected in 9 samples of Oh, 12 of Sc and 7 of Ta.
    1. Sc showed the highest Cyp1A1 and Cyp 2B induction followed by Oh, Ta and Tt. KW test showed an overall significant difference among species in Cyp1A1 (KW=13.86, df = 3, p = 0.003) and Cyp2B (KW=12.45, df = 3, p = 0.006) induction (Table 1).
    2. A subsequent post hoc test, adjusted with Bonferroni correction, showed significant differences only when the three target species were compared to Tt (Table 2).

- PAHs were extracted from all samples but, at the moment quantified only for 3 samples of humpback and snubfin dolphins and 2 for bottlenose dolphins.
    1. Mean levels of PAHs in Australian samples (Mean = 77,552 ng/g l.w., 95%CI = 0-201,146) was higher compared to Mediterranean samples (Mean 24,385 ng/g l.w., 95%CI =0-143,284). A two samples randomization test with Monte-Carlo simulation, showed that PAHs levels in Australian samples were not smaller or higher than in Mediterranean samples (MCM: Mean = 0.99, Var. = 1.41 p > 0.05).
    2. Results from the analysis on cangerogenic PAHs were similar. Mean level of cangerogenic PAHs was higher in samples from Australia (Mean QLD = 12,563 ng/g l.w., Mean MS = 3,095 ng/g l.w.) however randomisation test was not significant (MCM: Mean = 1, Var. = 0.3, p = 0.7).

- OCs were extracted from all samples but quantified from 4 humpback dolphins, 3 snubfin dolphins and 5 bottlenose dolphins.
    1. Overall OCs levels in QLD samples were substantially lower compared to MS samples. Within QLD samples a simple visual comparison showed that OCs levels are higher in Oh than in Sc and Ta.
    2. Results from a two samples randomization tests showed that DDT levels in MS samples are higher than in QLD samples (DDT: Mean = 1.20, Var. = 1.99, p = 0). On contrary, while PCBs levels are in average larger in MS than in QLD samples, the difference is not significant (Mean = 0.98, Var. = 1.18, p = 0.09).

CONCLUSION

Due to the small sample size (preliminary results) and high variance we are aware of the limited statistical power of the analyses presented in this report. Nevertheless these are the first data on anthropogenic contaminants levels derived in biopsy samples collected from free ranging Australian inshore dolphins species and indicate an existing problem related to water coastal pollution. Of particular concern are the high PAH levels found in samples from the Fitzroy River, considered the site with medium-low human impact. The high PAHs levels are further underlined by the significantly higher levels of Cyp 1A1, found in samples from Australia compared to MS samples. The cytochrome 1A1 is induced by planar PAHs which indicate an anthropogenic provenience.

On contrary organochlorines were found in substantially lower levels, however PCBs level in QLD samples were not significant less than in MS samples.

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CAPTIONS

Table 1 - Mean values of CYP1A1 and CYP2B detected in 28 biopsy samples collected from Snubfin dolphins (Oh = 9), humpback dolphins (Sc = 12), Indo-pacific bottlenose dolphins (Ta = 7). Data from 14 samples of bottlenose dolphin (Tt) were added as out group. Semi-quantitative analysis was performed for each WB (in triplicate) with Quantity One software (BioRad, 1-D Analysis Software) using Adjusted Volume (Intensity *mm^2) as quantitative parameter.

Species	Mean Cyp 1 A1	95%CI		Mean Cyp 2B	95%CI	
Oh	271.88	194.28	316.35	138.1578	122.67	138.28
Sc	297.87	251.35	341.42	169.8364	148.06	195.74
Ta	242.61	172.14	243.50	146.4529	111.16	176.18
Tt	143.43	112.11	187.88	96.6532	89.68	111.93

Table 2 Interspecific differences in cytochromes Cyp 1A1 and 2B induction in the three Australian inshore dolphins species. Snubfin dolphins (Oh = 9), humpback dolphins (Sc = 12), Indo-pacific bottlenose dolphins (Ta = 7). Data from 14 samples of bottlenose dolphin (Tt) were added as out group. In this table are presented results from a Post-Hoc test following a Kruscall-Wallis Test for multiple groups comparison. Groups are specified as described in the method section. Samples from Tt were added as out group.

Species	Cyp1A1			Cyp2B		
Groups	**Difference**	**T. Statistics**	**p**	**Difference**	**T. Statistics**	**P**
Oh vs Sc	-25.9943	0.4693	0.6	-31.6786	1.0571	0.3
Oh vs Ta	29.2625	0.4308	0.7	-8.2951	0.3781	0.7
Oh vs Tt	128.4457	3.9417	0.0007	41.5045	2.8886	0.008
Sc vs Ta	55.2569	0.7620	0.4	23.3835	0.6566	0.5
Sc vs Tt	154.4400	3.8528	0.0008	73.1831	3.0053	0.006
Ta vs Tt	99.1831	2.0442	0.05	49.7996	2.6836	0.01

Figure 1 Overall study areas showing the three sampling sites with a red star, Port Curtis, Fitzroy River and Port Clinton, along the Central Queensland coast, Australia.