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Anthropogenic contaminants in Indo-Pacific humpback and Australian snubfin dolphins from the central and southern Great Barrier Reef

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ABSTRACT

We present the first evidence of accumulation of organochlorine compounds (DDTs, PCBs, HCB) and polycyclic aromatic hydrocarbons (PAHs) in Indo-Pacific humpback and Australian snubfin dolphins from the central and southern Great Barrier Reef. These dolphins are considered by the Great Barrier Marine Park Authority to be high priority species for management. Analyses of biopsy samples, collected from free ranging individuals, showed PAHs levels comparable to those reported from highly industrialized countries. DDTs and HCB were found at low levels, while in some individuals, PCBs were above thresholds over which immunosuppression and reproductive anomalies occur. These results highlight the need for ongoing monitoring of these and other contaminants, and their potential adverse effects on dolphins and other marine fauna. This is particularly important given the current strategic assessment of the Great Barrier Reef World Heritage Area being undertaken by the Australian Government and the Queensland Government.

1. Introduction

Coastal marine water quality is declining around the world as a result of increasing pressure from human population growth, intensifying land use, urbanization and industrial development (Lotze et al., 2006). In Australia the negative effects of anthropogenic contaminants on tropical marine coastal ecosystems are of increasing concern as human populations expand adjacent to these communities (Esther et al., 1997). Accordingly, within the Great Barrier Reef Marine Park the highest levels of pesticides, organochlorine compounds and polycyclic aromatic hydrocarbons were detected in sites adjacent to human activities (Haynes and Johnson, 2000; Haynes et al., 2000; Smith et al., 2012). River discharge, urban stormwater, and agricultural and industrial runoff have been identified as the primary sources of anthropogenic contaminants into the Great Barrier Reef lagoon (Schaffelke et al., 2012).

Inshore dolphins are particularly vulnerable to the exposure and accumulation of contaminants as a result of their high site fidelity, long life span, high trophic level and large fat repositories that can serve as deposits for anthropogenic contaminants (Bossart, 2011). As a result high levels of various contaminants have been reported in dolphins around the globe (Vos et al., 2004). In Australia data on contaminant levels in cetaceans are scarce and entirely obtained from opportunistic studies on stranded animals (Gaus et al., 2005; Weijs et al., 2013). Within the Great Barrier Reef and in southern Queensland, a range of organohalogen compounds have been detected in the blubber of four stranded bottlenose dolphins (*Tursiops truncatus*), one common dolphin (*Delphinus delphis*), and several dugongs (*Dugong dugon*) (Haynes et al., 2005; Hermanussen et al., 2008; Vetter et al., 2001). While no information is available for two species of major conservation concern, such as the Indo-Pacific humpback (*Sousa chinensis)* and the Australian snubfin (*Orcaella heinsohni*)

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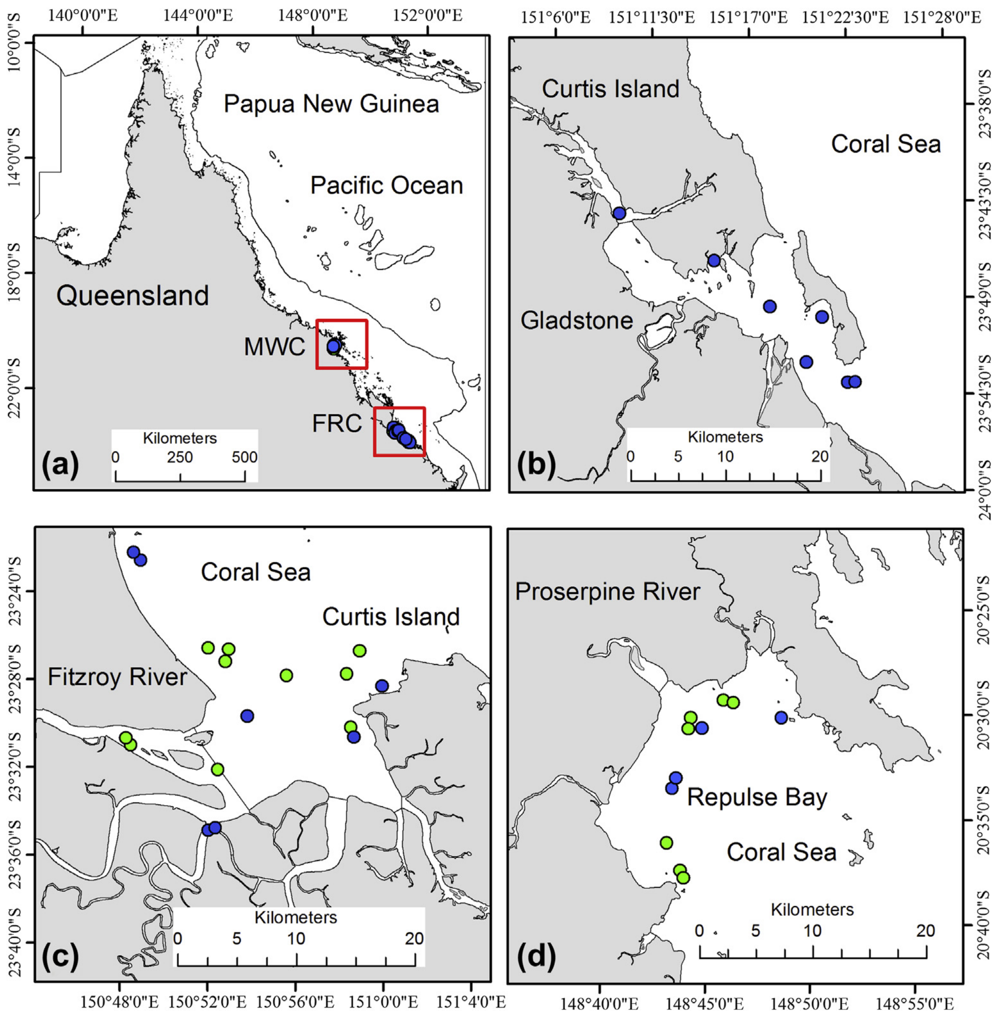

Fig. 1. Map showing the sampling locations of biopsy samples collected from humpback dolphins (n = 18, blue dots) and snubfin dolphins (n = 17, green dots) in three estuaries of central and southern Great Barrier Reef (a): Port Curtis (b) Fitzroy River (c) and Proserpine River (d).

dolphins (hereafter referred to as humpback and snubfin dolphins respectively).

Humpback and snubfin dolphins are classified as “Near Threatened” in the Queensland Nature Conservation Act 1992 and are considered by the Great Barrier Reef Marine Park Authority as high priority for management (GBRMPA, 2007). Due to their coastal distribution and strong site fidelity (Cagnazzi et al., 2011, 2013; Parra, 2006; Parra et al., 2006) snubfin and humpback dolphins are likely to be exposed on a daily basis to a wide range of contaminants.

The present study assessed the levels of dichlorodiphenyltrichloroethane (DDT) and its metabolites, polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) and polycyclic aromatic hydrocarbons (PAHs) in biopsy samples of humpback and snubfin dolphins collected from the Mackay–Whitsundays (MWC) and the Fitzroy River (FRC) catchments of Great Barrier Reef Marine Park, Queensland. In these catchments the export of sediments, nutrients, pollutants and modern pesticide residues into the Great Barrier Reef lagoon has increased at alarming levels as a result of extensive land modification, agriculture, grazing and industrial activities combined with increased rainfall (Chiew and McMahon, 2002; Kroon et al., 2012). Both catchments are used for industrial, agricultural and grazing activities.

2. Material and methods

2.1. Survey procedures and sampling

Biopsy samples were collected in 2009 and 2010 during boat-based surveys of inshore dolphins near the Proserpine River in the MWC, and in Port Curtis and near the Fitzroy River within the FRC (Fig. 1). Biopsy samples were collected from adult dolphins not accompanied by calves using the PAXARMS biopsy system (Krützen et al., 2002). Sampled individuals were photo-identified to limit the risk of resampling the same individuals. Additional data collected included sampling location, location of the biopsy sample in relation to the dorsal fin and dolphin reaction level. Samples were preserved in liquid nitrogen immediately after collection and stored in a -80 °C freezer once on land.

Overall, during the study we collected a total of 35 full size (>0.5 g of blubber and epidermal layers) biopsy samples (humpback dolphins: FRC = 13, MWC = 5; snubfin dolphins: FRC = 8, MCW = 9) (Fig. 1).

2.1.1. Analytical procedures

Analysis for HCB, DDTs and PCBs was performed according to methods of the U.S. Environmental Protection Agency (EPA) 8081/8082 modified by Marsili and Focardi (1997). PAHs were analysed by HPLC with fluorescence detection as described in Marsili et al. (1997). Full protocols are described in Appendix A.

In summary, total PCBs ($\sum$PCBs) were quantified as the sum of 30 congeners (IUPAC no. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206), total DDTs ($\sum$DDTs) as the sum of the op’ and pp’, forms of DDT, DDD and DDE, while total PAHs ($\sum$PAHs) and carcinogenic PAHs ($\sum$carPAHs) as the sum of all fourteen PAH metabolites and of the six carcinogenic PAHs (Non carcinogenic: Naphthalene,

Table 1
Descriptive statistics for total and carcinogenic PAHs, PCBs, DDTs and HCB in biopsy samples of humpback and snubfin dolphins, presented using mean ± standard error and range (minimum and maximum values) at species level and only with mean and range at catchment level. Values are expressed in ng/g of lipid weight (l.w.). Values in dry and fresh weight are available in Appendix B, Table B1.

Contaminants	Overall by species	Fitzroy river Catchment	Mackay–Whitsundays
Indo-Pacific humpback dolphins (*n total* = *18*)			
∑PCBs	10 382 ± 5308 (776–93 522)	13 143.9 (776.75–93 522.5)	3753.9 (1382.4–7431.9)
DDTs	2132 ± 450 (308–6163)	2616.6 (491.99–6163.6)	969.2 (308.4–2452.2)
HCB	6.3 ± 3.1 (<0.1–45.5)	6.5 (0–45.5)	5.6 (0–28.3)
∑PAHs	51 035 ± 5227 (12 552–86 711)	50 879.9 (11 039.3–86 711.1)	41 543.5 (33 023.1–50 311.2)
Car∑PAHs	2983 ± 1012 (598–17 050)	3338.5 (180.1–17 050.1)	1129.4 (914.2–1512.8)
Australian snubfin dolphins (*n total* = *17*)			
∑PCBs	5249 ± 1551 (168–21 424)	6674.3 (1382.4–19 135.4)	3894.3 (168.6–21 424.6)
DDTs	2979 ± 1026 (178–16 073)	5285.3 (178.4–16 073.4)	930.6 (231.1–2121.9)
HCB	15.6 ± 4.1 (<0.1–47.7)	25.55 (0–47.7)	6.79 (0–27.2)
∑PAHs	32 296 ± 3152 (8682–61 921)	40 782.4 (29 286.6–61 921.3)	24 753.7 (8682.7–40 512.5)
Car∑PAHs	1705 ± 387 (188–6717)	2374.9 (666.9–6717.5)	1110.1 (188.6–2826.5)

In the table ∑PCBs = IUPAC no. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206; DDTs = the sum of the op' and pp', forms of DDT, DDD and DDE; ∑PAHs = sum of Naphthalene, Acenaphtene, Fluorene, Phenantrene, Anthracene, Fluoranthene, Pyrene, B(g,h,i)perylene, B(k)fluoranthene, B(a)pyrene, B(a)anthracene. Crysene, B(b)fluorantene, DiB(a,h)anthracene; Car∑PAHs = sum of B(k)fluoranthene, B(a)pyrene, B(a) anthracene. Crysene, B(b)fluorantene, DiB(a,h)anthracene.

Acenaphtene, Fluorene, Phenantrene, Anthracene, Fluoranthene, Pyrene, B(g,h,i) perylene; Carcinogenic: B(k)fluoranthene, B(a)pyrene, B(a)anthracene. Crysene, B(b)fluorantene, DiB(a,h)anthracene). The mean Extracted Organic Material (EOM %) was 26.3% (SD = 9,03) for snubfin dolphins and 26.09% (SE = 12.52) for humpback dolphins.

Data are summarised by catchment (FRC and MWC) and within catchment by species (Hd = humpback and Sd = snubfin) resulting in four groups. However, due to the small and geographically uneven sample sizes, statistical comparisons were not attempted. Contaminant levels were reported using Mean, standard error (SE) and range (minimum and maximum values).

3. Results and discussion

∑PAHs, ∑carPAHs, ∑PCBs, DDTs and HCB levels are summarised by group in Table 1. The PAHs fingerprint was highly similar across groups (Fig.2); the most abundant PAHs were those with lower molecular weight, which are also the most water-soluble and bioavailable. Among these, naphthalene and pyrene were the most dominant chemicals, and each accounted for at least 25% of the ∑PAHs in each group, whereas ∑carPAHs varied between 2.8% and 6.4% of ∑PAHs (Fig. 2). Overall ∑PAHs recorded in this study are amongst the higher found in the scientific literature (Appendix B Table B1).

HCB and DDTs were found at low levels (Table 1, Appendix B Table B2 and B3); the major constituent of DDTs was pp'-DDE accounting for at least 78% of the total composition (Fig. 3). On contrary in 6 samples (Hd-FRC = 4, Sd-FRC = 1, Sd-MWC = 1) individual ∑PCBs values exceeded the threshold value (∑PCBs = 11 000 ng/g l.w.), derived for bottlenose dolphins in relation to foetal and neonatal mortality associated with maternal PCBs exposure (Appendix B Table B2) (Schwacke et al., 2002). Among these, two snubfin dolphins (FRC = 1 and MWC = 1) exceeded also the proposed threshold value (∑PCBs = 17 000 ng/g l.w.) for adverse health effects in marine mammals (Jepson et al., 2005; Kannan et al., 2000) while one humpback (FRC = 1) showed ∑PCBs levels higher than those associated with carcinoma (∑PCBs = 77 000 ng/g l.w.) in California sea lions (*Zalophus californianus*) (Ylitalo et al., 2005).

Although no statistical comparisons were attempted, there was evidence of geographic differences in contaminant levels between groups of the same species. Mean levels for all contaminants were higher in samples of humpback and snubfin dolphins collected from the Fitzroy River Catchment compared to samples of the same species collected in the Mackay–Whitsunday Catchment (Table 1);

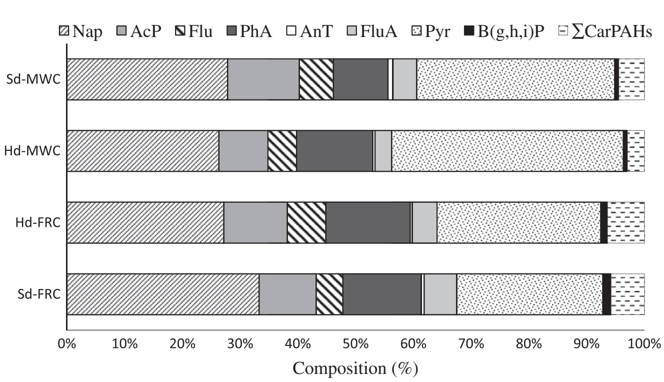

Fig. 2. Percentage composition of the fourteen PAHs compounds analysed in biopsy samples of humpback (Hd) and snubfin (Sd) dolphins collected from the Fitzroy River (FRC) AND THE Mackay–Whitsundays (MWC) catchments of the Great Barrier Reef Marine Park. The following abbreviations were used: Naphthalene (Nap), Acenaphtene (Acp), Fluorene (Flu), Phenantrene (PhA), Anthracene (AnT), Fluoranthene (FluA), Pyrene (Pyr), B(g,h,i)perylene (B(g,h,i)P), B(k)fluoranthene + B(a)pyrene + B(a) anthracene + Crysene + B(b)fluorantene + DiB(a,h)anthracene (∑CarPAHs).

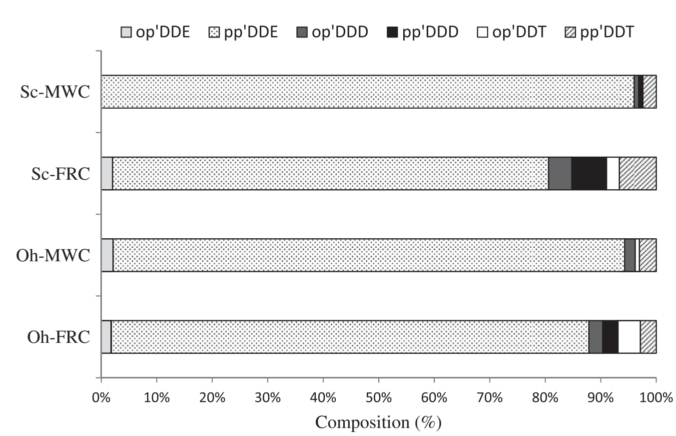

Fig. 3. Percentage composition of the op' and pp', forms of DDT, DDD and DDE in biopsy samples of humpback (Hd) and snubfin (Sd) dolphins collected from the Fitzroy River (FRC) and the Mackay–Whitsundays (MWC) catchments of the Great Barrier Reef Marine Park.

while humpback dolphins from the FRC showed higher average levels among all groups.

4. Conclusion

This study provides initial data on the concentrations of common persistent organic pollutants in biopsy samples of humpback and snubfin dolphins from the central and southern Great Barrier Reef, as well as raises concerns about the adverse health effects these and the new generation of contaminants could be having on local populations of these important top order predators.

Overall the low levels of HCB and DDTs detected in this study corroborate available information which indicates that these contaminants still persist in the water, sediments and biota of the Great Barrier Reef, but at levels of no concern to marine organisms (Haynes et al., 2005; Mortimer, 2000). DDTs and HCB were banned from Australia by 1987; therefore the low levels detected in this study are likely to indicate residuals from historical sources of these products. This observation is supported by the high average pp'-DDE/$\sum$DDTs ratio for both species (humpback dolphins = 0.87; snubfin dolphins = 0.89) showing a degradation of the initial product rather than new sources of DDTs into the environment (Aguilar, 1984).

On contrary, in a few samples PCBs levels exceeded suggested thresholds for small cetaceans. It must be noticed that these thresholds should not be used as an absolute value but, rather, as a guide to determine whether levels of PCB exposure in individual animals are likely to exert significant adverse biological effects.

These results are in accordance to higher values of PCBs found in the Great Barrier Reef particularly near port facilities such as Townsville, Mackay and Gladstone, and in the proximity of rivers such as the Burdekin and Fitzroy, where samples for this study were taken (Brodie et al., 2012; Haynes and Johnson, 2000; Müller et al., 1999; Shaw and Muller, 2005; Van Oosterom et al., 2010). Although the importation of PCBs to Australia has been largely restricted since 1987, PCBs can still be imported to Australia under consent of Minister for Justice and Customs, therefore near industrial areas new sources of PCBs may still be entering the marine environment.

Average $\sum$PAHs levels were very high compared to the few available studies on small odontocetes (Appendix B Table B1); however these comparisons must be taken with caution as analytical methods varied across studies and thresholds values for PAHs have not been proposed yet. The cytotoxic, genotoxic, immunotoxic, and carcinogenic potential of PAHs are well documented, but the complex relationship between PAHs and adverse health effects is still under investigation (Fossi and Marsili, 2003; Godard et al., 2006; Marsili et al., 2012; Martineau et al., 2002; Menzie et al., 1992; Villeneuve et al., 2002). For example only recently naphthalene, the most abundant PAH found in this study, has been reclassified under the International Agency for Research on Cancer and the US Environmental Protection Agency as potentially carcinogenic to humans (NTP, 2000, 2011).

Sources of anthropogenic contaminants along the central and southern sections of the Great Barrier Reef Marine Park are likely to increase in the future, as a result of a widespread contamination of several new pesticides in the catchment area of the Great Barrier Reef (Smith et al., 2012), increasing annual runoff (Chiew and McMahon, 2002), and the rapid urban and industrial development along the coast (DSDIP, 2012).

These results suggest that humpback and snubfin dolphins have the potential to accumulate PCBs at levels potentially dangerous for their health. In recent years numerous fatalities of humpback and snubfin dolphins have been reported within the southern Great Barrier Reef region, without an apparent cause of death (DEHP, 2012). Most of these deaths occurred following large floods and major industrial coastal developments (DEHP, 2011). The immunosuppressive effect of contaminants may have played an important role in these deaths.

In this study we presented preliminary information of a larger study which aims to assess interspecific differences and geographic variation in contaminant levels, as well as to develop and validate a suite of non-lethal biomarkers for contaminant exposure, reproduction alteration and genotoxicity. The overall aim of this project is to evaluate the potential of using inshore dolphins as sentinel species for the health status of the coastal ecosystem (Bossart, 2011). The advantages of this approach are that skin biopses from dolphins can be obtained in a non-lethal way, allows the screening of a large number of samples with little disturbance to the animals and are suitable for the assessment of toxicological status (Fossi et al., 2004; Panti et al., 2011). While the lack of consideration for the variability in contaminant levels among different body parts and through the blubber layer is the main limitation of this approach.

Further biopsy sampling of free ranging and stranded individuals, combined with long-term monitoring of known individuals and when possible the collection of target organs and morphometric data from dead stranded animals, is essential to increase our understanding of the potential deleterious effects that these contaminants may have on the health of inshore dolphins and the survival of their populations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.08.008.

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