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Baseline

Investigation of trophic level and niche partitioning of 7 cetacean species by stable isotopes, and cadmium and arsenic tissue concentrations in the western Pacific Ocean

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ABSTRACT

A total of 24 stranded or bycatch cetaceans, including *Balaenoptera omurai*, *Lagenodelphis hosei*, *Kogia sima*, *Stenella attenuata*, *Grampus griseus*, *Neophocaena phocaenoides*, and *Sousa chinensis,* were collected from 2001 to 2011 in Taiwan. Using the muscular $\delta^{13}C$ and $\delta^{15}N$ data, three ecological groups were identified as the oceanic baleen whale, the neritic, and the coastal toothed whale groups, coinciding with their taxonomy, feeding habits and geographical distribution. A horizontal inshore to offshore distribution was found for the sympatric neritic toothed dolphins, *G. griseus*, *K. sima*, *S. attenuata*, and *L. hosei* in the outermost offshore waters, accompanying their growth. For the first time we identify Taiwan's Chinese white dolphin, *S. chinensis,* as an exclusive fish eater. Cd and As bioaccumulated in the *G. griseus*, *L. hosei* and *S. attenuata* increase as they grow. Prey-derived As- and Cd-induced health threats were found in *L. hosei*, and *G. griseus*.

Analysis of muscular $\delta^{13}C$ and $\delta^{15}N$ is widely applied to gain insights into the food web structure of ecosystems (e.g., Post, 2002; Praca et al., 2011; Aurioles-Gamboa et al., 2013). It has also been applied in the study of cetaceans' ecological roles (e.g., Das et al., 2000, 2003), of how they share resources with other top marine predators (Das et al., 2000), their dietary shifts (Riccialdelli et al., 2010), and the identification of their migratory routes (e.g., Praca et al., 2011; Riccialdelli et al., 2012). Since $\delta^{15}N$ values generally increase through the food chain, the $\delta^{15}N$ of a consumer's tissue can be used to identify its relative and absolute trophic position (Kelly, 2000; Newsome et al., 2010). However, the increase in $\delta^{13}C$ values in the food web are usually smaller and may reflect the origin of the primary production (Rau et al., 1982; Kelly, 2000; Newsome et al., 2010). As terrestrial and marine carbon sources differ in their $\delta^{13}C$ values (Kelly, 2000), $\delta^{13}C$ can indicate offshore/nearshore or benthic/pelagic contributions to food intake (Fry and Sherr, 1984; Cherel and Hobson, 2007).

Cetaceans are marine apex and cosmopolitan species distributed worldwide. Understanding their ecological roles in a marine ecosystem is an important issue for the conservation of marine mammals and their environments. Therefore, many scientists have used the analysis of muscular $\delta^{13}C$ and $\delta^{15}N$ as a tool to gain insights into the ecological niches of cetaceans (e.g., Das et al., 2000; Post, 2002; Praca et al., 2011; Aurioles-Gamboa et al., 2013). However, such information in the western Pacific Ocean tropical volcanic chain is scarce. Therefore, we took advantage of the location of Taiwan as a biodiversity hot spot. To the east of the island, the Kuroshio Current brings heat from the tropics (Su and Pu, 1986; Mensah et al., 2014), triggering many upwellings along its way (Udarbe-Walker and Villanoy, 2001), resulting in high primary production which creates an abundance of food resources for the top marine predators (Ku et al., 2014). Therefore, it has become an important habitat for migratory marine organisms, attracting cetaceans for foraging year round. So far, 31 cetacean species have been documented in the area (Chou, 2008). The 5 most dominant dolphin species which appear in eastern Taiwan are Risso's dolphins (*Grampus griseus*), pantropical spotted dolphins (*Stenella attenuata*), Fraser's dolphins (*Lagenodelphis hosei*), dwarf sperm whales (*Kogia sima*) and spinner dolphins (*Stenella longirostris*). In contrast, in western Taiwan, where there is dense

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industrial development along the coastal area, only finless porpoises (*Neophocaena phoconoides*) and Chinese white dolphins (*Sousa chinensis*, eastern Taiwan Strait subpopulation) can be found (Chou, 2008). With such a diverse composition of species, it is worth investigating their ecological role and habitat usage for the conservation of cetaceans in the future.

Cadmium is a non-essential element, but arsenic is recognized as a micronutrient for cetaceans, playing a role in the activities of enzymes (Shibata et al., 1992). Cadmium (Cd) is not easy for any animal to eliminate (Eisler, 1985, 1988), and certain concentrations of these elements in the body would be toxic (Eisler, 1985, 1988). However, due to the longer half-life of the metal residue in the consumer, the non-essential elements can be applied as a tracer in the study of predators' feeding habits and prey identification, e.g. renal Cd concentration has been used as a tracer for high cephalopod consumption in dolphins and seals (Endo et al., 2008). As is a metalloid, showing both metallic and non-metallic characteristics, and is capable of forming both cationic and anionic salts (Shibata et al., 1992). It exists in the marine environment globally, and transfers to cetaceans through the food chain (Kubota et al., 2001). Therefore, hepatic As has also been applied in the study of dolphins' feeding habits and location in relation to prey high in As from anthropogenic sources (Bellante et al., 2012). Therefore, we also analyzed the dolphins' tissue concentrations of Cd and As to identify any possible dietary shift throughout their growth in relation to their foraging area.

Furthermore, the combined use of stable isotopes and heavy metal analyses can be a useful tool for studying marine mammal ecology (Das et al., 2000, 2003), not only to understand whether the transfer process of toxic heavy metals to a certain level has a harmful health effect on the top predator in a marine environment (Das et al., 2003), but also to further reveal its ecological niche (Capelli et al., 2008). However, the literature investigating the ecological niches and feeding habitats of cetaceans using these tools is limited, in particular, in the western Pacific Ocean. Therefore, the aims of this study are to use 6 stranded dolphins, i.e. *G. griseus, K. sima, L. hosei, N. phoconoides, S. chinensis, S. attenuate,* and one baleen whale (Omura's whales, *Balaenoptera omurai*), (1) to study their ecological niches by the analysis of muscular $\delta^{13}C$ and $\delta^{15}N$, (2) to examine the relationship between their body size, stable isotopes and their Cd and As tissue concentrations to understand the possible changes in their feeding habitats and resource partitioning, and finally (3) to combine all of this information to assess the possible health threats derived from their natural habitats.

A total of 24 stranded or bycatch individuals of 7 cetacean species, including 1 Omura's whale, 3 Fraser's dolphins, 5 dwarf sperm whales, 4 pantropical spotted dolphins, 8 Risso's dolphins, 1 finless porpoise and 2 Chinese white dolphins, were collected from 2001 to 2011 in Taiwan. Muscle tissues, livers and kidneys were collected by the Taiwanese Cetacean Stranding Network, Taiwan Cetacean Society, with many volunteers from the Cetacean Laboratory (Prof. Lien-Siang Chou), the Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, and the National Museum of Marine Biology and Aquarium (Dr. Chiou-Ju Yao), Taichung.

The tissue samples collected for cadmium and arsenic analyses were firstly trimmed off their outer layer by stainless steel scalpel. Only the inner part of the metal-free tissue samples were then put into zip lock plastic bags and stored at −20 °C as analytical samples. Before analysis, about 10 g of each tissue sample were homogenized and freeze-dried for at least 72 h. The samples were divided into two portions for cadmium and arsenic analyses, and isotope analysis, respectively.

Due to the depletion in $\delta^{13}C$ value from lipids (Tieszen et al., 1983; Sweeting et al., 2006), lipid-extraction was performed on the muscle tissues before isotope analysis. Approximately 0.5 g per sample was rinsed with 5 ml chloroform and 2.5 ml methanol (Merck, GR grade) (2:1 v/v) added in a tube. It was then well-mixed and vibrated for 5–10 min, and stood for 1 h. The mixture was filtered through Büchner funnel filtration with a 90 mm glass fiber filter (Whatman, GF/A). Then, 3 ml chloroform was added to the residue which was filtered again and dried by evaporation. The dried samples were ground into fine powder with a mortar and pestle, and then stored in Eppendorf at −20 °C until analyzed.

Carbon and nitrogen isotopes were measured by the Plant Physiology Laboratory (Prof. Wen-Yuan Kao) of the Department of Life Sciences, National Taiwan University, performed on a V.G. Optima IRMS (Thermo Scientific, DELTA V Advantage) coupled with an N–C elemental analyzer (Thermo Scientific, FlashEA 1112 series).

The samples for the cadmium and arsenic analyses were digested following the method established in M.-H. Chen's lab (Chen, 2002). Approximately 0.3 g of homogenized freeze-dried non-lipid extracted sample was used for the analysis. At the same time, the standard reference materials, DOLT-2 (dogfish liver) and DORM-2 (dogfish muscle) from the National Research Council of Canada were used to verify the analytical quality.

Arsenic and cadmium were measured by graphite furnace atomic absorption spectrometry (Hitachi Z-5000, tube type: 7JO-8885). Cadmium was measured using the standard addition method to avoid unknown interferences. In this method, each unmeasured sample is mixed with 0, 2, 4 $\mu g\, l^{-1}$ of 1 $\mu g\, ml^{-1}$ cadmium standard solution. Arsenic measurements were taken by adding 10 µl of 1000 $\mu g\, ml^{-1}$ palladium in concentrated nitric acid as the matrix modifier for a 20 µl sample. The recovery of the standard materials of DORM-2 and DOLT-2 with four replicates (vs certified value) were 0.048 ± 0.006 (vs 0.043 ± 0.008) and 19.8 ± 0.69 (vs 20.8 ± 0.50) for Cd, and 17.6 ± 1.1 (vs 18.0 ± 1.1) and 15.0 ± 1.40 (vs 16.6 ± 1.10) for As. Our data presented here are $\mu g\, g^{-1}$ dry weight, and use a conversion factor of 4.5 to transfer the wet weight data for comparison with the literature.

Non-parametric ANOVA (Kruskal–Wallis) using the Dunn Test as a post hoc test was used to test the species-specific differences in the isotopes and heavy metal concentrations ($p < 0.05$). All of the statistical analyses were performed using SAS® Version 9.3 (SAS Institute Inc., Cary, NC, USA).

The matrix of the $\delta^{13}C$ and $\delta^{15}N$ isotope values plot can be distinguished as three different ecological groups, namely the oceanic baleen whale group, and the neritic and the coastal toothed whale groups (Fig. 1). The first group, consisting only of the one Omura's whale, had the lowest $\delta^{13}C$ and $\delta^{15}N$ values, −16.93‰ and 10.92‰,

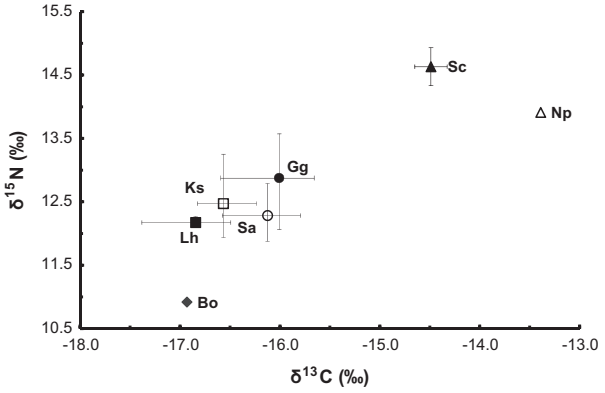

Fig. 1. The muscular $\delta^{13}C$ and $\delta^{15}N$ plot for the seven cetaceans in Taiwanese waters from 2001 to 2011. Bo = Omura's whales (*Balaenoptera omurai*), Lh = Fraser's dolphins (*Lagenodelphis hosei*), Ks = dwarf sperm whales (*Kogia sima*), Sa = pantropical spotted dolphins (*Stenella attenuata*), Gg = Risso's dolphins (*Grampus griseus*), Np = finless porpoises (*Neophocaena phocaenoides*) and Sc = Chinese white dolphins (*Sousa chinensis*).