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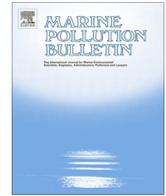


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Investigation of trophic level and niche partitioning of 7 cetacean species by stable isotopes, and cadmium and arsenic tissue concentrations in the western Pacific Ocean



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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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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*.

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Analysis of muscular $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is widely applied to gain insights into the food web structure of ecosystems (e.g., Post, 2002; Praca et al., 2011; Auriolles-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 $\delta^{15}\text{N}$ values generally increase through the food chain, the $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values (Kelly, 2000), $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as a tool to gain insights into the ecological niches of cetaceans (e.g., Das et al., 2000; Post, 2002; Praca et al., 2011; Auriolles-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 phocaenoides*) 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. phocaenoides*, *S. chinensis*, *S. attenuata*, and one baleen whale (Omura's whales, *Balaenoptera omurai*), (1) to study their ecological niches by the analysis of muscular $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, (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 $-20\text{ }^{\circ}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $-20\text{ }^{\circ}\text{C}$ 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 $\mu\text{g l}^{-1}$ of $1\text{ }\mu\text{g ml}^{-1}$ cadmium standard solution. Arsenic measurements were taken by adding 10 μl of $1000\text{ }\mu\text{g ml}^{-1}$ 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 $\mu\text{g g}^{-1}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, -16.93‰ and 10.92‰ ,

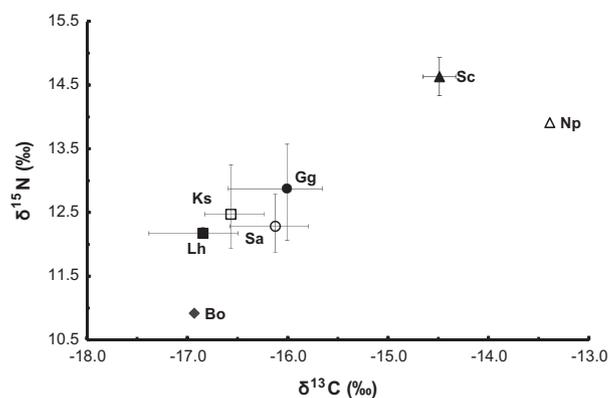


Fig. 1. The muscular $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ plot for the seven cetaceans in Taiwanese waters from 2001 to 2011. Bo = Omura's whales (*Balaenoptera omurai*), Lh = Fraser's dolphins (*Lagenodelphis hosei*), Ks = dwarf sperm whales (*Kogia sima*), Sa = pantropical spotted dolphins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

respectively. The second group, which had intermediate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, included the Fraser's dolphins ($-16.84 \pm 0.47\text{‰}$ and $12.17 \pm 0.09\text{‰}$), the dwarf sperm whales ($-16.57 \pm 0.24\text{‰}$ and $12.47 \pm 0.54\text{‰}$), the pantropical spotted dolphins ($-16.12 \pm 0.39\text{‰}$ and $12.28 \pm 0.38\text{‰}$) and the Risso's dolphins ($-16.01 \pm 0.30\text{‰}$ and $12.87 \pm 0.56\text{‰}$). The highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values occurred in the finless porpoises (-13.39‰ and 13.91‰) and the Chinese white dolphins ($-14.49 \pm 0.23\text{‰}$ and $14.63 \pm 0.42\text{‰}$); thus, they were classified as the last group. Due to only having one Omura's whale sample for the oceanic baleen whale group, we did not include it in the statistical test. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the neritic toothed whale group were significantly lower than those of the coastal toothed whale group ($p < 0.05$). The $\delta^{15}\text{N}$ value of Omura's whale had the lowest value, while the Chinese white dolphins had the highest value. Apart from these two, the $\delta^{15}\text{N}$ of the remaining 5 species of dolphin were highly overlapping (Fig. 1).

The grouping of the three ecological niche groups matches their taxonomy, feeding habits and geographical distribution. Omura's whale (*B. omurai*) is the only species which falls into the oceanic baleen whale group, as it differs significantly from the other toothed whales. According to its $\delta^{15}\text{N}$ value, it is a marine planktonic filter feeder and has a trophic level of 3 in the marine ecosystem (Aurioles-Gamboa et al., 2013). It feeds on marine zooplankton and epipelagic small fishes (Bannister, 2001). As a secondary consumer, its $\delta^{15}\text{N}$ falls within the muscular $\delta^{15}\text{N}$ data of Balaenoptera, ranging from 8.4‰ to 13.9‰ (Ryan et al., 2013), also similar to the minus 3‰ for the adjustment of $\delta^{15}\text{N}$ measured from the bone or dentin tissues of blue whales and fin whales (14.0–15.3‰) in the northwestern Pacific Ocean, which is significantly lower than the $\delta^{15}\text{N}$ range for toothed whales (14.5–23.2‰) in the same area (Aurioles-Gamboa et al., 2013).

Omura's whale used to be identified as Bryde's whale due to their similarity in size (Yamada, 2009). It was newly named Omura's whale after mtDNA identification (Wada et al., 2003; Sasaki et al., 2006). Here we firstly report its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, as well as its Cd and As tissue concentrations. We found that this whale is situated at the lowest trophic level with the lowest $\delta^{13}\text{C}$ value, representing that it inhabits the open ocean, and has very low tissue concentrations of both Cd and As.

The group of neritic toothed whales, including Fraser's dolphins, dwarf sperm whales, pantropical spotted dolphins, and Risso's dolphins, grouped together reflect their natural appearance off the coast of Eastern Taiwan (Yeh, 2001). Among the four, Fraser's dolphin shows the lowest $\delta^{13}\text{C}$ value, very similar to that of the Omura's whale, representing that, of the four, they live furthest from the shore (Yamada, 2009; Botta et al., 2011). They are tropical open oceanic dolphins (Huggins, 2011), inhabiting an area off the ridge of the continental shelf in water depths of more than 1000 m (Louella and Dolar, 2009), in agreement with Yeh's (2001) field observation in southeastern Taiwan. Due to the topographical feature of eastern Taiwan, Fraser's dolphins live not far from the shore, similar to the Fraser's dolphins which have been found close to the shore where the water is deep (Louella and Dolar, 2009). On the other hand, Risso's dolphins revealed the highest $\delta^{13}\text{C}$ value, representing that they inhabit the continental waters closer to the shore (Bearzi et al., 2011). The $\delta^{13}\text{C}$ value of the other two dolphins indicates that they tend to live in between, supporting the finding of Yeh (2001) that they have a highly overlapping habitat with the other two species. Furthermore, our results agree with the findings of Aurioles-Gamboa et al. (2013), who also documented a lower mean $\delta^{13}\text{C}$ value of dwarf sperm whales than that of Risso's dolphins in the Gulf of California, as they might feed farther from the shore.

The coastal toothed whale group includes finless porpoises and Chinese white dolphins, which are well known as shallow

nearshore cetacean species (Parsons, 1997). They inhabit the western coast of Taiwan (Wang et al., 2004, 2007; Yeh, 2011). However, our $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results show that their ecological niches are different. The finless porpoises prefer foraging in deeper, cleaner and more saline waters compared with the Chinese white dolphins which prefer foraging near shallower, murky, brackish estuaries (Parsons, 1997; Barros et al., 2004). The habitat of the Taiwanese subpopulation of Chinese white dolphins is limited to the coastal waters with a water depth of less than 17 m in western Taiwan (Wang et al., 2007; Yeh, 2011). Its $\delta^{15}\text{N}$ value was higher than that of finless porpoises, due to the Chinese white dolphins feeding exclusively on fishes, in particular Sciaenids (e.g., *Johnius* sp., *Collichthys lucida*) and *Thryssa* spp. (Barros et al., 2004), whereas finless porpoises forage for a more diverse diet, not only feeding on croakers (Sciaenidae), but also consuming a large proportion of cephalopods, e.g. pencil squids (Loliginidae) (Wang, 2003; Barros et al., 2004). Therefore, here we provide one more example to confirm that the muscular $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of cetaceans can be a powerful tool for distinguishing their ecological niches.

The highest concentrations of As were generally found in the liver tissues, except for the Omura's whale and the dwarf sperm whales, while the highest Cd concentrations were found in the kidney tissues, also excluding the Omura's whale (Table 1). Among the 7 cetacean species, the highest mean muscular, hepatic and renal As concentrations were measured in the Risso's dolphins, while the lowest were in the Omura's whale, which was under the detection limit of $0.32 \mu\text{g g}^{-1}$ dry weight. On the other hand, the highest mean hepatic and renal Cd concentrations were found in the Fraser's dolphins, and once again the lowest was in the Omura's whale ($0.006 \mu\text{g g}^{-1}$ dry weight) (Table 1). However, due to the limited sample size and huge data variation, no statistically significant intraspecific difference could be found in the As and Cd concentrations for the three tissues (Table 1, $p > 0.05$).

The high tissue concentrations of Cd reflect the amount of cephalopod intake of cetaceans (Das et al., 2000, 2003). The more cephalopods consumed, the higher Cd concentration in their tissues (Das et al., 2000, 2003). Oceanic cephalopods are common prey species for cetaceans (Bustamante et al., 1998; Praca et al., 2011). They can concentrate Cd in their digestive gland, giving their predators a higher Cd concentration in their tissues through their dietary intake (Bustamante et al., 1998). Therefore, in our study, the data for the Chinese white dolphins and the Omura's whale, which had lower Cd concentrations, further support the findings that Chinese white dolphins do not feed on oceanic cephalopods, but prey mainly on fishes (Barros et al., 2004), while Omura's whales feed on zooplankton and epipelagic small fishes (Nicol et al., 2010; Ryan et al., 2013). On the other hand, cetaceans with higher Cd concentrations such as Risso's dolphins, feed on a relatively higher proportion of oceanic cephalopods compared with pantropical spotted dolphins (Wang et al., 2002, 2003, 2003, 2012). The high concentration of Cd found in the Fraser's dolphins not only seems to come from the amount of cephalopods (18.8 N%) they consume, but may also come from their major food source, hatchfishes (50.2 N%) (Wang et al., 2012). It seems that hatchfishes may contain a high concentration of Cd similar to cephalopods, thus making them the main Cd source for their predators. To confirm this hypothesis, the Cd concentrations in hatchfishes need to be analyzed in the future. Such high Cd tissue concentrations were also found in the muscles and livers of the Fraser's dolphins and Risso's dolphins.

The relationships between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes and body length in the same species of cetacean show a species-dependent trend. The relationships for pantropical spotted dolphins show an increasing trend for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as they grow. On the other hand, the $\delta^{15}\text{N}$ values of Risso's dolphin displayed a

Table 1
Muscular (M), hepatic (L), and renal (K) As and Cd concentrations ($\mu\text{g g}^{-1}$ dry weight) of cetaceans worldwide and this study.

Species name	Location	n	As-M		As-L		As-K		Cd-M		Cd-L		Cd-K		Reference
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
<i>Balaenoptera omurai</i> , Omura's whales	E. Taiwan, W Pacific O.	1	<0.1	-	<0.1	-	1.29	-	0.0038	-	0.0067	-	0.0067	-	This study
<i>Ziphius cavirostris</i> , Curier's beaked whales	Italian coasts	3	0.90	(<0.5–2.2)	0.5	(<0.5–2.2)	-	(<0.5–17.2)	0.367	(0.1–0.9)	2.40	(0.1–0.9)	-	(27.1–164.7)	Bellanté et al., 2012
<i>Kogia sima</i> , Dwarf sperm whales	E. Taiwan, W Pacific O.	5	0.66 ± 0.35	(<0.32–0.99)	0.90 ± 0.69	(0.16–1.85)	1.03 ± 0.34	(0.50–1.42)	0.17 ± 0.15	(0.05–0.42)	14.8 ± 4.23	(8.46–18.74)	34.2 ± 25.7	(8.44–73.18)	This study
<i>Lagenodelphis hosei</i> , Fraser's dolphins	E. Taiwan, W Pacific O.	3	0.30 ± 0.12	(<0.32–0.38)	3.38 ± 3.93	(0.75–7.89)	0.77 ± 0.26	(0.48–1.00)	0.25 ± 0.22	(0.11–0.50)	50.7 ± 30.0	(22.79–81.59)	267 ± 112	(151.5–374.4)	This study
<i>Stenella coeruleoalba</i> , Striped dolphins	Italian coasts	10	1.565	(<0.5–5.1)	4.1 ± 3.5	(<0.5–10.7)	-	(<0.5–10.2)	0.120	(<0.1–0.3)	4.84	(0.2–16.4)	-	(<0.1–84.8)	Bellanté et al., 2012
	E. Adriatic Sea	5	1.49 ± 1.13	(0.41–3.38)	8.42 ± 8.60	(1.26–22.77)	2.97 ± 1.67	(0.81–5.04)	0.018 ± 0.009	(0.032–0.126)	9.86 ± 5.36	(0.54–14.09)	27.45 ± 29.66	(2.48–79.2)	Billandzic et al., 2012
<i>Stenella attenuata</i> , Pantropical spotted dolphins	E. Taiwan, W Pacific O.	4	0.77 ± 0.60	(<0.32–1.29)	2.57 ± 1.99	(0.60–4.47)	1.73 ± 1.24	-	0.17 ± 0.29	(<0.01–0.61)	7.51 ± 9.37	(0.41–21.03)	55.0 ± 63.6	(3.59–146.6)	This study
<i>Tursiops truncatus</i> , Bottlenose dolphins	E. Adriatic Sea	14	1.17 ± 1.04	(0.09–3.24)	6.12 ± 10.85	(0.50–40.28)	2.84 ± 2.66	(0.54–8.37)	0.050 ± 0.045	(0.009–0.18)	2.84 ± 5.22	(0.34–20.16)	12.83 ± 12.96	(0.054–45.45)	Billandzic et al., 2012
	S. Carolina, US	10	1.915	(<0.5–4.7)	3.3 ± 3.6	(<0.5–9.6)	3.53	(<0.5–10.2)	0.075	(<0.1–1)	2.97	(<0.1–12.5)	17.0	(<0.1–84.8)	Bellanté et al., 2012
	Indian R. Florida, US	12	-	-	1.70 ± 1.34	(0.628–5.22)	-	-	-	-	0.266 ± 0.364	(0.003–1.09)	-	-	Stavros et al., 2011
	Hong Kong, S. China Sea	3	-	(1.73–23.0)	0.821 ± 0.271	-	-	(<0.9–4.29)	-	-	0.142 ± 0.228	(0.002–0.948)	-	-	Stavros et al., 2011
	E. Taiwan, W Pacific O.	8	2.04 ± 1.72	(<0.32–3.91)	4.40 ± 3.24	(0.44–8.23)	3.12 ± 2.28	(0.16–6.07)	0.58 ± 0.75	(0.02–1.67)	39.1 ± 49.8	(0.42–125.1)	50.84 ± 58.1	(2.06–169.5)	This study
<i>Grampus griseus</i> , Risso's dolphins	Italian coasts	2	<0.5	-	4.20 ± 2.70	-	4.5	(2.0–7.0)	0.40	-	22.10	(5.1–39.1)	36.3	(26.3–46.3)	Bellanté et al., 2012
	E. Mediterranean, Israel	1	-	-	-	-	-	0.765	-	75.60	-	39.87	-	-	Shoham-Frider et al., 2014
	E. Mediterranean, Israel	1	-	-	-	-	-	2.745	-	64.35	-	34.34	-	-	Shoham-Frider et al., 2002
	Ligurian Sea	3	-	-	-	-	-	0.315	-	20.43	-	38.16	-	-	Capelli et al., 2008
	S. Adriatic Sea	2	-	-	-	-	-	0.675	-	32.45	-	52.20	-	-	Storelli et al., 1999
	E. Adriatic Sea	4	5.45 ± 2.34	(3.69–8.91)	10.85 ± 7.83	(2.02–21.11)	8.06 ± 5.94	(4.01–16.88)	0.39 ± 0.21	(0.25–0.72)	23.22 ± 21.42	(2.57–53.55)	67.05 ± 7.2	(58.5–75.6)	Billandzic et al., 2012
<i>Neophocaena phocaenoides</i> , Finless porpoises	Western Taiwan	1	0.51	-	0.74	-	0.59	-	0.10	-	6.20	-	26.50	-	This study
	Hong Kong	15	-	-	-	(<0.67–40.25)	-	(<0.66–20.13)	-	-	0.62	(<0.37–2.86)	3.4	(<0.63–19.6)	Parson, 1999
<i>Sousa chinensis</i> , Chinese white dolphins	Western Taiwan	2	0.36	-	-	-	0.85	(0.16–1.53)	0.007	-	-	-	2.62	(0.94–4.30)	This study
	Hong Kong	11	-	-	2.30 ± 4.50	(<0.36–12.94)	5.97 ± 4.39	(<0.6–12.12)	-	-	2.18 ± 6.97	(<0.36–23.17)	12.08 ± 29.14	(<0.6–84.10)	Parsons, 1998, 1999

slightly decreasing trend as they grow. Apart from this, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and body length of the other cetaceans do not show any significant change with their growth (Fig. 2). Furthermore, the $\delta^{15}\text{N}$ values of the Risso's dolphins could be divided into two subgroups, a group of three larger males (body length = BL > 270 cm) and a mixed gender group of five smaller dolphins (BL < 200 cm). The subgroup of larger samples has a significant lower mean $\delta^{15}\text{N}$ value ($12.21 \pm 0.26\text{‰}$) than that ($13.27 \pm 0.24\text{‰}$) of the smaller group ($p < 0.05$).

The four neritic toothed whales are sympatric odontocetes on the eastern coast of Taiwan (Wang et al., 2012) which have a trophic level of 3–4 in the marine ecosystem (Auriolles-Gamboja et al., 2013). They have overlapping ecological niches, and may forage at different water depths, partitioning the food sources. Wang et al. (2012) reported that Risso's dolphins have the narrowest dietary niche width, feeding mainly on enoploteuthid squid, *Enoploteuthis chunii* (family Enoploteuthidea, 95.5% in total number of prey (N%)), whereas pantropical spotted dolphins have the widest dietary niche width, and Fraser's dolphins lie in the middle. Fraser's dolphins prefer the mesopelagic prey living at a depth of 200–1000 m and do not have any surface dwelling prey (Huggins, 2011). They feed on a greater variety of food sources, not only squid (*Enoploteuthis chunii*, 13.5 N%), but also many epipelagic and mesopelagic fishes, mainly hatchetfishes (Sternoptychidae), i.e. *Polyipnus stereope* (50.2 N%). The pantropical spotted dolphins mainly feed on lanternfishes (Myctophidae) and squid (Enoploteuthidae), constituting 49.8 N% and 25.8 N% of their diet, respectively (Wang et al., 2012). Moreover, the results of stomach analysis of 5 dwarf sperm whales showed that the whales feed exclusively on cephalopods, e.g. *Enoploteuthis chunii* (58.5 N%), *Taonius pavo* (11.4 N%) and *Histioteuthis miranda* (10.6 N%) (Wang et al., 2002).

Among the 7 cetaceans, the pantropical spotted dolphins, Fraser's dolphins and Risso's dolphins show positive relationships between hepatic/renal Cd concentrations and body length. For the other four species, the relationship is not clear (Fig. 3(A) and (B)). In the case of hepatic/renal As, only the Risso's dolphins showed a negative trend for both hepatic/renal As and body length, while the pantropical spotted dolphins showed a negative trend only for renal As and body length. No relationship could be found

between As and body length in the other species (Fig. 3(C) and (D)). In particular, there were significant differences between the small and large Risso's dolphins. Significant lower Cd concentrations of the three tissues were found in the small Risso's dolphins compared with the large ones (Muscle: 0.04 ± 0.04 vs 1.48 ± 0.16 ; Liver: 4.67 ± 3.01 vs 96.41 ± 27.73 ; Kidney: 13.78 ± 8.74 vs $112.61 \pm 50.10 \mu\text{g g}^{-1}$ dry weight ($p < 0.05$)), whereas the case of As was the opposite (Muscle: 3.12 ± 1.14 vs 0.24 ± 0.14 ; Liver: 6.23 ± 2.54 vs 1.34 ± 1.13 ; Kidney: 4.49 ± 1.63 vs $0.86 \pm 0.63 \mu\text{g g}^{-1}$ dry weight for the small and large groups, respectively ($p < 0.05$)).

Combining the trend of renal Cd/isotope values, $\delta^{13}\text{C}/\delta^{15}\text{N}$ value, and body length may pinpoint their feeding location and the trophic level change as they grow. This can also show the habitat and food source partitioning of the neritic toothed whales. The Fraser's dolphins, pantropical spotted dolphins and Risso's dolphins' hepatic/renal Cd tissue concentrations increase with their growth. In combination with the relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and their body length, three types of inshore–offshore living styles emerged. First, the concentration of renal/hepatic Cd increases dramatically, and is combined with a decrease in their $\delta^{13}\text{C}$ value as the Fraser's dolphins grow. This implies that they migrate from neritic waters to oceanic waters when they become larger (BL > 240 cm) and are able to dive deeper for deep-sea hatchetfishes. The young Fraser's dolphins (BL < 206 cm) inhabit continental waters and then therefore highly overlap with dwarf sperm whales and pantropical spotted dolphins (Yeh, 2001). Secondly, on the contrary, pantropical spotted dolphins move closer to the shore as they grow, and are able to forage for inshore fishes and squids. Therefore, these two species of dolphins both show increasing Cd concentrations as they grow, but from different sources. Finally, the remaining two dolphins show no inshore–offshore migration as they grow. In the case of Risso's dolphins, the three large males (BL > 270 cm) contained significantly higher hepatic and renal Cd than the smaller ones. According to their $\delta^{13}\text{C}$ values, there is no sign of inshore–offshore movement as they grow; however, the larger males showed much lower $\delta^{15}\text{N}$ value than the smaller ones. Accordingly, we suggest that the larger male has the deep diving ability to harvest exclusively deep-sea and lower trophic cephalopods. Such a negative relationship between renal Cd concentration and $\delta^{15}\text{N}$ value of cetaceans was also found in the study of Das et al. (2003). They found that striped dolphins, common dolphins, harbor porpoises and white-beaked dolphins on the Irish and French Channel coasts, NE Atlantic Ocean, contained tissue Cd concentrations which changed with the amount of cephalopod intake, and displayed lower $\delta^{15}\text{N}$ values as the dolphins grow. However, the dwarf sperm whales, a cephalopod exclusive feeder (Wang et al., 2002), which showed constant $\delta^{13}\text{C}$, as well as hepatic and renal Cd concentrations, but with a slight increase of $\delta^{15}\text{N}$ values as they grow, appear to have a more varied diet of squids (Wang et al., 2002) which may contain lower Cd concentrations and be situated at higher trophic levels.

The As concentration in the tissue of the cetaceans mostly did not show any intraspecific difference by size, except for the Risso's dolphins which had the highest As concentrations in all three tissue types, and showed a significant size difference. The small Risso's dolphins had higher As concentrations than the large ones. Hepatic As levels in marine mammals vary by species and depend on feeding habits (Kunito et al., 2008). Our results echo the finding of Kunito et al. (2008) that species feeding on cephalopods tend to contain higher As concentrations than those feeding on fish. This can be seen in the cephalopod-loving Risso's dolphins and pantropical spotted dolphins vs the plankton-feeding Omura's whales and the fish-eating Chinese white dolphins. However, the high concentrations of As found in the Fraser's dolphins suggests that their As source may also be the hatchetfishes they consume.

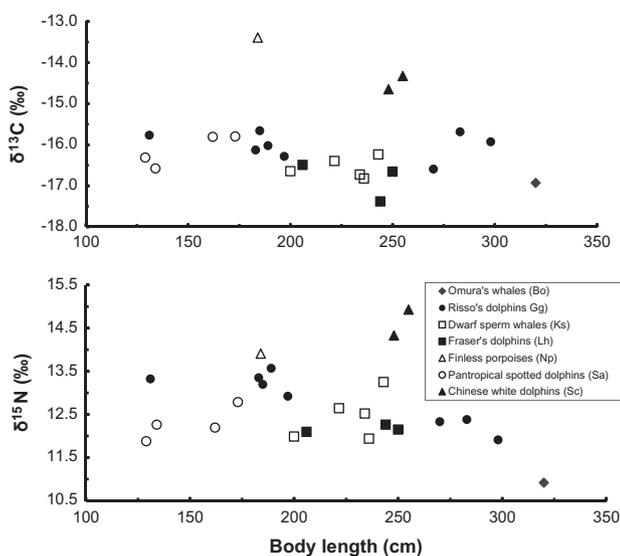


Fig. 2. The relationships between body length (cm) and the muscular $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the seven cetaceans from Taiwanese waters. Bo = Omura's whales (*Balaenoptera omurai*), Lh = Fraser's dolphins (*Lagenodelphis hosei*), Ks = dwarf sperm whales (*Kogia sima*), Sa = pantropical spotted dolphins (*Stenella attenuata*), Gg = Risso's dolphins (*Grampus griseus*), Np = finless porpoises (*Neophocaena phocaenoides*) and Sc = Chinese white dolphins (*Sousa chinensis*).

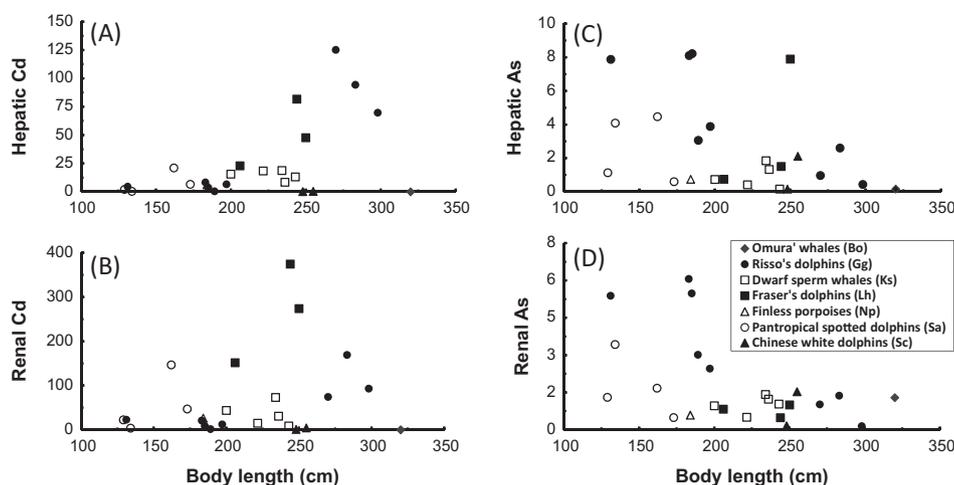


Fig. 3. The relationships between the body length (cm) and hepatic/renal Cd and As concentrations ($\mu\text{g g}^{-1}$ dry weight) for seven cetaceans from Taiwanese waters. Bo = Omura's whales (*Balaenoptera omurai*), Lh = Fraser's dolphins (*Lagenodelphis hosei*), Ks = dwarf sperm whales (*Kogia sima*), Sa = pantropical spotted dolphins (*Stenella attenuata*), Gg = Risso's dolphins (*Grampus griseus*), Np = finless porpoises (*Neophocaena phocaenoides*) and Sc = Chinese white dolphins (*Sousa chinensis*).

Distinct differences in As tissue concentration and $\delta^{15}\text{N}$ value were found in the two groups of Risso's dolphins. Hepatic/renal As concentrations and $\delta^{15}\text{N}$ values were higher in the smaller Risso's dolphin group than in the larger group. The two subgroups of Risso's dolphins could be divided by age: 15 year-old and older male adults (270–298 cm of body length, $n = 3$) and 0–4 year-old immature individuals (131–197 cm of body length, $n = 5$) (Chen et al., 2011). Therefore, it is suggested that the sexually-mature male adults feed on more deep-sea cephalopods, which may contain higher Cd concentrations and have a lower trophic level, while immature individuals feed on diverse prey species in pelagic waters, in which the prey contain higher As concentrations from natural and anthropogenic sources.

The tissue concentrations of As and Cd of the 7 Taiwanese cetaceans mostly fall within the range of the tissue As and Cd concentrations of the same species worldwide (Table 1). However, two large Fraser's and Risso's dolphins, as well as three small Risso's dolphins, contained elevated renal Cd and hepatic As concentrations, respectively, which would pose a health threat resulting from the two metals. Omura's whales and Chinese white dolphins do not feed on cephalopods at all so they have very low Cd tissue concentrations. The remaining 5 dolphins all had mean renal Cd concentrations higher than that of a healthy person ($19 \mu\text{g g}^{-1}$ dry weight) (Barregard et al., 1999), showing a higher Cd intake from their diet.

Several renal dysfunction diagnosis standards are considered: (1) Caurant's (2013) suggestion of a renal Cd concentration threshold for European small cetaceans of $50 \mu\text{g g}^{-1}$ wet weight ($\sim 225 \mu\text{g g}^{-1}$ dry weight), (2) in humans, renal Cd concentrations above 200–400 $\mu\text{g g}^{-1}$ dry weight can lead to renal damage (Piotrowski and Coleman, 1980), and (3) Fujise et al. (1988) noted that renal dysfunction can occur in cetaceans when hepatic concentrations exceed $20 \mu\text{g g}^{-1}$ wet weight ($\sim 90 \mu\text{g g}^{-1}$ dry weight). The two large Fraser's dolphins (244 cm female and 250 cm male BL) contained renal Cd exceeding $225 \mu\text{g g}^{-1}$ dry weight (374 and $274 \mu\text{g g}^{-1}$ dry weight for renal Cd, and 82 and $48 \mu\text{g g}^{-1}$ dry weight for hepatic Cd, respectively), and the two large male Risso's dolphins (270 cm and 283 cm BL) contained hepatic Cd exceeding $90 \mu\text{g g}^{-1}$ dry weight (125 and $94 \mu\text{g g}^{-1}$ dry weight for hepatic Cd, and 75 and $169 \mu\text{g g}^{-1}$ dry weight for renal Cd, respectively), showing significant Cd contamination (Eisler, 1985) that would pose a kidney dysfunction health problem (Elinder and Jarup, 1996).

In the case of hepatic As concentrations, the background level for Indian people is $0.16 \mu\text{g g}^{-1}$ dry weight, in comparison with

the hepatic concentration for chronic arsenicosis which is $1.46 \mu\text{g g}^{-1}$ dry weight (Santra et al., 1999). It seems that more than half of our cetaceans have hepatic and renal As concentrations higher than $1.5 \mu\text{g g}^{-1}$ dry weight (Fig. 3(C) and (D)). Once again, as with Cd, such high As tissue concentrations found in the toothed cetaceans most likely come from their cephalopod-eating habit (Kunito et al., 2008). Organisms that have As tissue residues exceeding $1.3 \mu\text{g g}^{-1}$ wet weight ($\sim 5.85 \mu\text{g g}^{-1}$ dry weight.) are therefore considered to be As polluted (Eisler, 1988). Moreover, three small Risso's dolphins (131 cm female, 183 cm male, and 185 cm male BL) contained hepatic As concentrations of 7.88, 8.11 and $8.23 \mu\text{g g}^{-1}$ dry weight, respectively, and one Fraser's dolphin (250 cm male BL, hepatic As concentration = $7.89 \mu\text{g g}^{-1}$ dry weight) broke the highest hepatic As record held by a harp seal, *Pagophilus groenlandicus*, recorded as $7.68 \mu\text{g g}^{-1}$ dry weight (Kunito et al., 2008). It seems that these four dolphins are likely to have suffered from As-induced health problems.

To summarize, using the muscular $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data of the cetaceans, we can categorize the ecological niches of cetaceans in Taiwanese waters into three groups according to their ecological niches and geographic distribution. Moreover, via the correlation analysis between the Cd and As tissue concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data, and body length, we can further understand the resource partitioning of sympatric odontocetes in the neritic zone, showing their interspecific diet habits and intraspecific diet shifts. Moreover, a spatiotemporal distribution pattern can be emerged that the pantropical spotted dolphins migrate inshore as they grow, whereas the Fraser's dolphins move off the neritic waters as they grow. They share the space and food resources with Risso's dolphins and dwarf sperm whales in the very narrow sublittoral zone of Eastern Taiwan. Furthermore, the extremely high renal or hepatic As and Cd concentrations found in the large Fraser's dolphins and Risso's dolphins is assumed to pose a health threat derived from their prey sources that is hatchetfishes for Fraser's dolphins and Enoploteuthid squid for Risso's dolphins. It would be worth further investigating the As and Cd concentrations in those prey organisms to better understand the food chain pathways of the two metals.

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