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Trace element concentrations, risks and their correlation with metallothionein genes polymorphism: A case study of narrow-ridged finless porpoises (*Neophocaena asiaeorientalis*) in the East China Sea

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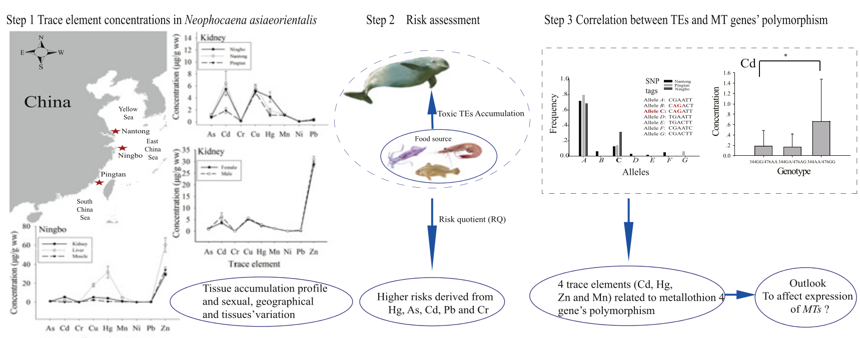
HIGHLIGHTS

- Trace elements (TEs) and metallothionein gene in finless porpoise were analyzed.
- TEs concentration was positively correlated with body length, and varied in sexes.
- TEs concentration is likely related to local environment pollution levels.
- Nantong and Ningbo porpoises could face health risks due to Hg, As, Cd, Pb, and Cr exposure.
- Two polymorphic metallothionein gene sites related to accumulations of Cd, Hg, Zn and Mn were detected.

GRAPHICAL ABSTRACT



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ABSTRACT

The concentration of trace elements (TEs) and their risk to narrow-ridged finless porpoises (*Neophocaena asiaeorientalis*) are still unclear. The present study determined the concentration of typical TEs in liver, kidney, and muscle tissues from porpoises in the East China Sea, assessed potential health risk of TEs to porpoises, and explored the relationship between TE concentration and metallothionein genes (*MTs*) polymorphism. It was found that Zn, Cu, Mn, Cd and Hg were highly accumulated in liver, and Cd was highly accumulated in kidney. The concentrations of Cr, As, Pb and Ni were very low in all three tissues. TE concentrations showed significant positive correlation with body length, and sexual variation. The levels of most TEs were higher in tissues of porpoises in Ningbo and Nantong than in Pingtan, which is likely related to the local environment pollution level. The risk assessment showed that porpoises from Nantong and Ningbo could face health risks due to Hg, As, Cd, Pb, and Cr exposure. Moreover, two polymorphic sites on the *MT4* gene were found to be significantly associated with increased levels of Hg, Cd, Zn and Mn. Whether these two polymorphic sites are involved in expression of *MTs*, or other functional processes, needs further research.

1. Introduction

Trace elements (TEs) are present in trace concentrations in various environmental matrices (Tchounwou et al., 2012). Chronic exposure to non-essential TEs such as As, Cd, Hg, and Pb even at relatively

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low levels could cause negative impacts on health of humans and aquatic mammals (Llobet et al., 2003; Lavery et al., 2009; Maanan et al., 2015). Some essential TEs, such as Cu and Zn, at excessive levels could also invoke toxicity (Rosa et al., 2008, Jović and Stanković, 2014).

Small cetaceans have been regarded as indicator species for assessing nearshore pollution due to their long-term accumulation of TEs and to being apex predators (Das et al., 2003, Bellante et al., 2012, Cáceres-Saez et al., 2013). The accumulation of TEs has been recorded in >60 cetacean species in the last three decades (O' Shea, 1999, Lavery et al., 2009, Cáceres-Saez et al., 2013), however, reports about toxic effects on wild marine mammals are still rare (Lavery et al., 2008), because toxicity studies on live cetaceans are largely ethically prohibited. The toxicological risk that TEs pose is an issue that researchers have focused on (Llobet et al., 2003; Liu et al., 2015); currently evaluation of the toxicological effects of pollutants on marine mammals is often estimated via derivation models/indices (Sample et al., 1996; Hung et al., 2004; Randhawa et al., 2015).

In order to assess the chronic impact of pollutants on the marine environment, a suite of biomarkers has been developed. Biomarkers, especially cytochrome P4501A enzyme induction, acetylcholinesterase inhibition, DNA integrity and metallothionein induction have received special attention (Sarkar et al., 2006). For example, the up-regulation of metallothioneins (MTs) has been correlated with exposure to toxic metal pollutants, and thus, has proved to be a useful health assessment tool for mammals (Das et al., 2002; Lavery et al., 2008; Lavery et al., 2009). However, metallothionein extraction depends on fresh tissue samples, which is usually impractical for marine mammals. Instead, we may evaluate protein-relative metallothionein genes. Furthermore, the single nucleotide polymorphism sites (SNPs) located in functional genes or near encoding regions could serve as useful tools for correlation analysis between genetic variation and function (Chambers et al., 2008; Chen et al., 2010). Because different SNPs within metallothionein genes may present different responses to toxicity of TEs (Kita et al., 2006), the relationships between TE accumulation and SNPs at *MT* gene sites might provide an index of health in cetaceans.

Narrow-ridged finless porpoises (*Neophocaena asiaeorientalis*, NFPs) are one of the most common cetacean species in northeastern Asia, including Chinese coastal waters (Wang and Reeves, 2012). The East China Sea (ECS) is a key distribution area of NFPs, but the TEs research there was deficient (Zhou et al., 1994). The ECS is consistently contaminated by land-based pollutants discharged by the Yangtze River and Qiantang River (Asante et al., 2008; Chen et al., 2014). For instance, the amounts of anthropogenic heavy metals delivered into the ECS from the Yangtze River have been increased from 5000 tons in 2002 to 36,200 tons in 2012 (NBO, 2003–2013). This increase highlights the urgent need for research to understand the potential health risks for ECS NFPs. Outside of ECS, some studies have been conducted in TE levels in finless porpoises from other Chinese waters, such as Bohai, Hong Kong, Beibu Gulf (Zhou, 1986; Yang et al., 1988; Zhang et al., 1995; Parsons, 1999; Dong et al., 2006; Hung et al., 2007; Wang et al., 2008; Murphy et al., 2010). Therefore, our research would fill the information gap.

Here, we analyze samples of NFPs from ECS to: 1) describe concentration characteristics of TEs in typical tissues; 2) compare pollutant concentrations among porpoises from three geographical populations; 3) assess the health risk of being exposed to TEs; and 4) explore the correlation between the tissue TEs accumulation and *MTs* polymorphism. Furthermore, these populations' ecological parameters such as population size, habitat use was unavailable, and the conservation status was unknown. This study would have important conservation implications from the perspective of TEs' influence on porpoise health.

2. Materials and methods

2.1. Tissue samples

A total of 61 NFPs with 166 samples (57 kidneys, 56 livers and 53 muscles) were used for TEs determination. All the tissue samples were collected during 2008–2011 from Nantong, Ningbo, and Pingtan along coasts of ECS (Table 1, Fig. 1). Most animals died from fisheries bycatch, and there were no obvious pathological features and fatal injuries were found. Our samples with an approximately equal sex ratio (female: male, Nantong 12:11, Ningbo13:11, and Pingtan 7:7). To minimize the effect of age-related bioaccumulation on TE concentrations (Borrell et al., 2014), body length was used as a covariate during analysis. In order to evaluate the risk from consuming contaminated food items (see Section 2.4), 29 potential prey species (Chen et al., 1979, Zhou et al., 1993, Barros et al., 2002, Shirakihara et al., 2008, B Chen's unpublished data) were also sampled (Appendix A). For a better population genetic analysis, additional NFPs samples were also sequenced, consequently 63 and 76 DNA templates were used respectively for *MT2* and *MT4* (Table 2). Finally, 56 (Nantong, 23; Ningbo, 19; Pingtan, 14) of the genetic samples sequenced had TEs quantified.

2.2. Trace element determinations

Determination of trace elements followed the procedures of Tu et al. (2012). Generally, 0.1978–0.2122 (g) tissue samples were used. Concentrations of nine TEs (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn) were measured by ICP-MS (Inductively Coupled Plasma Mass Spectrometry, Aglient 7700, USA) with scandium, germanium and rhodium as the internal standards. Methodological accuracy was determined by the duplicated measurements through blank and sample spikes. The standard solutions (GSB 04-1767-2004, GSB 04-1729-2004, Aglient) were obtained from the National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials (NCATN, Beijing, China) for matrix spikes. The recovery was satisfactory for all the elements of interest (As: 101.9%, Cd: 101.4%, Cu: 90%, Cr: 94.2%, Hg: 109.9%, Mn: 86%, Ni: 90.4%, Pb: 100.5%, Zn: 122%). Furthermore, the reference material GBW (E) 080193 (bovine liver) was also utilized to optimize the conditions. Determinations of all tissue trace elements were conducted by trained professionals in Nanjing Normal University Center for Analysis & Testing and laboratory of Jiangsu Sinography Testing Company Limited.

2.3. Test of TEs variation on body length, sex and geographical populations

Relationships between body length or sex and TE accumulation were analyzed using parametric regression and covariance analysis (ANCOVA) respectively by the SPSS.

ANCOVA was also used to reveal accumulation difference of TEs among three geographical populations (Jarić et al., 2011). The age was not available, so we used body length as covariate in ANCOVA (Parsons, 1999; Das et al., 2003). The followed pair-wise comparisons among three populations were conducted by the least significant difference (LSD) method. Comparing the TE concentrations between tissues, for each population, non-parametric tests were performed by InStat 3 software (Kruskal-Wallis test followed by Dunn's Multiple Comparisons Test).

2.4. Risk assessments indices for toxicity

The risk assessment methods *RfD*- (Reference Dose) and *TRV*- (Toxicity Reference Values) based risk quotient (RQ) were employed which had been used for cetaceans, (*S. chinensis* and *N. phocaenoides*)

Table 1
Sampling information for finless porpoises examined in this study.

Population	Total	Number (sex)	Kidney	Liver	Muscle
Ningbo	24	13 (female), 11 (male)	24	24	23
Nantong	23	12 (female), 11 (male)	20	19	16
Pingtan	14	7 (female), 7 (male)	13	13	14
Total	61	32 (female), 29 (male)	57	56	53