Science of the Total Environment 575 (2017) 628–638

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

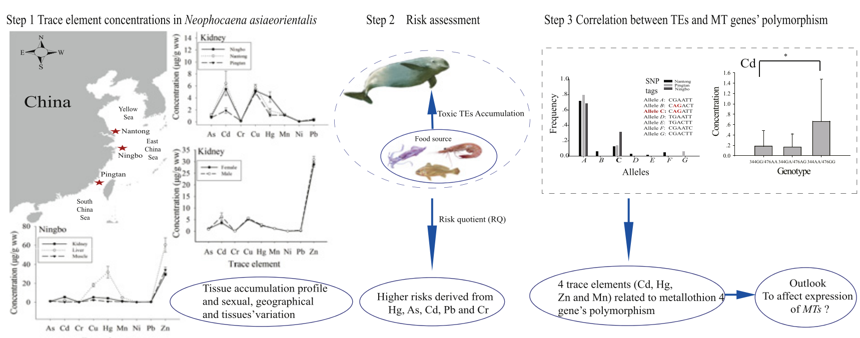
Jun Liu [a], Bingyao Chen [a,*], Thomas Allen Jefferson [b], Hui Wang [a], Guang Yang [a,*]

[a] Jiangsu Key Laboratory for Biodiversity and Biotechnology, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210023, China
[b] Clymene Enterprises, Yerba Valley Way, Lakeside, CA 92040, USA

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



ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form 8 September 2016
Accepted 8 September 2016
Available online 30 September 2016

Editor F.M. Tack

Keywords:
Finless porpoises
Heavy metals
Metallothionein
Risk assessment
Tissue trace element

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

* Corresponding authors.
E-mail addresses: chby2632@163.com (B. Chen), gyang@njnu.edu.cn (G. Yang).

http://dx.doi.org/10.1016/j.scitotenv.2016.09.062
0048-9697/

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

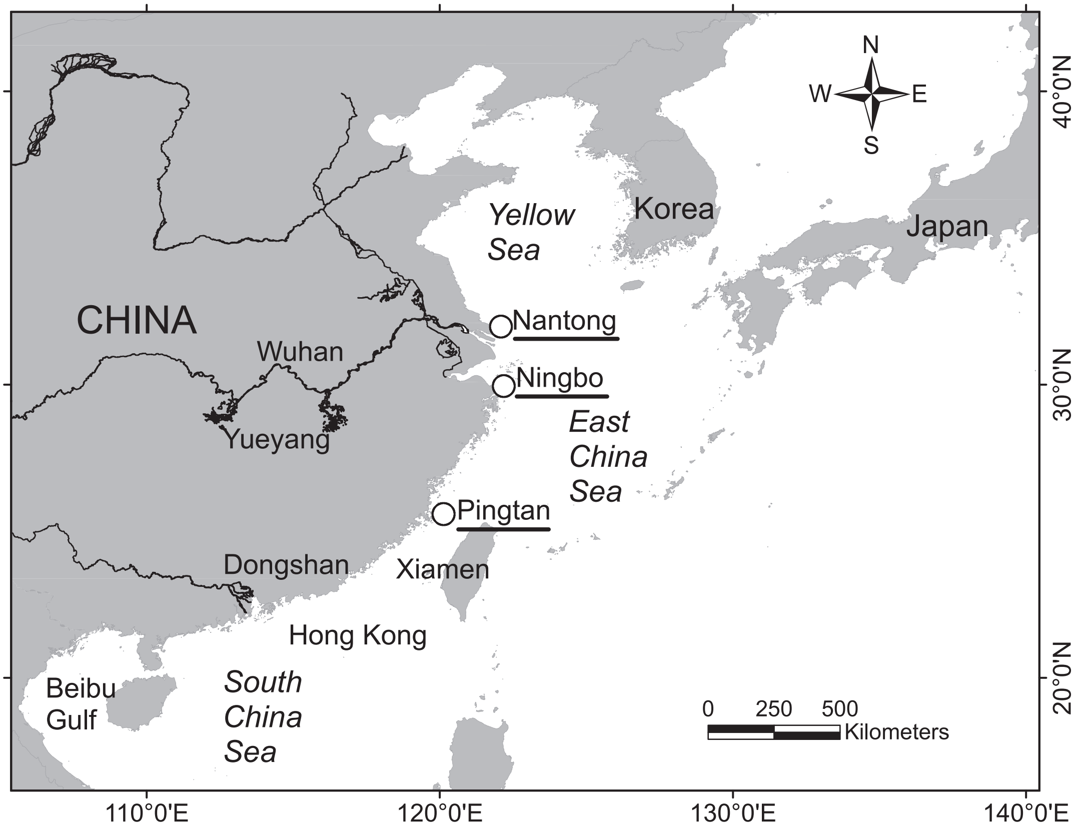


Fig. 1. The sampling location of narrow-ridged finless porpoises in East China Sea.

(Hung et al., 2004; Hung et al., 2007). The RQ was calculated by formula below (Hung et al., 2007):

$$RQ = \text{Concentration of TEs } in \text{ food}/MAC_{RfD}(\text{or}MAC_{TRV})$$

$$MAC_{RfD}(\text{or } MAC_{TRV}) = (RfD\ (\text{or } TRV) \times BW \times AT)/(IR \times FI \times EF \times ED)$$

where, most parameter values were obtained from references mentioned in Hung et al. (2007). The values of RfD and TRV were summarized in Table 3. AT: averaging time (period over which exposure is averaged in days), 10,220 days; BW, body weight, we used 40 kg in present study; IR: gestation rate, 3 kg/day; FI: fraction ingested from contaminated source, unitless, 0.9; EF: exposure frequency, 365 day/year; ED = exposure duration, 28 years (Hung et al., 2004, 2007).

With respect to the concentration of TEs in food, 50th and 95th percentile data was used for RQ. When RQ was larger than unit (value = 1) it would mean that there was a chance that the contaminant did pose a risk to the animal (Hung et al., 2004). Further, concentrations of TEs in some other small cetaceans were also reviewed for comparison.

2.5. DNA extraction and SNPs identification

Metallothionein-2 binds various heavy metals, Metallothionein-4 seems to bind zinc and copper (Skutkova et al., 2012) (see in the database GeneCards). The Metallothionein-2 gene (*MT2*) and Metallothionein-4 gene (*MT4*) were chosen to explore the correlation between genetic polymorphism and TE accumulations in NFPs.

Extraction of genomic DNA from muscle samples followed the standard protocol described by Sambrook et al. (1989). A fragment of *MT2* was amplified by Polymerase chain reaction (PCR) using the following primers designed according to known sequences from Ensembl (species, *Tursiops truncutus*): forward: 5′-ACCCGCCTCTATTCTAAGTT-3′; and reverse: 5′-TTGTCCTGGTTGCTCTATTT-3′. *MT4* forward: 5′-CAGCATC

Table 2
Genetic characteristics of *MT2* and *MT4* of narrow-ridged finless porpoises in the East China Sea.

Genes	Populations	Number of individuals	Number of sequences	Number of sites	Number of haplotypes (H)	Nucleotide diversity (π)	Tajima' s D	Fu's Fs
MT2	Nantong	27	54	4	5	0.001	−1.144	−2.378
	Ningbo	17	34	2	3	0.001	−1.444	−0.391
	Pingtan	19	38	2	3	0.001	−0.902	−1.088
	Total	63	126	6	7	0.001		
MT4	Nantong	32	64	5	6	0.002	−0.239	−0.946
	Ningbo	19	38	2	2	0.001	1.655	3.232
	Pingtan	25	50	3	3	0.001	−0.195	0.797
	Total	76	152	6	7	0.001		

Table 3
Parameters for health risk assessment of narrow-ridged finless porpoises in the East China Sea.

Trace elements	RfD[a] (μg/g·ww/day)	MAC_{RfD}[b] (μg/g·ww)	TRV[c] (μg/g·ww/day)	MAC_{TRV}[b] (μg/g·ww)
As	0.0003	0.004	0.46	6.14
Cd	0.001	0.01	0.30	3.39
Cr	0.003	0.04	837.10	11,161.32
Cu	0.02	0.27	4.65	62.03
Hg	0.0007	0.01	0.40	5.35
Mn	0.14	1.87	26.91	358.86
Ni	0.02	0.27	12.23	163.12
Pb	–	–	2.45	32.62
Zn	0.3	4	48.94	652.47

[a] Obtained from Table 3 of Hung et al., 2007, references including Integrated Risk Information System, USEPA (IRIS) (http://www.Epa.gov/iris), ATSDR, 1995, WHO, 1996.
[b] 40 kg of average body weight from our data were used.
[c] Obtained from Sample et al., 1996.

TTCAACCTCCTGTCCATC-3′; and reverse: 5′-CCAACTCCTCGACTAATG-3′. Eight to16 clones from each individual were sequenced and two for each individual were picked up. For more accurate identification of SNPs in *MTs*, variable sites that appeared >3 times in all clones from the whole population were kept through sequence blasting using the MEGA 5 software (Tamura et al., 2011).

In order to investigate the polymorphism of *MTs* in NFPs, the indexes of the number of polymorphic sites (S) and haplotypes (h), nucleotide diversity (π), Fu's *Fs* value (Fu, 1997) and Tajima's D value (Tajima, 1989) were calculated using DnaSP V5 software package (Librado and Rozas, 2009). The correlation analysis between SNP genotypes and accumulation of trace elements in liver, kidney and muscle was performed using the LSD method and Mann-Whitney U test. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Accumulation profile of TEs in typical tissues

The results (Fig. 2) indicated that: 1) Concentrations of Zn, Cu and Mn in liver were significantly higher than in kidney for all populations; 2) Concentrations of Cd, Hg, Mn and Zn were significantly higher in liver than in muscle for all populations; 3) Concentrations of Cd, Cu and Mn were significantly higher in kidney than in muscle for all populations; 4) The accumulation level of Pb did not show any difference in three tissues of NFPs between the three populations; 5) Cr was accumulated to

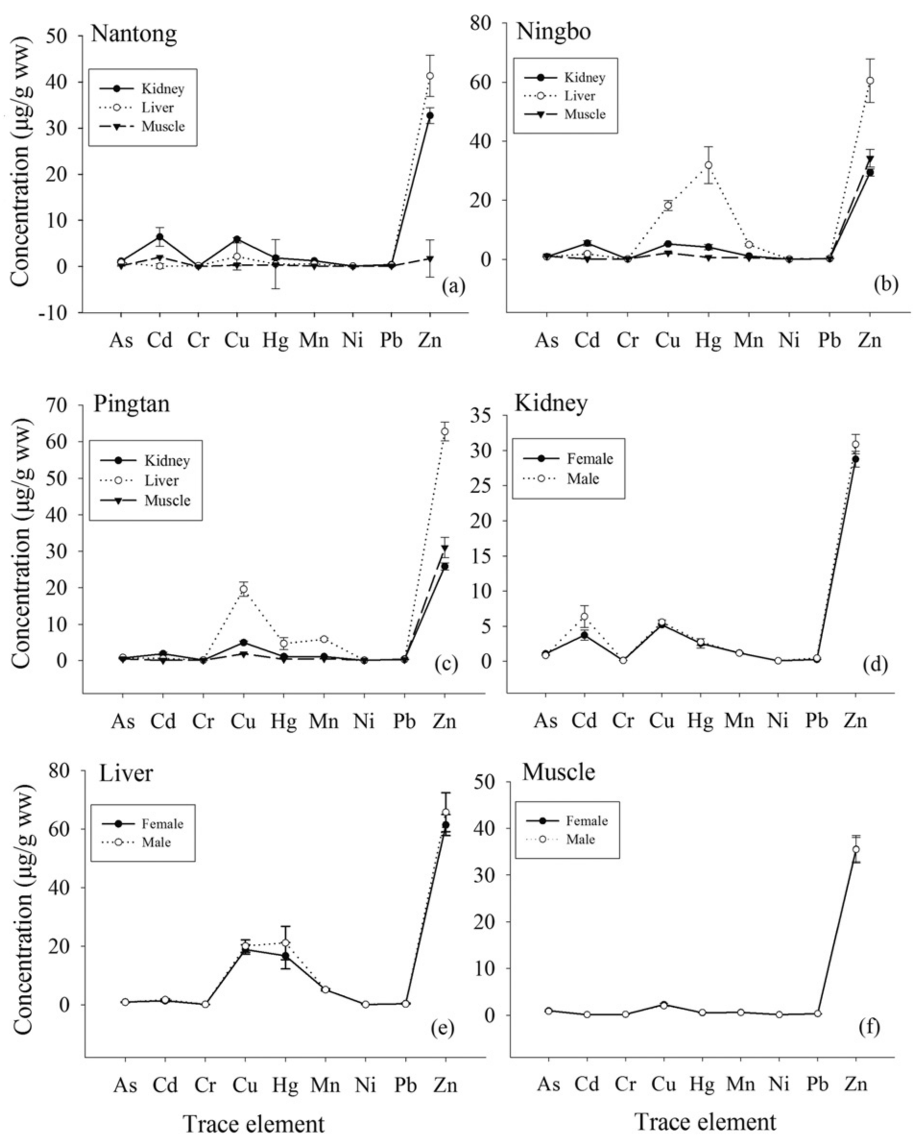


Fig. 2. The variation of TEs concentration among individuals by sex, geographical populations and tissues.

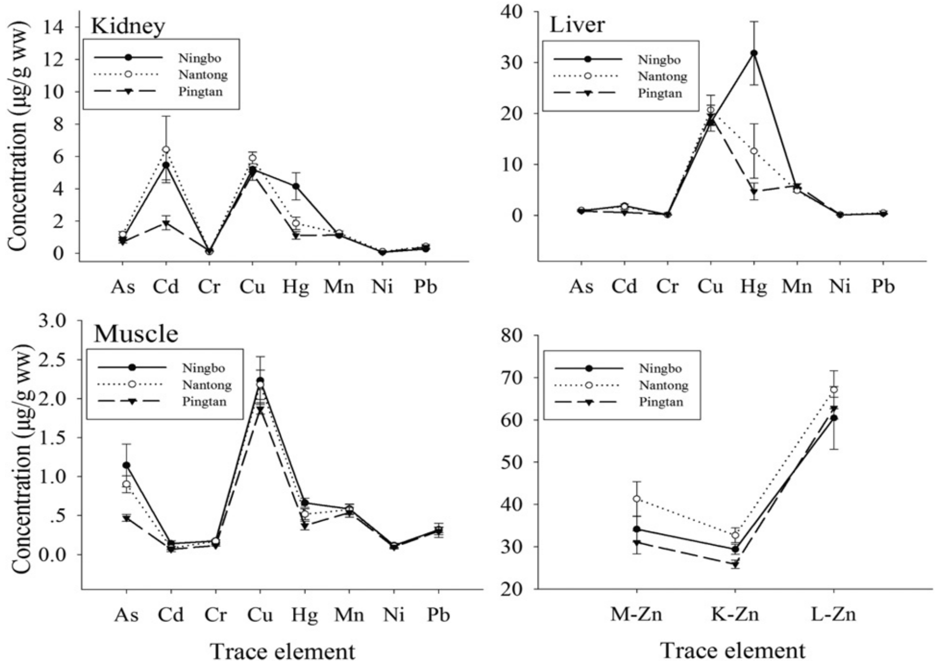


Fig. 3. Variance of TEs among three populations.

higher levels in the muscle than in other tissues for the locations Nangtong and Ningbo, but there was no significance difference in concentrations levels between tissues in Pingtan NFPs. The concentration of As in muscle was significantly lower than that in kidneys and livers of Pingtan population.

3.2. Difference in accumulation of TEs among populations, by body length and sex

ANCOVA analysis showed statistically significant differences of TEs in certain tissues, between the three populations: As ($F_{(2, 53)} = 9.014$, $p = 0.000$), Cu ($F_{(2, 53)} = 3.540$, $p = 0.036$), Hg ($F_{(2, 54)} = 6.223$, $p = 0.004$) and Zn ($F_{(2, 53)} = 4.585$, $p = 0.015$) in kidneys; Cr ($F_{(2, 43)} = 4.946$, $p = 0.012$), Hg ($F_{(2, 52)} = 7.548$, $p = 0.001$) and Mn ($F_{(2, 52)} = 3.947$, $p = 0.025$) in livers; As ($F_{(2, 49)} = 4.350$, $p = 0.018$), Cr ($F_{(2, 37)} = 3.529$, $p = 0.040$) and Hg ($F_{(2, 48)} = 4.581$, $p = 0.015$) in muscles.

The following LSD test revealed the main difference was between Ningbo or Nantong and Pingtan (Fig. 3). Between Ningbo and Pingtan, significantly higher levels of TE concentration were found in Ningbo NFPs for As, Hg and Zn in kidneys, Hg in livers, As, Cr and Hg in muscles; Between Nantong and Pingtan, significantly higher levels of TE concentration were found in Nantong NFPs for As and Zn in kidneys, Mn in livers and As in muscles; Between Ningbo and Nantong populations, significantly higher levels of Hg in the three tissues were found in Ningbo NFPs (See details in Appendix B).

Some TEs in the tissues of each population showed a significant positive correlation with body length (all $p < 0.05$), i.e. for the Ningbo population, Cd ($r^2 = 0.31$, $p = 0.00$), Hg ($r^2 = 0.39$, $p = 0.00$) and Zn ($r^2 = 0.19$, $p = 0.03$) in the liver tissue, Cd ($r^2 = 0.36$, $p = 0.00$) in the kidney tissue, and Hg ($r^2 = 0.43$, $p = 0.00$), Pb ($r^2 = 0.18$, $p = 0.04$), Zn ($r^2 = 0.20$, $p = 0.03$) in the muscle; for the Nantong population, As ($r^2 = 0.29$, $p = 0.02$), Cd ($r^2 = 0.41$, $p = 0.00$), Hg ($r^2 = 0.50$, $p = 0.00$), Mn ($r^2 = 0.25$, $p = 0.03$) in the liver tissue, Cd ($r^2 = 0.37$, $p = 0.00$), Hg ($r^2 = 0.50$, $p = 0.00$) in the kidney tissue and Hg ($r^2 = 0.45$, $p = 0.01$) in the muscle tissue; for the Pingtan population, Cd ($r^2 = 0.43$, $p = 0.01$), Pb ($r^2 = 0.40$, $p = 0.02$) in the liver tissue and Hg ($r^2 = 0.62$, $p = 0.00$) in the kidney tissue (Fig. 4).

For Pb (male, 0.46 ± 0.10 μg/g·ww; female, 0.27 ± 0.03 μg/g·ww) and Cd (male, 6.39 ± 1.57 μg/g·ww; female, 3.73 ± 0.72 μg/g·ww) in kidneys, male NFPs had significantly higher concentrations than female NFPs (ANCOVA, $p < 0.05$) (Fig. 2).

3.3. Risk assessment of TEs

The concentrations and variance of nine TEs among 29 prey species indicated that the highest concentration for TEs accumulated was for Zn, followed by concentrations of Cu and Mn (Appendix A). The *RfD*-based RQ showed relatively high risk from seven elements (Table 4), i.e. As presented the potentially highest level of risk, followed by Cd, Cr, and Cu, and then Hg, Mn, and Zn. *TRV*-based RQ results indicated low risk from most of the TEs. But, RQ of As at 95th percentile ranged from 1 to 10 (Table 4) indicating that As level could pose a potential risk to NFPs (Hung et al., 2004).

3.4. MTs polymorphism and its correlation with tissue TEs concentration

A total of 602 bps of *MT2*, and 885 bps of *MT4* were amplified, cloned and then sequenced. The same numbers of polymorphic sites (*S*), alleles (*h*), nucleotide diversity (π) were 6, 7, and 0.001 respectively, for both *MT2* and *MT4* (Table 2). No significantly negative values for Fu's *Fs* and Tajima's *D* were determined, indicating non-neutral evolution. Based on SNPs characters of both *MT2* and *MT4*, a total seven alleles (*A*–*G*) were defined. Most alleles, except for allele C, exhibited a much lower level of allelic frequency (Fig. 5). Allele *C* was shared in all populations with the relative high frequency of 12.5% for Nantong, 14% for Ningbo, and 31.5% for Pingtan (Fig. 5). Allele *C* has two obvious SNPs of *MT4* at loci 344 and 476 (between exon 2 and 3, at the second intron).

Ningbo, Nantong and Pingtan populations had similar genotype frequency (Fig. 5), i.e. main genotype of 344GG/476AA (58.3%, 60.9%, and 78.6% respectively), secondary genotype of 344GA/476AG (25%, 21.7%, and 21.4%) and 344AA/476GG (16.7%, 17.4%, and 0%).

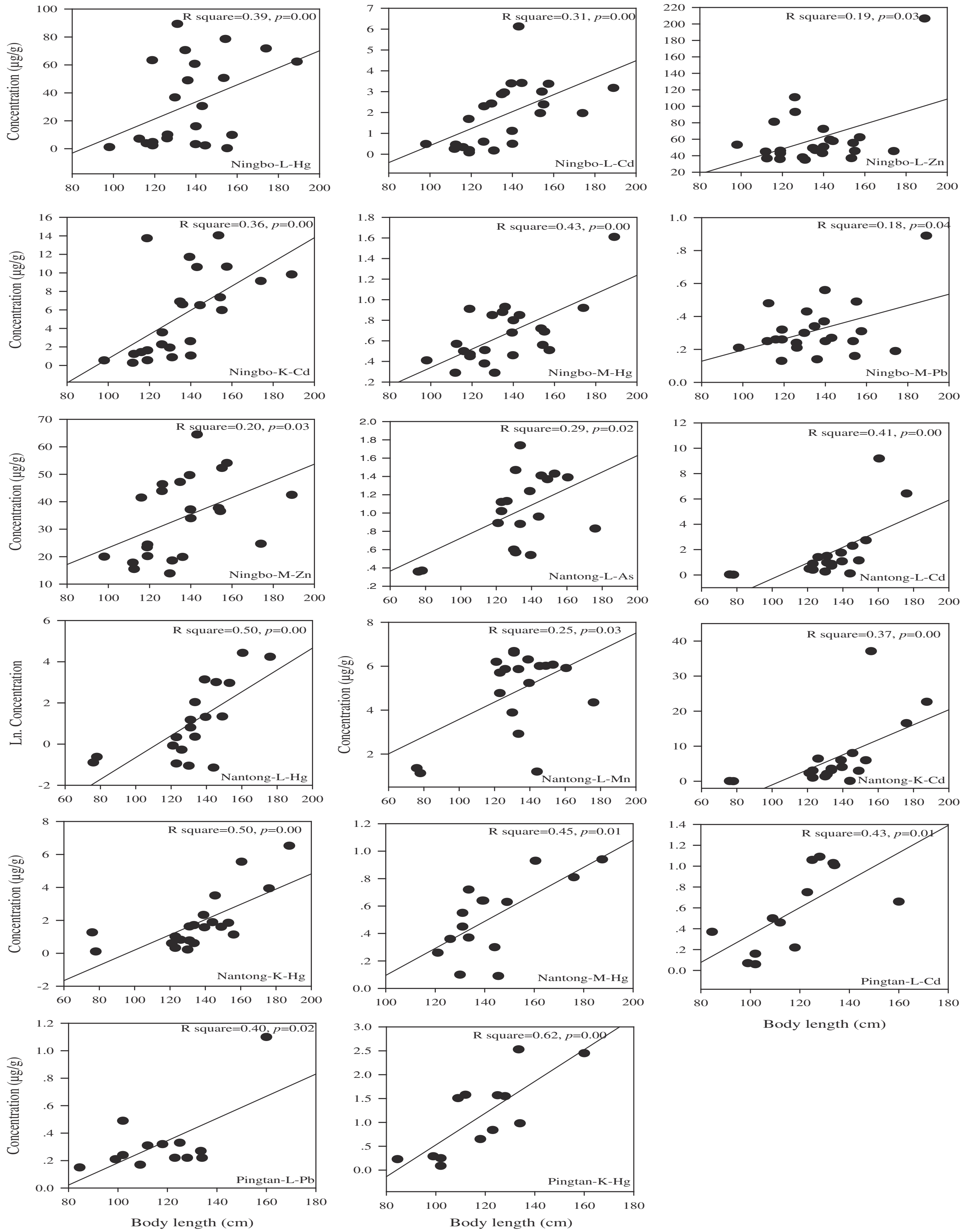


Fig. 4. Regression analysis between concentrations of tissue TEs and body length.

Females and males had also similar frequency of the genotype 344GG/476AA (65.6%, 65.5%), 344GA/476AG (25%, 20.1%) and 344AA/476GG (9.4%, 17%). For females and males, the sexual variation on genotype (Fig. 6) was not significant.

The SNPs of MT4 at loci 344 and 476 were used as variables for analyzing the correlation between SNPs frequency tissue TEs concentration. The types 344 GG/476 AA showed higher frequency in the individuals that accumulated more Zn and Mn in the liver tissue (Fig. 7). A higher

Table 4
RQ values estimated for the Nantong population using 50th and 95th percentile concentration data.

Contaminants	RQ (50th) <1	1–10	10–100	>100	RQ (95th) <1	1–10	10–100	>100
MAC_{RfD}								
As				612.5				3252.5
Cd			15.0					248.3
Cr			53.3				79.3	
Cu			16.9					106.5
Hg		9.6					18.2	
Mn		2.0					12.2	
Ni	0.8					2.6		
Zn		4.0					13.4	
Pb								
MAC_{TRV}								
As	0.40					2.12		
Cd	0.05				0.62			
Cr	0.0002				0.0003			
Cu	0.07				0.46			
Hg	0.02				0.03			
Mn	0.01				0.06			
Ni	0.001				0.004			
Zn	0.02				0.08			
Pb	0.003				0.01			

content of Zn in the kidney and, Cd and Hg in the muscle were closely related to the rare genotypes 344AA/476GG (Fig. 7).

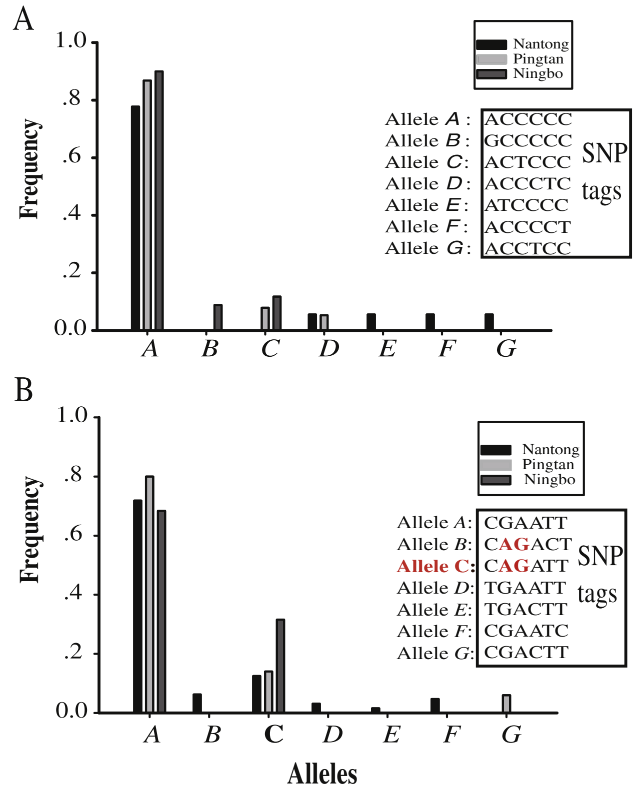


Fig. 5. Allele frequency and SNP tags for *MT2* (A) and *MT4* (B) in three populations.

4. Discussion

4.1. Distributions of trace elements in tissues

Higher concentrations of Zn, Cu, Mn and Hg accumulated in livers than in kidneys and muscles (Fig. 2), possibly because of the essential biochemical function of liver (WHO, 1996; Tchounwou et al., 2012), its role in the immune response and clearance of toxic components (Jirillo et al., 2002). High concentrations of Cd in kidneys might lead to more Cd in urinary organs, which was correlated with renal dysfunction (Babula et al., 2012) and might be harmful to the kidney. On the other hand, highest Cd concentrations are usually higher in kidney than other organs (Parsons, 1999; Seixas et al., 2007; Bellante et al., 2012; Shoham-Frider et al., 2014) due to the presence of metal binding proteins (Das et al., 2003).

4.2. Geographical, body length, and sexual variation

Previous work has shown that levels of tissue TEs are affected by geographical location, sex, and age (Das et al., 2003), and these findings were supported by the present study. TEs concentrations in Nantong and Ningbo populations were found to be significantly higher than that in Pingtan (Fig. 3). Comparing with NFPs along coasts of China, the general order from high to low in terms of TE level (especially Hg and Cd) in NFPs, is as follows: Bohai Sea > East China Sea > South of East China Sea (Appendix B). Actually, TE levels in sediment were also consistent with the pollution trend, according to comparison of Pb, As, Ni and Cr content (Table 5).

In our research, toxic TEs (As, Cd, Hg and Pb) were found to be positively related with body length (Fig. 4). An accumulation of toxic TEs might reflect a low excretion rate of these TEs (Das et al., 2003). Furthermore, the concentrations of Pb and Cd in kidneys of female porpoises were statistically significantly lower than in males. This may be related to the transference of TEs from females to calves via lactation or across the placenta (Das et al., 2003; Parsons, 2004; Kubota et al., 2005; Yang et al., 2008; Kamel et al., 2014; Noël et al., 2016).

4.3. Risk assessments of TEs

TRV-based and *RfD*-based RQ results differed in our study (Table 4). Considering that *RfD* is generally used for human health risk assessment and *TRV* derived from toxicological studies on terrestrial mammals (Sample et al., 1996; Hung et al., 2004, 2007), the results might not completely represent the actual risks of TEs on NFPs. However, due to the high value of *RfD*-based RQ, As, Cd, Cr, Cu, Hg, Mn and Zn should be considered as possible contaminants of concern.

Immune and reproductive systems of marine mammals could be impacted by TEs (Reijnders, 1980; Reijnders et al., 1999; Sonne, 2010; Desforges et al., 2016). Some threshold levels were preliminarily determined, e.g. suppression of lymphocyte proliferation was determined to be 0.002-1.3 μg/g for Hg, 0.1–2.4 μg/g for cadmium in blood (Desforges et al., 2016). In this study, we noticed concentrations of As in the liver of Nantong NFPs were close to the level of about 3.767 μg/g·dry weight (1.017 μg/g·ww), at which the porpoise would be considered to be contaminated, in comparison with the hepatic concentration of 1.46 μg/g dry weight for chronic arsenicosis (Liu et al., 2015). The concentration of Hg in livers of Ningbo NFPs (31.85 μg/g·ww) were close to that in *Sousa chinensis* (35.43 μg/g·ww, Appendix B) reported by Parsons (1998) who considered the level to be potentially health threatening. Besides, this level is higher than 20.0 μg/g wet weight level of mercury above which it was found that harbor porpoises (*Phocoena phocoena*) were more susceptible to die of infectious disease (Bennett et al., 2001). These suggested there might be a potential health risk associated with exposure to As and Hg (Bennett et al., 2001; Liu et al., 2015; Monk et al., 2014).

Mean concentration of Pb in liver of Nantong porpoises (Appendix B) is close to that of *S. chinensis* from Hong Kong waters (Parsons, 1998; Parsons, 1999) and *S. chinensis* from Xiamen waters (Chen et al., 2007), but higher than those of harbor porpoises that died from infectious disease (Bennett et al., 2001).

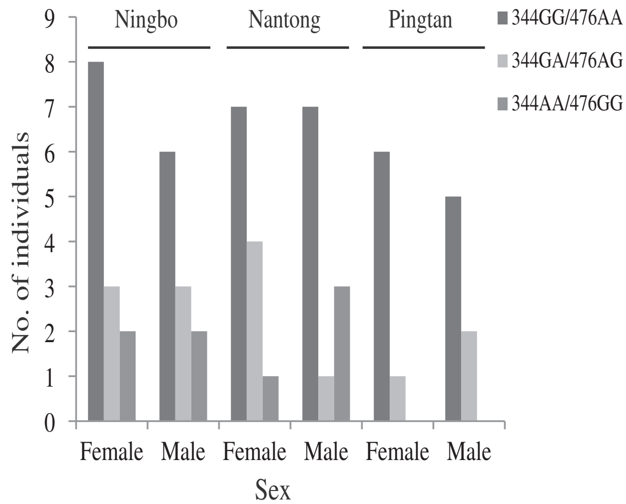


Fig. 6. Frequency of three genotypes in females and males.

The concentrations of Cd were at the threshold (20 μg/g·ww), above which signs of renal dysfunction can occur (Parsons, 1999). Moreover, levels were higher than those recorded in the 1980s (Yang et al., 1988). These concentrations may mean that current levels of Pb and Cd in NFP tissues, may pose a health risk to these animals.

Additionally, the concentrations of Cr, Zn, Mn, Cu, and Ni in tissues of NFPs were within the general range of TE concentration recorded in cetaceans (Appendix B).

4.4. Comparison with other cetaceans

We compared the present research with previous studies on cetaceans (Appendix B). The concentrations of As in all tissues of kidney, liver and muscle in three populations of Ningbo, Nantong and Pingtan NFPs were slightly higher than in *Sousa chinensis* from Xiamen waters (Chen et al., 2007) and *N. phocaenoides* from Beibu Gulf (Wang et al., 2008). The concentrations of Hg in livers of NFPs in Nantong and Ningbo were similar to those of *N. phocaenoides*, *S. chinensis* from Hong Kong waters (Parsons, 1998, Parsons, 1999, Parsons, 2004,) and *T. truncatus* (Parsons and Chan, 2001) but lower than NFPs from the Bohai Sea (Zhang et al., 1995). Cd concentrations were highest in NFPs from Bohai Sea than other populations (Yang et al., 1988; Zhou et al., 1994; Zhang et al., 1995; Dong et al., 2006). Meanwhile concentrations of Cr, Pb, Cu, Mn, Ni and Zn in the same tissue were similar to other cetacean populations (Yang et al., 1988; Zhou et al., 1994; Zhang et al., 1995; Parsons, 1998; Parsons, 1999; Parsons and Chan, 2001; Parsons, 2004; Chen et al., 2007; Wang et al., 2008).

4.5. Correlation between MTs polymorphism and tissue TEs concentrations

Low genetic polymorphism of *MT2* ($\pi = 0.001$) and *MT4* ($\pi = 0.001$) was found in the present study, and two polymorphic sites were

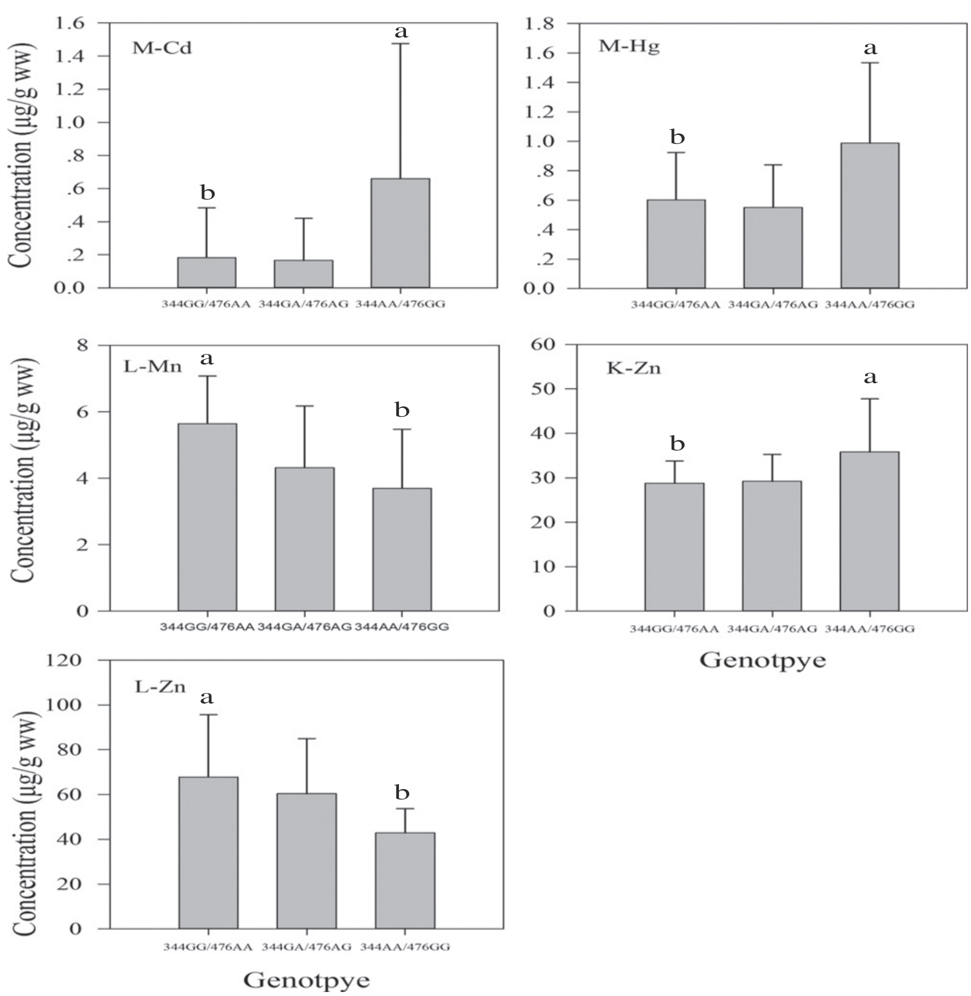


Fig. 7. Boxplot of the TEs concentration at different SNP genotypes. The different lowercase letters show significant difference at 0.05 levels.

Table 5
Mean concentrations of TEs in sediments from the East China Sea, South of the East China Sea and the Bohai Sea. Data are expressed as μg/g dry weight.

Areas	Cr	Mn	Ni	Cu	Zn	As	Cd	Hg	Pb	Reference
Ningbo, East China Sea	19.5 ± 3.31	143.9 ± 6.21	7.5 ± 0.51	22.3 ± 0.33	157.2 ± 71.01	–	0.2 ± 0.04	0.41 ± 0.01	6.81 ± 0.24	This study
Nantong, East China Sea	24.9 ± 1.65	226.5 ± 12.81	11.2 ± 0.58	6.2 ± 0.47	33.6 ± 4.50	6.9 ± 0.50	0.12 ± 0.02	0.15 ± 0.02	19.7 ± 1.52	This study
Pingtan, South of East China Sea	0.16 ± 0.05	0.04 ± 0.03	n.d.	4.42 ± 0.34	57.9 ± 13.39	7.3 ± 2.42	0.02 ± 0.01	0.05 ± 0.00	0.04 ± 0.01	This study
Liaodong Bay, Bohai Sea	46.4	–	22.5	19.4	71.7	8.3	–	0.04	31.8	Hu et al., 2013
Coastal Bohai Bay, China	101	–	40.7	38.5	131.1	–	34.7	–	–	Gao and Chen, 2012
Yangtze River Estuary, East China Sea	146.2	–	–	13	69	–	0.06	0.12	22.2	Zhao et al., 2008
Dapeng Bay, Southeast China	24			5	86		0.2	0.03	9.4	Huang et al., 2005

associated with accumulations of Zn, Mn, Cd and Hg (Fig. 7). Polymorphic sites on genes may affect functions of the protein (Kita et al., 2006), so further research is needed to determine whether these two polymorphic sites were involved in expression of metallothioneins and/or other functional process.

The application of population genetics is actually complex (Przeworski et al., 2005). Zn and Mn were highly accumulated in individuals with the main genotype (344 GG/476 AA), which may indicate the adaptation of the homozygous genotype according to a standard selective sweep model (Przeworski et al., 2005). Zn, Hg and Cd were highly accumulated in kidneys and muscles of porpoises with the rare genotype (344 AA/476 GG), which suggests that higher concentrations of the toxic metal occur in conjunction with this rare allele (G) (Kayaalti et al., 2010). This could be explained by a model of directional selection on standing variation, and that means selection does not always act on a new allele (Przeworski et al., 2005). In this situation, the allele associated with the adaptive phenotype would be kept at a low frequency, e.g. 0.2% and 3.8% in two marine populations of threespine sticklebacks (*Gasterosteus aculeatus*) (Przeworski et al., 2005). Both sites 344 A/G and 476 G/A found in the present research need to be further verified.

4.6. Conservation implication

The species *N. asiaeorientalis* has been designated as “Vulnerable” by the IUCN (Wang and Reeves, 2012), and the Yangtze finless porpoise sub-species (*N. a. asiaeorientalis*) has been listed as “Critically Endangered” (Wang et al., 2013).

To date, important factors that determine conservations status, such as population size, survival rate, habitat selection, home range, and threats to porpoises in Ningbo, Nantong, and Pingtan, remain unknown because a lack of systematic surveys in these regions. Therefore, their current conservation status remains unknown. The present study found that all population had low nucleotide diversity of metallothionein genes, and that TEs, especially non-essential TEs, were relatively high in the Ningbo and Nantong populations, and different among populations. These findings together with the high mortality rates of this species (Yang et al., 1999; Wang and Reeves, 2012), in particular, highlight the need to provide immediate protection for these likely threatened populations especially the Ningbo and Nantong populations. Moreover, due to high mortality, possibility induced by fisheries bycatch of the species, the Ningbo and Nantong populations may be threatened, and should be protected immediately. It is urgent that surveys and other studies be conducted to ascertain the true conservation status, and population trends, of Chinese narrow-ridged finless porpoise populations.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (grant no. 31300456, 31325025), the National Key Programme of Research and Development, Ministry of Science and Technology (Grant no. 2016YFC0503200), University Science Research Project of Jiangsu Province (11KJBl80003), the Priority Academic Program Development of Jiangsu Higher Education Institutions, and the Jiangsu Provincial Innovation Project for Scientific Research of Graduate Students in Universities (grant no. CXZZ13_0409). We are thankful to Susan J Chivers and Amiée R Lang for their improvements to the earlier manuscript; we also deeply appreciate two anonymous reviewers for their helpful comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.09.062.

References

Asante, K.A., Agusa, T., Mochizuki, H., Ramu, K., Inoue, S., Kubodera, T., Takahashi, S., Subramanian, A., Tanabe, S., 2008. Trace elements and stable isotopes (δ^{13}C and δ^{15}N) in shallow and deep-water organisms from the East China Sea. Environ. Pollut. 156, 862–873.

ATSDR, 1995. Agency for Toxic Substances and Disease Registry. Public health assessmenthttp://www.atsdr.cdc.gov/HAC/PHA/nwpipe/npctoc.html.

Babula, P., Masarik, M., Vojtech, A., 2012. Mammalian metallothioneins: properties and functions. Metallomics 4, 739–750.

Barros, N.B., Jefferson, T.A., Parsons, E.C.M., 2002. Food habits of finless porpoises (*Neophocaena phocaenoides*) in Hong Kong waters. Raffles B Zool. (Supplement 10), 115–123.

Bellante, A., Sprovieri, M., Buscaino, G., Buffa, G., Stefano, V.D., Manta, D.S., Barra, M., Filiciotto, F., Bonanno, A., Mazzola, S., 2012. Distribution of Cd and As in organs and tissues of four marine mammal species stranded along the Italian coasts. J. Environ. Monit. 14, 2382.

Bennett, P.M., Jepson, P.D., Law, R.J., Jones, B.R., Kuiken, T., Baker, J.R., Rogan, E., Kirkwood, J.K., 2001. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environ. Pollut. 112, 33–40.

Borrell, A., Aguilar, A., Tornero, V., Drago, M., 2014. Concentrations of mercury in tissues of striped dolphins suggest decline of pollution in Mediterranean open waters. Chemosphere 107, 319–323.

Cáceres-Saez, I., Guevara, S.R., Dellabianca, N.A., Goodall, R.N.P., Cappozzo, H.L., 2013. Heavy metals and essential elements in Commerson's dolphins (*Cephalorhynchus C. commersonii*) from the southwestern South Atlantic Ocean. Environ. Monit. Assess. 185, 5375–5386.

Chambers, J.C., Paul, E., Delilah, Z., Zhang, W., Li, Y., Froguel, P., Balding, D., Scott, J., Kooner, J.S., 2008. Common genetic variation near MC4R is associated with waist circumference and insulin resistance. Nat. Genet. 40, 716–718.

Chen, P., Liu, P., Liu, R., Lin, K., Pilleri, G., 1979. Distribution, ecology, behaviour and conservation of the dolphins of the middle reaches of Changjiang (Yangtze) river (Wuhan-Yueyang). Invest. Cetacea. 9, 87–103.

Chen, B.Y., Gu, S.R., Zhang, F.F., Xu, X.R., Yang, G., 2007. A preliminary analysis on heavy metal concentrations in the Chinese white dolphins in Xiamen. Chinese Journal of Zoology 42, 102–105 In Chinese.

Chen, H.I., Chiu, Y.W., Hsu, Y.K., Li, W.F., Chen, Y.C., Chuang, H.Y., 2010. The association of metallothionein-4 gene polymorphism and renal function in long-term lead-exposed workers. Biol. Trace Elem. Res. 137, 55–62.

Chen, B., Fan, D., Li, W., Wang, L., Zhang, X., Liu, M., Guo, Z., 2014. Enrichment of heavy metals in the inner shelf mud of the East China Sea and its indication to human activity. Cont. Shelf Res. 90, 163–169.

Das, K., Jacob, V., Bouquegneau, J.M., 2002. White-sided dolphin metallothioneins: purification, characterization. Comp. Biochem. Physiol. C 131, 245–251.

Das, K., Debacker, V., Piller, S., JM, B., 2003. Heavy metals in marine mammals. In: JG, V., GD, B., Fournier, M., TJ, O.''.S. (Eds.), Toxicology of Marine Mammals. Taylor & Francis, Washington D.C, pp. 135–167.

Desforges, J.P.W., Sonne, C., Levin, M., Siebert, U., Guise, S., Rune, D., 2016. Immunotoxic effects of environmental pollutants in marine mammals. Environ. Int. 86, 126–139.

Dong, W.W., Xu, Y., Wang, D., Hao, J.Y., 2006. Mercury concentrations in Yangze finless porpoises (*Neopocaena phocaenoides asiaeorientalis*) from Eastern Dongting Lake, China. Fresenius Environ. Bull. 15, 441–447.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147, 69–74.

Gao, X., Chen, C.T.A., 2012. Heavy metal pollution status in surface sediments of the coastal Bohai Bay. Water Res. 46, 1901–1911.

Hu, B., Li, J., Zhao, J., Yang, J., Bai, F., Dou, Y., 2013. Heavy metal in surface sediments of the Liaodong Bay, Bohai Sea: distribution, contamination, and sources. Environ. Monit. Assess. 185, 5071–5083.

Huang, X.Q., Zhang, S.Z., Huo, Z.H., 2005. Heavy metal distribution in seawater in Dapeng Bay of Shenzhen and the Pearl River Estuary. Trans. Oceanol. Limnol. 4, 38–44 In Chinese.

Hung, C.L.H., So, M.K., Connell, D.W., Fung, C.N., Lam, M.H.W., Nicholson, S., Richardson, B.J., Lam, P.K.S., 2004. A preliminary risk assessment of trace elements accumulated in fish to the Indo-Pacific humpback dolphin (*Sousa chinensis*) in the Northwestern waters of Hong Kong. Chemosphere 56, 643–651.

Hung, C.L.H., Lau, R.K.F., Lam, J.C.W., Jefferson, T.A., Hung, S.K., Lam, M.H.W., Lam, P.K.S., 2007. Risk assessment of trace elements in the stomach contents of Indo-Pacific humpback dolphins and finless porpoises in Hong Kong waters. Chemosphere 66, 1175–1182.

Jarić, I., Višnjić-Jeftić, Ž., Cvijanović, G., Gačić, Z., Jovanović, L., Skorić, S., Lenhardt, M., 2011. Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (*Acipenser ruthenus*) from the Danube River in Serbia by ICP-OES. Microchem. J. 98, 77–81.

Jirillo, E., Caccavo, D., Magrone, T., Piccigallo, E., Amati, L., Lembo, A., Kalis, C., Gumenscheimer, M., 2002. The role of the liver in the response to LPS: experimental and clinical findings. J. Endotoxin Res. 8, 319–327.

Jović, M., Stanković, S., 2014. Human exposure to trace metals and possible public health risks via consumption of mussels *Mytilus galloprovincialis* from the Adriatic coastal area. Food Chem. Toxicol. 70, 241–251.

Kamel, L.D., Yahia, B., Mohammed, B., Zitouni, B., 2014. Heavy metals in soft tissues of short beaked common dolphins (*Delphinus delphis*) stranded along the Algerian west coast. Open Journal of Marine Science 4, 110–117.

Kayaalti, Z., Mergrn, G., Soylemezoglu, T., 2010. Effect of metallothionein core promoter region polymorphism on cadmium, zinc and copper levels in autopsy kidney tissues from a Turkish population. Toxicol. Appl. Pharmacol. 245, 252–255.

Kita, K., Miura, N., Yoshida, M., Yamazaki, K., Ohkubo, T., Imai, Y., Naganuma, A., 2006. Potential effect on cellular response to cadmium of a single-nucleotide A → G polymorphism in the promoter of the human gene for metallothionein IIA. Hum. Genet. 120, 553–560.

Kubota, R., Kunito, T., Fujihara, J., Tanabe, S., Yang, J., Miyazaki, N., 2005. Placental transfer of arsenic to fetus of Dall's porpoises (*Phocoenoides dalli*). Mar. Pollut. Bull. 51, 845–849.

Lavery, T.J., Butterfield, N., Kemper, C.M., Reid, R.J., Sanderson, K., 2008. Metals and selenium in the liver and bone of three dolphin species from South Australia, 1988–2004. Sci. Total Environ. 390, 77–85.

Lavery, T.J., Kemper, C.M., Sanderson, K., Christopher, G.S., Peter, C., James, G.M., Laurent, S., 2009. Heavy metal toxicity of kidney and bone tissues in South Australian adult bottlenose dolphins (*Tursiops aduncus*). Mar. Environ. Res. 67, 1–7.

Librado, P., Rozas, J., 2009. DnaSP v5: Aa software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Liu, J.Y., Chou, L.S., Chen, M.H., 2015. 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. Mar. Pollut. Bull. 93, 270–277.

Llobet, J.M., Falcoä, G., Casas, C., Teixidoä, A., Domingo, J.L., 2003. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. J. Agric. Food Chem. 51, 838–842.

Maanan, M., Saddik, M., Maanan, M., Chaibi, M., Assobhei, O., Zourarah, B., 2015. Environmental and ecological risk assessment of heavy metals in sediments of Nador lagoon, Morocco. Ecol. Indic. 48, 616–626.

Monk, A., Charlton-Robb, K., Buddhadasa, S., Thompson, R.M., 2014. Comparison of mercury contamination in live and dead dolphins from a newly described species, *Tursiops australis*. PLoS One 9, e104887.

Murphy, M.B., Lin, L., Lam, P.K.S., 2010. Temporal trends of organochlorines and trace elements in marine mammals from Hong Kong. In: Isobe, T., Nomiyama, K., Subramanian, A., Tanabe, S. (Eds.), Interdisciplinary Studies on Environmental Chemistry-Environmental Specimen Bank. TERRAPUB, Tokyo, pp. 103–110.

NBO, 2003–2013. National Bureau of Oceanography of China, Bulletin of Marine Environmental Quality. http://www.soa.gov.cn.

Noël, M., Jeffries, S., Lambourn, D.M., Telmer, K., Macdonald, R., Ross, P.S., 2016. Mercury accumulation in harbour seals from the northeastern Pacific Ocean: the role of transplacental transfer, lactation, age and location. Arch. Environ. Contam. Toxicol. 70, 56–66.

O' Shea, T.J., 1999. Environmental contaminants and marine mammals. In: JE, R., SA, R. (Eds.), Biology of Marine Mammals. Smithsonian Institution, Washington DC, pp. 485–563.

Parsons, E.C.M., 1998. Trace metal pollution in Hong Kong: implications for the health of Hong Kong's Indo-Pacific hump-backed dolphins (*Sousa chinensis*). Sci. Total Environ. 214, 175–184.

Parsons, E.C.M., 1999. Trace elements concentrations in the tissues of cetaceans from Hong Kong's territorial waters. Environ. Conserv. 26, 30–40.

Parsons, E.C.M., 2004. The potential impacts of pollution on humpback dolphins, with a case study on the Hong Kong population. Aquat. Mamm. 30, 18–37.

Parsons, E.C.M., Chan, H.M., 2001. Organochlorine and trace element contamination in bottlenose dolphins (*Tursiops truncatus*) from the South China Sea. Mar. Pollut. Bull. 42, 780–786.

Przeworski, M., Coop, G., Wall, J.D., 2005. The signature of positive selection on standing genetic variation. Evolution 59, 2312–2323.

Randhawa, N., Gulland, F., Ylitalo, G.M., DeLong, R., Mazet, J.A.K., 2015. Sentinel California sea lions provide insight into legacy organochlorine exposure trends and their association with cancer and infectious disease. One Health 1, 37–43.

Reijnders, P.J.H., 1980. Organochlorine and heavy metal residues in harbour seals from the Wadden Sea and their possible effects on reproduction. Neth. J. Sea Res. 14, 30–65.

Reijnders, P.J.H., Aguilar, A., Donovan, G.P., 1999. Chemical pollutants and cetaceans. Journal of Cetacean Research & Management Spec. (special issue 1), 1–273.

Rosa, C., Blake, J.E., Bratton, G.R., Dehn, L.A., Gray, M.J., O'Hara, T.M., 2008. Heavy metal and mineral concentrations and their relationship to histopathological findings in the bowhead whale (*Balaena mysticetus*). Sci. Total Environ. 399, 165–178.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. In: 2nd Ed. Cold Spring Harbor Laboratory, New York.

Sample, B.E., Opresko, D.M., Suter, G.W., 1996. Toxicological Benchmarks for Wildlife. Oakridge National Laboratory, Oakridge.

Sarkar, A., Ray, D., Shrivastava, A.N., Sarker, S., 2006. Molecular biomarkers: their significance and application in marine pollution monitoring and potential role. Ecotoxicology 15, 333–340.

Seixas, T.G., Kehrig, H.A., Fillmann, G., DiBeneditto, A.P., Souza, C.M.M., Secchi, E.R., Moreira, I., Malm, O., 2007. Ecological and biological determinants of trace elements accumulation in liver and kidney of *Pontoporia blainvillei*. Sci. Total Environ. 385, 208–220.

Shirakihara, M., Seki, K., Takemura, A., Shirakihara, K., Yoshida, H., Yamazaki, T., 2008. Food habits of finless porpoises *Neophocaena phocaenoides* in western Kyushu, Japan. J. Mammal. 9, 392–406.

Shoham-Frider, E., Kerem, D., Roditi-Elasar, M., Goffman, O., Morick, D., Yoffe, O., Kress, N., 2014. Trace elements in tissues of cetacean species rarely stranded along the Israeli Mediterranean coast. Mar. Pollut. Bull. 83, 376–382.

Skutkova, H., Babula, P., Stiborova, M., Eckschlager, T., Trnkova, L., Provaznik, I., Hubalek, J., Kizek, R., Adam, V., 2012. Structure, polymorphisms and electrochemistry of mammalian metallothioneins—a review. Int. J. Electrochem. Sci. 7, 12415–12431.

Sonne, C., 2010. Health effects from long-range transported contaminants in Arctic top predators: an integrated review based on studies of polar bears and relevant model species. Environ. Int. 36, 461–491.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–598.

Tchounwou, P.B., Yedjou, C.G., Paylolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. In: Luch, A. (Ed.), Molecular, Clinical and Environmental Toxicology, Experiential Supplentum 101. Springer Basel AG, Switzerland, pp. 133–164.

Tu, N.P.C., Ha, N.N., Matsuo, H., Tuyen, B.C., Tanabe, S., Takeuchi, I., 2012. Biomagnification profiles of trace elements through the food web of an integrated shrimp mangrove farm in Ba Ria Vung Tau, South Vietnam. Am. J. Environ. Sci. 8, 117–129.

Wang, J.Y., Reeves, R., 2012. *Neophocaena asiaeorientalis*. The IUCN Red List of Threatened Species e.T41754A17599353.

Wang, Y., Fang, Z.Q., Zhou, H.Y., Dong, A., 2008. Concentrations and distribution of heavy metals in finless porpoise in coast of the Beibu gulf. Mar. Environ. Sci. 27, 63–66 In Chinese.

Wang, D., Turvey, S.T., Zhao, X., Mei, Z., 2013. *Neophocaena Asiaeorientalis* ssp. *asiaeorientalis*. The IUCN Red List of Threatened Species e.T43205774A45893487.

WHO, 1996. Trace Elements in Human Nutrition and Health. World Health Organization, Geneva.

Yang, L., Yu, D., Lu, P., 1988. Studies on the levels of some metals and organichlorine compounds in *Lipotes vexillifer* and *Nephocaena phocaenoides*. Acta Theriologica Sinica 8, 122–127 In Chinese.

Yang, G., Zhou, K., Xu, X., 1999. A survey on the incidental catches of small cetaceans in coastal waters of China. Chin. J. Appl. Ecol. 10, 713–716 In Chinese.

Yang, J., Kunito, T., Anan, Y., Tanabe, S., Miyazaki, N., 2008. Subcellular distribution of trace elements in kidney of a mother-fetus pair of Dall's porpoises (*Phocoenoides dalli*). Chemosphere 70, 1203–1210.

Zhang, W., Zhou, R., Zhou, K., Kamitani, B., 1995. The preliminary study on the concentration of Hg in finless porpoise (*Nephocaena phocaenoides*) from Bohai Sea. Mar. Environ. Sci. 14, 33–38 In Chinese.

Zhao, B.G., Shao, M.H., Bao, Y.E., 2008. The features of concentration and dispersion of the heavy metals and their changing patterns in surface sediment of East China Sea. Chin. J. Dalian Marit. Univ. 3, 13–16 In Chinese.

Zhou, K., 1986. An outline of marine mammalogical researches in China. Acta Theriolodical Sinica 6, 219–232 In Chinese.

Zhou, K., Gao, A., Sun, J., 1993. Notes on the biology of the finless porpoise in Chinese waters. IBI Reports 4, 69–74.

Zhou, K., Hou, Y., Gao, A., Kamiya, T., Tatsukawa, R., 1994. Heavy metals in tissues of finless porpoises in the East China Sea. Chinese Zoological Society 60th anniversary: Papers in memory of professor Chan Ching's 100th birthday. Chinese Zoological Society, Beijing, pp. 201–211.