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# Trace elements accumulation in the Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) – A threat to the endangered freshwater cetacean

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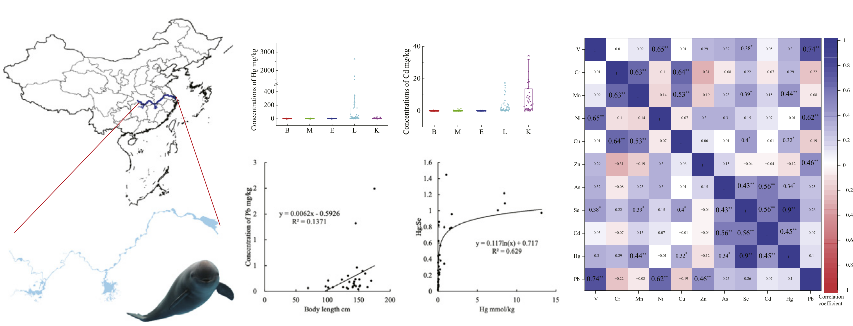
## HIGHLIGHTS

- Trace elements in the Yangtze River finless porpoise (YFP) were investigated.
- High concentrations of Hg and Cd have been found in YPF.
- The accumulation features of trace elements in different tissues have been revealed.
- Influences from body length, gender, and habitat have been revealed.

## GRAPHICAL ABSTRACT

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## ABSTRACT

As a freshwater cetacean with a population of only approximately 1000 individuals, the Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) is threatened by water pollution. However, studies of contaminants accumulated in the Yangtze finless porpoise remain limited. In this study, concentrations of 11 trace elements in different tissues sampled from 38 Yangtze finless porpoise individuals were determined. The elements V, Ni, Zn, and Pb were mostly accumulated in the epidermis, Cr, Mn, Cu, Se, and Hg were mostly accumulated in the liver, while As and Cd were mostly accumulated in the blubber and kidney, respectively. The results show that trace elements concentrations in the epidermis do not reliably indicate concentrations in internal tissues of the Yangtze finless porpoises. Positive correlations between different trace elements concentrations in tissues with the highest concentrations suggested the similar mechanism of metabolism or uptake pathway of those elements. Concentrations of As, Se, Cd, Hg, and Pb in the tissues with the highest concentrations were significantly positively correlated with the body length. Furthermore, significantly higher trace elements concentrations were measured in the reproductive organs of females (ovaries) than males (testis). However, no significant difference of trace elements concentrations between habitats was found. In consideration of higher Hg and Cd level in Yangtze finless porpoises compared to other small cetaceans, the potential risk of Hg (in particular) and Cd toxicity to Yangtze finless porpoises needs further attention.



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## 1. Introduction

As species at the top of aquatic food webs, cetacean tissues may have high contaminant concentrations due to bioaccumulation and biomagnification (Desforges et al., 2018; Gui et al., 2017; Parsons, 1998). Bioaccumulation of heavy metals and organic contaminants in cetaceans have previously been investigated, and have indicated the risks to cetacean populations (Betti and Nigro, 1996; Desforges et al., 2018; Gui et al., 2017; Jepson et al., 2016). However, most research has focus on marine cetaceans, and although the majority of cetaceans are marine, some cetaceans inhabit only freshwater environments (Veron et al., 2008). Freshwater cetaceans are more endangered than marine species due to their limited living spaces, increasingly scarce food resources, the high level of human interference, and water pollution (Braulik et al., 2015; Turvey et al., 2007; Wang, 2009). However, research on bioaccumulation of contaminants in freshwater cetaceans is limited, which inhibits robust assessment of the risks posed to them. Therefore, further research on the bioaccumulation of contaminants in freshwater cetaceans is essential.

The Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) is the only cetacean that lives in the Yangtze River catchment since the functional extinction of the Yangtze River dolphin (the baiji, *Lipotes vexillifer*) (Wang et al., 2006). As a critically endangered species on the International Union for Conservation of Nature (IUCN) red list (D. Wang et al., 2013), the Yangtze finless porpoise population has decreased rapidly during recent decades. The average annual decline rate of Yangtze finless porpoise populations from 2006 to 2012 was 13.7%, and the current population abundance of Yangtze finless porpoise is only approximately 1000 in its whole habitat (Mei et al., 2012, 2014).

Pollution is one of the critical threats from human activities to the survival of the Yangtze finless porpoise (Wang, 2009). The Yangtze River is the largest river in China and suffers a serious pollution problem due to the high population and rapid economic development in its catchment which has already affected the fish biodiversity in the river (Muller et al., 2008; Yang et al., 2012; Fu et al., 2003; Zhang et al., 2009). However, studies of contaminants in the Yangtze finless porpoise are limited and have only been conducted sporadically on individuals (Dong et al., 2006; Yang et al., 2008). Other studies of contaminants in marine finless porpoise have been conducted in the coastal area of China, Japan, and South Korea (Isobe et al., 2011; Lam et al., 2016; Liu et al., 2017; Park et al., 2010; Zhang et al., 2017). However, the pollution condition of the Yangtze River differs from the Eastern Asia coastal area. Furthermore, molecular biological evidence tends to classify Yangtze finless porpoise and marine finless porpoises into different species (Zhou et al., 2018), which makes the identification of factors that threaten the survival of the Yangtze finless porpoise especially important for the protection of this species.

Trace elements are common in the environment, and their accumulation in tissues of many cetacean species have been studied during the past few decades (Bennett et al., 2001; Gui et al., 2017; Kemper et al., 1994; Parsons, 1998). Chronic exposure to some trace elements (heavy metals such as As, Cd, Hg, and Pb) can have toxic effects on aquatic mammals even at relatively low concentrations (Bennett et al., 2001; Caceres-Saez et al., 2018; Parsons, 1998). Furthermore, some cetaceans are considered to be indicators for the trace elements pollution in particular habitats (Gui et al., 2017; Kucklick et al., 2011; Liu et al., 2017; Wise et al., 2009). Previous studies also reveal that different cetaceans' tissues have different accumulation characteristics for different trace elements (Sun et al., 2017). However, compared to previous studies of marine cetaceans, study of trace elements in the freshwater Yangtze finless porpoise is still very limited.

In the present study, concentrations of 11 trace elements in different tissues of Yangtze finless porpoises collected from different habitats were investigated to 1) assess trace elements accumulation in Yangtze finless porpoises and identify potential effects; 2) identify different accumulation characteristics of trace elements in Yangtze finless porpoises, and; 3) assess the factors affecting the trace elements accumulation in Yangtze finless porpoises. This study provides important new information for understanding the threat of trace element pollution to the Yangtze finless porpoise population and facilitating improved conservation of this freshwater cetacean species.

## 2. Materials and methods

### 2.1. Sample collection

Relying on the "Yangtze River Cetaceans Protection Network", 38 Yangtze finless porpoise individuals (19 males and 19 females) were collected from 2011 to 2014. This included 9 individuals from the Yangtze River, 25 individuals from the Poyang Lake, and 4 individuals from the Dongting Lake. All animals had died from stranding, bycatch, or physical trauma, and samples were frozen until postmortem. Gender and body length were determined during postmortem (Table S1).

Blubber ($n = 38$), muscle ($n = 38$), liver ($n = 38$), kidney ($n = 38$), and epidermis ($n = 31$) samples of Yangtze finless porpoises were collected during the dissection. Reproductive tissue samples (ovaries [$n = 11$] and testis [$n = 18$]) were also collected, if possible, for females and males, respectively. All tissue samples were frozen at −20 °C before analysis.

### 2.2. Sample preparation and chemical analysis

Two duplicates were processed during the analysis for each sample. Approximately 0.5 g of ground tissue samples were weighed into Teflon digestion tubes, soaked overnight in 4 mL of nitric acid, and then heated at 80 °C on a heating plate until there was no reddish-brown smoke. After pre-digestion, the digestion tubes were sealed and digested in a microwave digestion instrument (Anton Paar, Mltuwave 3000, Austria). Following microwave digestion, the cooled solutions were filtered through 0.45 μm filters and transferred into 50 mL volumetric flasks, and diluted with deionized (DI) water (≥18.2 MΩ).

The concentrations of 11 trace elements (V, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Hg, and Pb) in sample solutions were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Fisher, XSERIES 2, U.S.). Internal standards (Sc, Ge, In, and Bi) were used to correct the matrix effects and instrumental drift of the ICP-MS. Each sample was measured for three times to ensure the RSD < 5%. Performances to avoid ICP-MS interferences are provided in Supplementary information. All vessels used in the analysis were acid-washed and rinsed with DI water prior to use.

### 2.3. Quality assurance/quality control

Quality assurance/quality control (QA/QC) was performed using GBW10018 (GSB-9, chicken), GBW10051 (GSB-29, pork liver), GBW10050 (GSB-28, shrimp) obtained from National Research Center for Certified Reference Material of China and NIST2976 (mussel) from National Institute of Standards and Technology, U.S. Reference materials were processed under the same conditions as the tissue samples and results were in good agreement with the certified values (recovery rates of 82% to 119%); recovery of spiked blanks ranged from 73% to 115%. Limits of detection (LOD) was determined by the instrument as LOD = 3 s/b, while "s" was relative standard deviation of background and "b" was the slope of standard curve. Details of recoveries and limits of detection were present in Table S2. Method blanks were also processed and analyzed under the same conditions as the samples.

### 2.4. Data analysis

Trace element concentrations in this study are expressed as mg/kg based on wet weight (WW). Spearman correlation analysis and

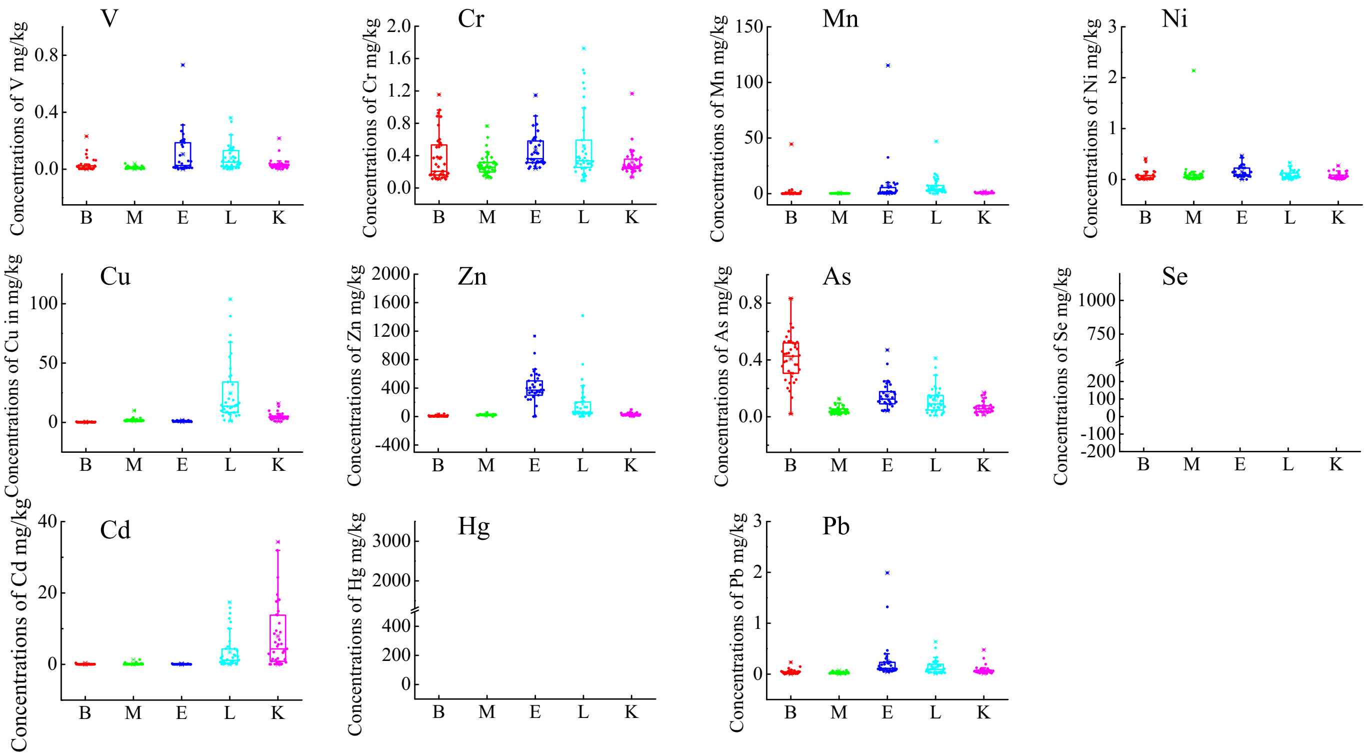


**Fig. 1.** Trace elements concentrations in different tissues (B – blubber, M – muscle, E – epidermis, L - liver, and K – kidney) of Yangtze finless porpoises.

regression analysis were used to evaluate relationships between different factors. Since the data of most trace elements concentrations were not normal and the homoscedasticity between groups could not pass the test, independent sample nonparametric test (Mann-Whitney *U* test or Kruskal-Wallis test depending on the number of group) was used to compare differences in trace element concentrations between samples from individuals of different genders and from different areas. Statistical analyses were performed using SPSS 20.0.

## 3. Results

### 3.1. Accumulations of trace elements in Yangtze finless porpoises

The highest concentrations of different trace elements were present in different tissues (Fig. 1, Table S3). Concentrations of V, Ni, Zn, and Pb were highest in the epidermis, with mean concentrations of 0.109 mg/kg, 0.154 mg/kg, 429 mg/kg, and 0.254 mg/kg, respectively. For Cr, Mn, Cu, Se, and Hg, the highest concentrations were in the liver, with mean concentrations of 0.521 mg/kg, 6.61 mg/kg, 24.7 mg/kg, 116 mg/kg, and 250 mg/kg, respectively. The highest concentrations of As and Cd were present in the blubber (0.407 mg/kg) and kidney (7.91 mg/kg), respectively. Overall, trace elements concentrations in Yangtze finless porpoise tissues having the highest concentrations decreased from Zn > Hg > Se > Cu > Cd > Mn > Cr > As > Pb > Ni > V.

Spearman correlation analysis showed the correlations between concentrations of trace elements in their highest-concentration tissues (Fig. 2). Not accounting for the trace elements present in the highest concentrations in the epidermis, concentrations of other trace elements in the epidermis were not significantly correlated with the concentrations in their highest-concentration tissues, with the exception of Hg (Table S4). Among all the analyzed trace elements, Hg and Se had the highest correlation coefficient (0.900, Fig. 2). Trace elements with the highest concentrations in the epidermis (V, Ni, and Pb) and liver (Mn, Cu, Se, and Hg) were significantly positively correlated with each other. However, Zn was significantly positively correlated only with Pb, and Cr was significantly positively correlated only with Mn and Cu. There were also significant positive correlations between As, Se, Cd, and Hg.

The Hg:Se ratios (molar ratio) were calculated to evaluate the potential detoxification of Hg by Se (Caceres-Saez et al., 2018). Most individual Yangtze finless porpoises had Hg: Se < 1, while some individuals with high Hg levels had Hg: Se > 1 (Fig. 3). The Hg:Se ratios significantly correlated with molar concentrations of Hg (Correlation Coefficient = 0.629, $p < 0.001$) and present logarithmic regression.

### 3.2. Differences of trace elements accumulation in Yangtze finless porpoises with different body length, genders, and from different areas

Concentrations of As (correlation coefficient = 0.341; $p < 0.05$), Se (correlation coefficient = 0.798; $p < 0.001$), Cd (correlation coefficient = 0.506; $p < 0.01$), Hg (correlation coefficient = 0.836; $p < 0.001$), and Pb (correlation coefficient = 0.395; $p < 0.05$) in their highest-concentration tissues were significantly positively correlated with the body length (Fig. 4).

There were no significant differences between trace elements concentrations in Yangtze finless porpoises of different genders except Ni, which was significantly higher in male porpoises (Table S5). However, concentrations of some trace elements (Ni, Cu, Se, Cd, Hg, and Pb) were significantly higher in the ovaries of females than in the testis of males (Fig. 5 and Table S6).

The mean Cr, Mn, Cu, Zn, As, Se, and Hg concentrations were highest in Yangtze finless porpoise samples from the Poyang Lake, V and Pb were highest in samples from the Yangtze River, and Ni and Cd were highest in samples from the Dongting Lake. However, no significant difference among habitats was found (Table S7).

## 4. Discussions

### 4.1. Trace elements accumulation in Yangtze finless porpoises

The trace elements measured in this study can be divided into essential elements (including V, Cr, Mn, Ni, Cu, Zn, and Se) and non-essential

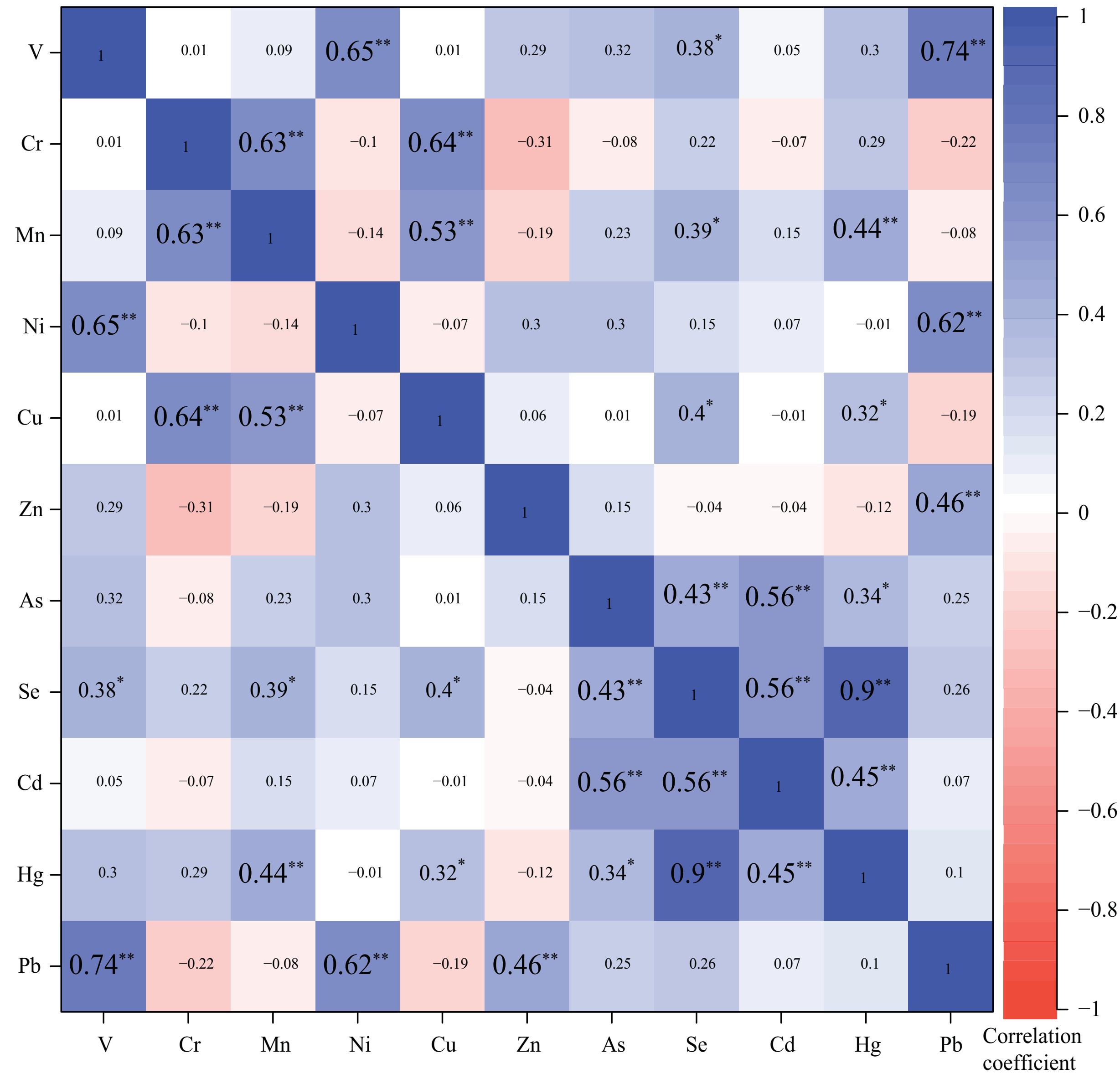


**Fig. 2.** Spearman correlations between concentrations of different trace elements in their highest-concentration tissues in Yangtze finless porpoises. Values are the Spearman correlation coefficients. The larger scale with * indicates $p < 0.05$, the largest scale with ** indicates $p < 0.01$.

elements (toxic heavy metals, including As, Cd, Hg, and Pb) (Sharma and Agrawal, 2005). Toxic heavy metals can lead to health risk to cetaceans. However, essential metals can also have toxic effects at certain concentrations (Tokar et al., 2012). In the North Sea, harbor porpoises (*Phocoena phocoena*) that died from infectious disease had significantly higher hepatic concentrations of Cd, Hg, Se and Zn compared to healthy porpoises that died from physical trauma in the North Sea (Mahfouz et al., 2014). Furthermore, an increased number of stranding events were reported in Indo-Pacific humpback dolphins (*Sousa chinensis*) from the Western Pearl River Estuary, China, in the same year that Cd concentrations peaked (Gui et al., 2017).

In this study, some tissue samples exceeded the Zn concentrations that are considered safe for marine mammals (80–400 mg/kg) (Law, 1996). Wounds caused by vessels or fishing equipment are common on the epidermis of the Yangtze finless porpoise, and the high Zn concentrations in the epidermis is likely to be related to wound repair; Zn is required for dermal cell proliferation and collagen deposition and so plays an important role in wound healing processes on the epidermis (Iwata et al., 1999; Lansdown et al., 2007). Similar results have previously been reported around the world, with the highest Zn levels measured in the epidermis of other cetaceans (Aubail et al., 2013; Borrell et al., 2015; Roditi-Elasar et al., 2003; Sun et al., 2017; Yang et al., 2002). However, in our Yangtze finless porpoises and Indo-Pacific humpback dolphins from Pearl River Estuary area (Sun et al., 2017), Zn concentrations exceeding the essential range have also been reported in liver tissue, suggesting that excess Zn may be causing toxicity to these species.

In this study, Se concentrations in Yangtze finless porpoises exceed the essential limitation (120 mg/kg) have also been found (Law, 1996). Yangtze finless porpoises with high liver Se concentrations also had high liver Hg concentrations. Among the non-essential elements measured in this study, Hg was measured at the highest mean concentrations in Yangtze finless porpoise tissue, and the bio-enrichment factor was between $10^4$ and $10^9$ compared with the Hg concentration in the Yangtze River water (Liu et al., 2015; Muller et al., 2008). Some individuals presented liver Hg concentrations over the range considered to pose a risk

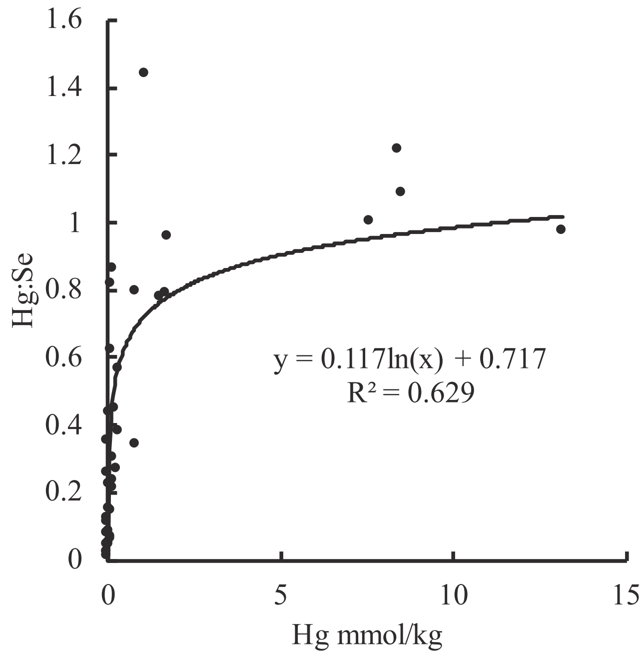


**Fig. 3.** Correlation and regression between Hg molar concentrations and Hg:Se ratios (molar ratios) in liver tissue of Yangtze finless porpoises.

of hepatic damage (400–1600 mg/kg) (Wagemann and Muir, 1984). Even for those individuals in which Hg concentrations did not exceed this range, chronic low-level exposure of Hg could also suppress immune and endocrine system of animals, and increase in diseases, infections, and other health impairments (Caceres-Saez et al., 2018; Stavros et al., 2011). However, Se can protect marine mammals from Hg toxicity through antioxidant properties, competition for binding sites, and the formation of non-toxic inert complexes (Cuvin-Aralar and Furness, 1991). The significant positive correlation and high correlation coefficient between Se and Hg in Yangtze finless porpoise tissues in this study suggest that the high Se concentrations could be a response of high Hg concentrations. Moreover, the high Hg:Se ratios in Yangtze finless porpoises with high Hg tissue concentrations suggests that the health of some Yangtze finless porpoises in the Yangtze River could be affected by Hg toxicity (Berry and Ralston, 2008; Khan and Wang, 2009).

Trace elements concentrations in Yangtze finless porpoise liver samples in this study are compared with those of other small cetaceans in previous studies in Table S8. Levels of Hg, Se, and Cd in this study are higher than those reported in most of other studies on finless porpoises and other small cetacean species. Environmental concentrations of Cd are reportedly high in some provinces in the middle reach of the Yangtze River (Cai et al., 1995; Li et al., 2010; Williams et al., 2009), which may be the cause of the high Cd concentration in Yangtze finless porpoise tissue seen in this study. For Hg, however, the concentration in the Yangtze River is relatively low compared with other water bodies, and previous studies on fish from this area have reported lower Hg tissue concentrations than in fish from coastal areas of China (Muller et al., 2008; Ullrich et al., 2001; Yi et al., 2011). This suggests that other mechanisms drive the accumulation of Hg in Yangtze finless porpoises, which requires further investigation.

Concentrations of most trace elements (except As and Pb) in this study are higher than or similar to those reported in finless porpoises from the East China Sea (which is in close proximity to the Yangtze River estuary), but lower than those in finless porpoises and Indo-

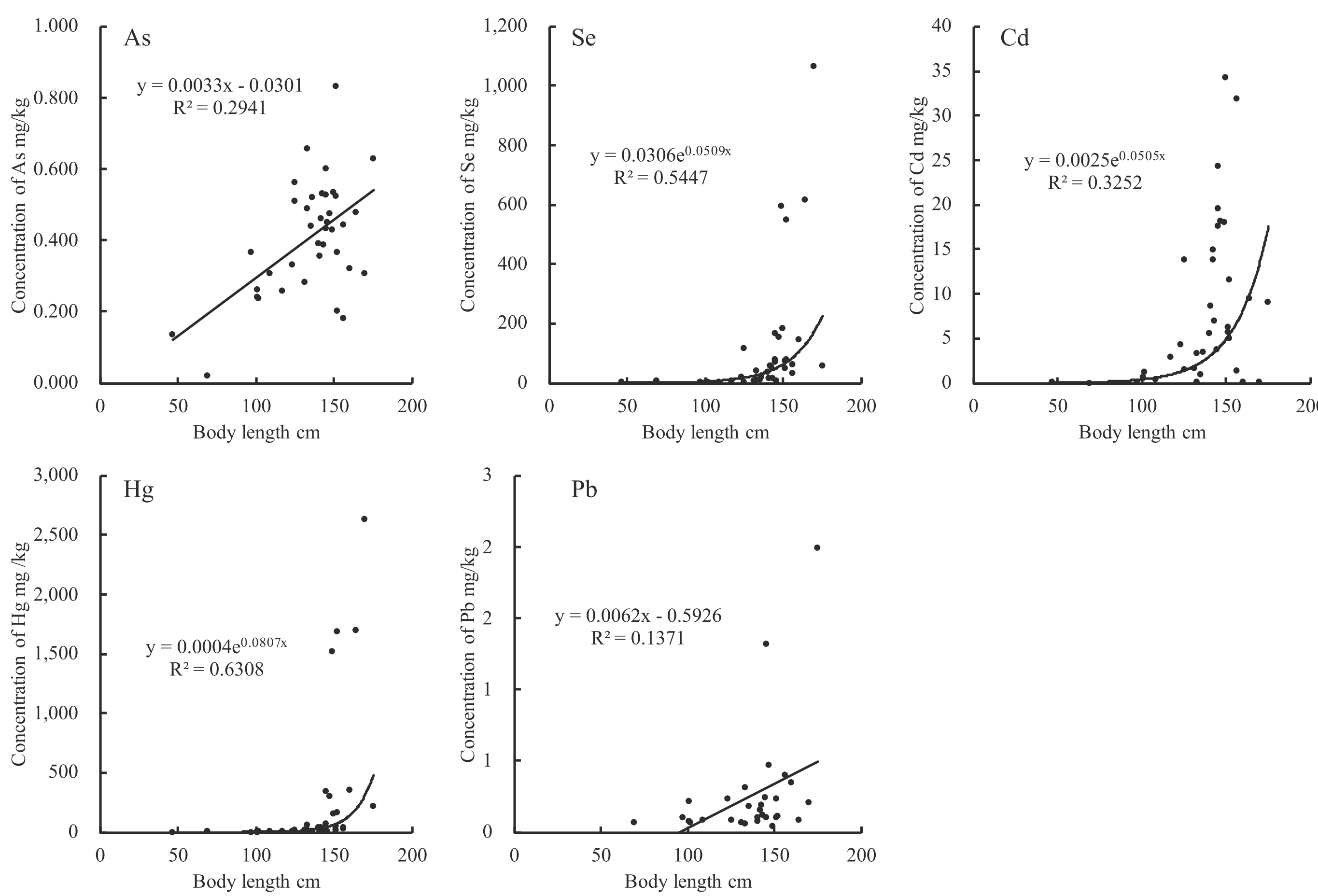


**Fig. 4.** Correlations and regressions between body length of Yangtze finless porpoises and concentrations of trace elements in the highest-concentration tissues.

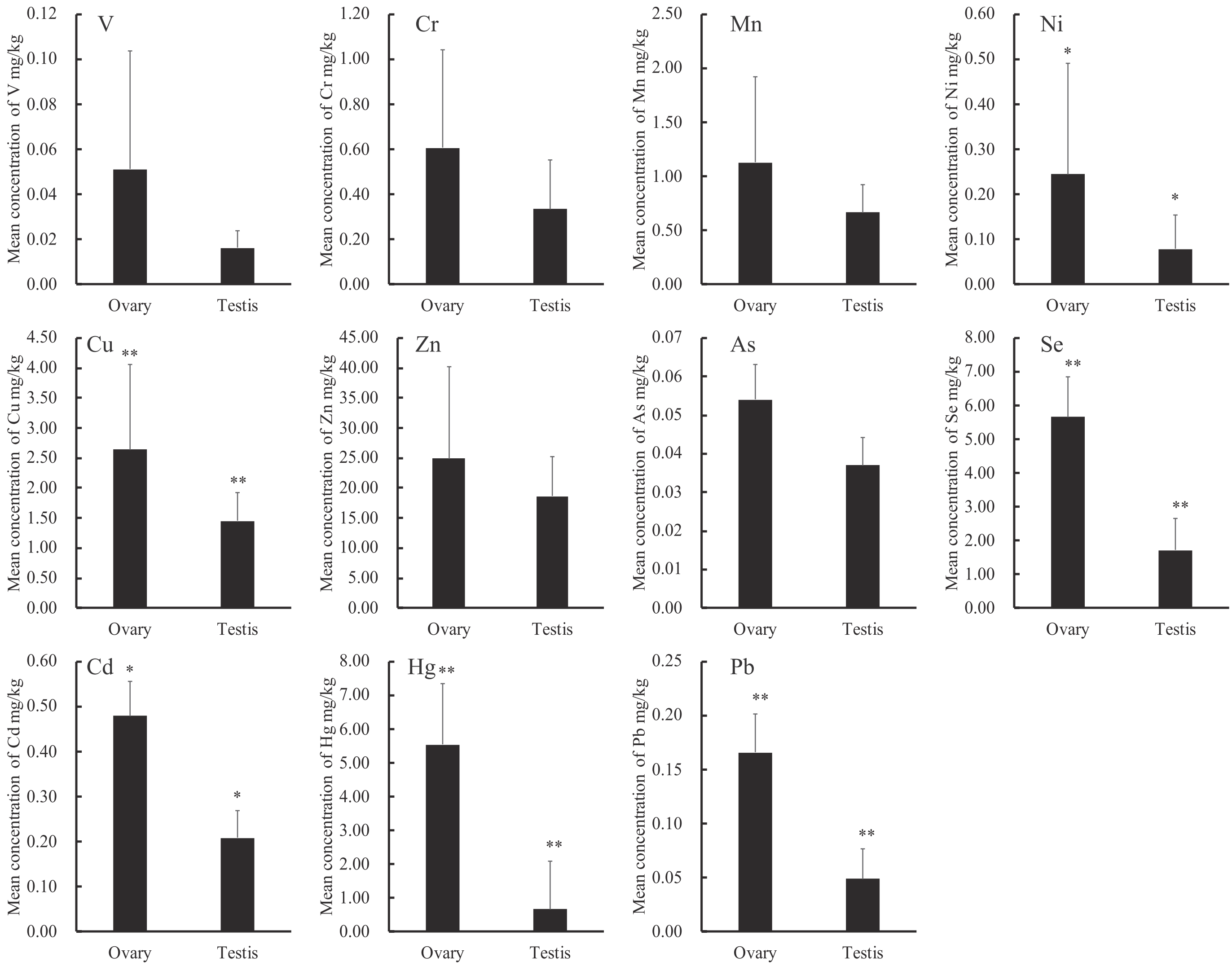


**Fig. 5.** Trace elements concentrations in reproductive tissues (ovary and testis) of Yangtze finless porpoises. Error bars represent the standard error. * indicates $p < 0.05$ and ** indicates $p < 0.01$.

Pacific Humpback Dolphins (*Sousa chinensis*) in the Pearl River Estuary (Gui et al., 2017; Liu et al., 2017; Sun et al., 2017; Zhang et al., 2017). These results are consistent with the characteristics of trace elements pollution levels in these regions (Muller et al., 2008; D. Wang et al., 2013, S.L. Wang et al., 2013; Yin et al., 2015), indicating that tissue concentrations in small cetaceans can reflect trace elements pollution status of water bodies.

### *4.2. Reasons for trace elements accumulations in different tissues of Yangtze finless porpoises*

Concentrations of some trace elements in the epidermis of small cetaceans are significantly positive correlated with the concentrations in internal tissues in previous studies (Yang et al., 2002; Stavros et al., 2011; Aubail et al., 2013; Borrell et al., 2015; Zhang et al., 2017). However, in the present study, such correlations were not found, suggesting that the epidermis might not be a reliable monitoring tool to characterize trace elements accumulations, which are more likely to be present in internal tissues in this species.

This study demonstrates significant correlations between some trace elements that are present in the highest concentrations in the same tissue, suggesting similar accumulation mechanisms of these elements. Accumulation of trace elements such as Hg, Cr, Mn, and Cu in the liver may partly be explained by detoxification process; detoxification process for many heavy metals and metabolism of essential trace elements occur in the liver (Campbell et al., 2005; Ferguson et al., 2001). Even for other trace elements such as Cd, Pb, Zn, and V, the second highest concentrations were also measured in the liver.

The epidermis of the Yangtze finless porpoise is the first (and therefore most direct) tissue exposed to trace elements ions in water. Gaskin (1986) reported that small 'Odontoceti' (Cetacea) assimilate 18% of water from drinking, 51% from food, and the remaining 31% through the skin. Hence, the epidermis is considered to play a nonnegligible role in trace elements assimilation in cetaceans (Augier et al., 1993; Cardellicchio et al., 2000), which suggests that trace elements accumulate in the epidermis of Yangtze finless porpoises may have different assimilation pathways compared with those that accumulated in internal tissues.

The tissues where the highest concentration of As accumulate may depend on the element species. Most As in aquatic organism tissues is in organic form (Rahman et al., 2012), which may lead to its accumulation in blubber, as was seen in this study. For Cd, toxic effects on kidneys (i.e., nephrotoxicity) are well known (Gallien et al., 2001), due to the reaction between Cd and sulfhydryl-rich protein or metallothionein to form highly stable and persistent complexes that are mainly stored in the kidneys (Sun et al., 2017).

### *4.3. Influences of gender and body length on trace element accumulation*

Similar to previous studies on small cetaceans (Gui et al., 2017; Sun et al., 2017; Zhang et al., 2017), no significant differences in most trace elements concentrations (in the highest-concentration tissue) were found between genders in Yangtze finless porpoises in this study. Previous studies have shown that breeding and feeding offspring are two important reasons for differences in accumulation of contaminants between female and male cetaceans (Das et al., 2003; Wagemann et al., 1983). This study indicates that the transmission of trace elements from mother to infant may not be considerable in Yangtze finless porpoises. The significantly higher concentrations of some trace elements (especially some toxic heavy metals) in the primary reproductive organ of female Yangtze finless porpoises is important, however, as trace elements such as Hg, Pb, and Ni are known to cause reproductive toxicity to cetaceans (Theron et al., 2012; Wise et al., 2019).

Significantly positive correlations between Yangtze finless porpoise body length and concentrations of As, Cd, Hg, Se, and Pb (in the highest-concentration tissue) found in this study agree with the results of Liu et al. (2017) in a study on finless porpoises in the East China Sea, and are similar to the results of several studies on other small cetaceans (Gui et al., 2017; Liu et al., 2017; Sun et al., 2017; Zhang et al., 2017). These trace elements are all non-essential, except Se for which the concentration correlates with Hg accumulation. Higher trace elements concentrations in larger individuals are a consequence of bioaccumulation and biomagnification of trace elements through the food chain (Borrell et al., 2015; Monteiro et al., 2016; Yang et al., 2006). In addition, some non-essential elements such as As and Hg can be transformed into organic species, which will enhance their enrichment in tissues rich in fat over an organism's lifespan (Caceres-Saez et al., 2018; Rahman et al., 2012). The positive correlations between some non-essential elements found in this study also suggest that they may have the similar accumulation mechanisms. The enrichment of toxic heavy metals, and particularly the exponential regression of Hg and Se with body length, suggests potential health effects to adult Yangtze finless porpoises, which may influence reproduction and therefore populations.

### 4.4. Spatial differences of trace elements accumulation in Yangtze finless porpoises

Spatial differences of trace elements accumulation resulting from the differences in industry development and pollutant discharge have been reported in small cetaceans both in the coastal area near the Yangtze River Estuary and the Pearl River Estuary (Gui et al., 2017; Liu et al., 2017). However, no significant spatial differences were found for most trace elements in this study. Although economic development and heavy metal pollution within the Yangtze River basin vary (Muller et al., 2008), the majority of Yangtze finless porpoise samples were collected from less developed areas. It has been reported that the Yangtze finless porpoise population tends to gather in particular areas of the river, where there is less impact from human activities such as shipping, bridges, ports, and fishing (Mei et al., 2014), and so to a certain extent Yangtze finless porpoises avoid areas with higher trace elements concentrations. However, our samples from the Yangtze River and the Dongting Lake are much less those from the Poyang Lake, which may affect the result of comparison. Further research on spatial difference should be performed after more samples were collected.

## 5. Conclusions

In conclusion, this study revealed the tendencies for different trace elements to accumulate in different tissues of Yangtze finless porpoises. The results showed that the measurement of trace elements concentrations in the epidermis does not provide a reliable indication of accumulation in internal tissues of Yangtze finless porpoises. Positive correlations between the trace elements accumulated in the same tissues suggested similar mechanisms of metabolism or detoxification pathways. Concentrations of As, Cd, Hg, Se, and Pb were significantly positively correlated with body length. No significant differences in most trace elements accumulations were found between genders. However, higher concentrations of some trace elements were present in the ovaries of female Yangtze finless porpoises than in the testis of male Yangtze finless porpoises, suggesting the higher potential reproductive risk of trace elements toxicity to females. Comparisons of trace elements accumulations in individuals from different major Yangtze finless porpoise habitats showed no significant geographical differences. Compared to previous studies on other small cetaceans, Hg and Cd concentrations were higher in Yangtze finless porpoises in this study, which suggests the potential risk of toxicity, especially for Hg, for this species.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.06.031.

## References

Aubail, A., Mendez-Fernandez, P., Bustamante, P., Churlaud, C., Ferreira, M., Vingada, J.V., Caurant, F., 2013. Use of skin and blubber tissues of small cetaceans to assess the trace element content of internal organs. Mar. Pollut. Bull. 76, 158–169.

Augier, H., Benkoel, L., Chamlian, A., Park, W., Ronneau, C., 1993. Mercury, zinc and selenium bioaccumulation in tissues and organs of Mediterranean striped dolphins Stenella coeruleoalba meyen. Toxicological result of their interaction. Cell. Mol. Biol. (Noisy-le-grand) 39, 621–634.

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.

Berry, M.J., Ralston, N.V.C., 2008. Mercury toxicity and the mitigating role of selenium. EcoHealth 5, 456–459.

Betti, C., Nigro, M., 1996. The comet assay for the evaluation of the genetic hazard of pollutants in cetaceans: preliminary results on the genotoxic effects of methyl-mercury on the bottle-nosed dolphin (Tursiops truncatus) lymphocytes in vitro. Mar. Pollut. Bull. 32, 545–548.

Borrell, A., Clusa, M., Aguilar, A., Drago, M., 2015. Use of epidermis for the monitoring of tissular trace elements in Mediterranean striped dolphins (Stenella coeruleoalba). Chemosphere 122, 288–294.

Braulik, G.T., Noureen, U., Arshad, M., Reeves, R.R., 2015. Review of status, threats, and conservation management options for the endangered Indus River blind dolphin. Biol. Conserv. 192, 30–41.

Caceres-Saez, I., Haro, D., Blank, O., Lobo, A.A., Dougnac, C., Arredondo, C., Cappozzo, H.L., Guevara, S.R., 2018. High status of mercury and selenium in false killer whales (Pseudorca crassidens, Owen 1846) stranded on southern South America: a possible toxicological concern? Chemosphere 199, 637–646.

Cai, S., Yue, L., Shang, Q., Nordberg, G., 1995. Cadmium exposure among residents in an area contaminated by irrigation water in China. Bull. W.H.O. 73, 359–367.

Campbell, L.M., Norstrom, R.J., Hobson, K.A., Muir, D.C.G., Backus, S., Fisk, A.T., 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Sci. Total Environ. 351, 247–263.

Cardellicchio, N., Giandomenico, S., Ragone, P., Di Leo, A., 2000. Tissue distribution of metals in striped dolphins (Stenella coeruleoalba) from the Apulian coasts, Southern Italy. Mar. Environ. Res. 49, 55–66.

Cuvin-Aralar, M.L.A., Furness, R.W., 1991. Mercury and selenium interaction - a review. Ecotoxicol. Environ. Saf. 21, 348–364.

Das, K., Debacker, V., Pillet, S., Bouquegneau, J.-M., 2003. Heavy metals in marine mammals. Toxicology of marine mammals 3, 135–167.

Desforges, J.P., Hall, A., McConnell, B., Rosing-Asvid, A., Barber, J.L., Brownlow, A., De Guise, S., Eulaers, I., Jepson, P.D., Letcher, R.J., Levin, M., Ross, P.S., Samarra, F., Vikingson, G., Sonne, C., Dietz, R., 2018. Predicting global killer whale population collapse from PCB pollution. Science 361, 1373–1376.

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

Ferguson, C.J., Wareing, M., Ward, D.T., Green, R., Smith, C.P., Riccardi, D., 2001. Cellular localization of divalent metal transporter DMT-1 in rat kidney. Am. J. Physiol-Renal. 280, F803–F814.

Fu, C., Wu, J., Chen, J., Wu, Q., Lei, G., 2003. Freshwater fish biodiversity in the Yangtze River basin of China: patterns, threats and conservation. Biodivers. Conserv. 12 (8), 1649–1685.

Gallien, I., Caurant, F., Bordes, M., Bustamante, P., Miramand, P., Fernandez, B., Quellard, N., Babin, P., 2001. Cadmium-containing granules in kidney tissue of the Atlantic white-sided dolphin (Lagenorhyncus acutus) off the Faroe Islands. Comp. Biochem. Phys. C 130, 389–395.

Gaskin, D., 1986. Kidney and water metabolism. In: Bryden, M.M., Harrison, R. (Eds.), Research on Dolphins, pp. 129–148.

Gui, D., Yu, R.Q., Karczmarski, L., Ding, Y.L., Zhang, H.F., Sun, Y., Zhang, M., Wu, Y.P., 2017. Spatiotemporal trends of heavy metals in Indo-Pacific Humpback Dolphins (Sousa chinensis) from the western Pearl River Estuary, China. Environ. Sci. Technol. 51, 1848–1858.

Isobe, T., Oshihoi, T., Hamada, H., Nakayama, K., Yamada, T.K., Tajima, Y., Amano, M., Tanabe, S., 2011. Contamination status of POPs and BFRs and relationship with parasitic infection in finless porpoises (Neophocaena phocaenoides) from Seto Inland Sea and Omura Bay, Japan. Mar. Pollut. Bull. 63, 564–571.

Iwata, M., Takebayashi, T., Ohta, H., Alcalde, R.E., Itano, Y., Matsumura, T., 1999. Zinc accumulation and metallothionein gene expression in the proliferating epidermis during wound healing in mouse skin. Histochem. Cell Biol. 112, 283–290.

Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, A., Borrell, A., Murphy, S., Barry, J., Brownlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., ten Doeschate, M., Esteban, R., Ferreira, M., Foote, A.D., Genov, T., Gimenez, J., Loveridge, J., Llavona, A., Martin, V., Maxwell, D.L., Papachlimitzou, A., Penrose, R., Perkins, M.W., Smith, B., de Stephanis, R., Tregenza, N., Verborgh, P., Fernandez, A., Law, R.J., 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Sci. Rep. 6.

Kemper, C., Gibbs, P., Obendorf, D., Marvanek, S., Lenghaus, C., 1994. A review of heavy-metal and organochlorine levels in marine mammals in Australia. Sci. Total Environ. 154, 129–139.

Khan, M.A.K., Wang, F.Y., 2009. Mercury-selenium compounds and their toxicological significance: toward a molecular understanding of the mercury-selenium antagonism. Environ. Toxicol. Chem. 28, 1567–1577.

Kucklick, J., Schwacke, L., Wells, R., Hohn, A., Guichard, A., Yordy, J., Hansen, L., Zolman, E., Wilson, R., Litz, J., Nowacek, D., Rowles, T., Pugh, R., Balmer, B., Sinclair, C., Rosel, P., 2011. Bottlenose dolphins as indicators of persistent organic pollutants in the Western North Atlantic Ocean and Northern Gulf of Mexico. Environ. Sci. Technol. 45, 4270–4277.

Lam, J.C.W., Lyu, J.L., Kwok, K.Y., Lam, P.K.S., 2016. Perfluoroalkyl substances (PFASs) in marine mammals from the South China Sea and their temporal changes 2002-2014: concern for alternatives of PFOS? Environ. Sci. Technol. 50, 6728–6736.

Lansdown, A.B.G., Mirastschijski, U., Stubbs, N., Scanlon, E., Agren, M.S., 2007. Zinc in wound healing: theoretical, experimental, and clinical aspects. Wound Repair Regen. 15, 2–16.

Law, R.J., 1996. Metals in marine mammals. Lewis Publishers. Florida, Boca Raton.

Li, J.H., Sun, Y.Y., Yin, Y., Ji, R., Wu, J.C., Wang, X.R., Guo, H.Y., 2010. Ethyl lactate-EDTA composite system enhances the remediation of the cadmium-contaminated soil by autochthonous willow (Salix x aureo-pendula CL 'J1011') in the lower reaches of the Yangtze River. J. Hazard. Mater. 181, 673–678.

Liu, J.Y., Liang, J., Yuan, X.Z., Zeng, G.M., Yuan, Y.J., Wu, H.P., Huang, X.L., Liu, J.F., Hua, S.S., Li, F., Li, X.D., 2015. An integrated model for assessing heavy metal exposure risk to migratory birds in wetland ecosystem: a case study in Dongting Lake Wetland, China. Chemosphere 135, 14–19.

Liu, J., Chen, B.Y., Jefferson, T.A., Wang, H., Yang, G., 2017. 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. Sci. Total Environ. 575, 628–638.

Mahfouz, C., Henry, F., Courcot, L., Pezeril, S., Bouveroux, T., Dabin, W., Jauniaux, T., Khalaf, G., Amara, R., 2014. Harbour porpoises (Phocoena phocoena) stranded along the southern North Sea: An assessment through metallic contamination. Environ. Res. 133, 266–273.

Mei, Z.G., Huang, S.L., Hao, Y.J., Turvey, S.T., Gong, W.M., Wang, D., 2012. Accelerating population decline of Yangtze finless porpoise (Neophocaena asiaeorientalis asiaeorientalis). Biol. Conserv. 153, 192–200.

Mei, Z., Zhang, X., Huang, S.-L., Zhao, X., Hao, Y., Zhang, L., Qian, Z., Zheng, J., Wang, K., Wang, D., 2014. The Yangtze finless porpoise: on an accelerating path to extinction? Biol. Conserv. 172, 117–123.

Monteiro, S.S., Torres, J., Ferreira, M., Marcalo, A., Nicolau, L., Vingada, J.V., Eira, C., 2016. Ecological variables influencing trace element concentrations in bottlenose dolphins (Tursiops truncatus, Montagu 1821) stranded in continental Portugal. Sci. Total Environ. 544, 837–844.

Muller, B., Berg, M., Yao, Z.P., Zhang, X.F., Wang, D., Pfluger, A., 2008. How polluted is the Yangtze River? Water quality downstream from the Three Gorges Dam. Sci. Total Environ. 402, 232–247.

Park, B.K., Park, G.J., An, Y.R., Choi, H.G., Kim, G.B., Moon, H.B., 2010. Organohalogen contaminants in finless porpoises (Neophocaena phocaenoides) from Korean coastal waters: contamination status, maternal transfer and ecotoxicological implications. Mar. Pollut. Bull. 60, 768–774.

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.

Rahman, M.A., Hasegawa, H., Lim, R.P., 2012. Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. Environ. Res. 116, 118–135.

Roditi-Elasar, M., Kerem, D., Hornung, H., Kress, N., Shoham-Frider, E., Goffman, O., Spanier, E., 2003. Heavy metal levels in bottlenose and striped dolphins off the Mediterranean coast of Israel. Mar. Pollut. Bull. 46, 503–512.

Sharma, R.K., Agrawal, M., 2005. Biological effects of heavy metals: An overview. J. Environ. Biol. 26, 301–313.

Stavros, H.C.W., Stolen, M., Durden, W.N., McFee, W., Bossart, G.D., Fair, P.A., 2011. Correlation and toxicological inference of trace elements in tissues from stranded and free-ranging bottlenose dolphins (Tursiops truncatus). Chemosphere 82, 1649–1661.

Sun, X., Yu, R.Q., Zhang, M., Zhang, X.Y., Chen, X., Xiao, Y.S., Ding, Y.L., Wu, Y.P., 2017. Correlation of trace element concentrations between epidermis and internal organ tissues in Indo-Pacific humpback dolphins (Sousa chinensis). Sci. Total Environ. 605, 238–245.

Theron, A.J., Tenting, G., Anderson, R., 2012. Harmful interactions of non-essential heavy metals with cells of the innate immune system. J. Clin. Toxicol. S 3 (00), 5.

Tokar, E.J., Boyd, W.A., Freedman, J.H., Waalkes, M.P., 2012. Toxic Effects of Metals, Casarett and Doull's Toxicology: The Basic Science of Poisons. 8e. McGraw-Hill Education, New York, NY.

Turvey, S.T., Pitman, R.L., Taylor, B.L., Barlow, J., Akamatsu, T., Barrett, L.A., Zhao, X.J., Reeves, R.R., Stewart, B.S., Wang, K.X., Wei, Z., Zhang, X.F., Pusser, L.T., Richlen, M., Brandon, J.R., Wang, D., 2007. First human-caused extinction of a cetacean species? Biol. Lett. 3, 537–540.

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit. Rev. Environ. Sci. Technol. 31, 241–293.

Veron, G., Patterson, B.D., Reeves, R., 2008. Global diversity of mammals (Mammalia) in freshwater. Hydrobiologia 595, 607–617.

Wagemann, R., Muir, D.C., 1984. Concentrations of Heavy Metals and Organochlorines in Marine Mammals of Northern Waters: Overview and Evaluation. Department of Fisheries and Oceans Canada, Western Region.

Wagemann, R., Snow, N.B., Lutz, A., Scott, D.P., 1983. Heavy-metals in tissues and organs of the Narwhal (Monodon-Monoceros). Can. J. Fish. Aquat. Sci. 40, 206–214.

Wang, D., 2009. Population status, threats and conservation of the Yangtze finless porpoise. Chin. Sci. Bull. 54, 3473–3484.

Wang, K.X., Wang, D., Zhang, X.F., Pfluger, A., Barrett, L., 2006. Range-wide Yangtze freshwater dolphin expedition: the last chance to see Baiji? Environ. Sci. Pollut. R. 13, 418–424.

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

Wang, S.L., Xu, X.R., Sun, Y.X., Liu, J.L., Li, H.B., 2013. Heavy metal pollution in coastal areas of South China: a review. Mar. Pollut. Bull. 76, 7–15.

Williams, P.N., Lei, M., Sun, G.X., Huang, Q., Lu, Y., Deacon, C., Meharg, A.A., Zhu, Y.G., 2009. Occurrence and partitioning of cadmium, arsenic and lead in mine impacted paddy rice: Hunan, China. Environ. Sci. Technol. 43, 637–642.

Wise, J.P., Payne, R., Wise, S.S., LaCerte, C., Wise, J., Gianios, C., Thompson, W.D., Perkins, C., Zheng, T.Z., Zhu, C.R., Benedict, L., Kerr, I., 2009. A global assessment of chromium pollution using sperm whales (Physeter macrocephalus) as an indicator species. Chemosphere 75, 1461–1467.

Wise, J.P., Wise, J.T.F., Wise, C.F., Wise, S.S., Zhu, C.R., Browning, C.L., Zheng, T.Z., Perkins, C., Gianios, C., Xie, H., Wise, J.P., 2019. Metal levels in whales from the Gulf of Maine: a one environmental health approach. Chemosphere 216, 653–660.

Yang, J., Kunito, T., Tanabe, S., Amano, M., Miyazaki, N., 2002. Trace elements in skin of Dall's porpoises (Phocoenoides dalli) from the northern waters of Japan: an evaluation for utilization as non-lethal tracers. Mar. Pollut. Bull. 45, 230–236.

Yang, J., Miyazaki, N., Kunito, T., Tanabe, S., 2006. Trace elements and butyltins in a Dall's porpoise (Phocoenoides dalli) from the Sanriku coast of Japan. Chemosphere 63, 449–457.

Yang, F.X., Zhang, Q.H., Xu, Y., Jiang, G.B., Wang, Y.W., Wang, D., 2008. Preliminary hazard assessment of polychlorinated biphenyls, polybrominated diphenyl ethers, and polychlorinated dibenzo-p-dioxins and dibenzofurans to Yangtze finless porpoise in Dongting Lake, China. Environ. Toxicol. Chem. 27, 991–996.

Yang, H., Xie, P., Ni, L., Flower, R.J., 2012. Pollution in the Yangtze. Science 337, 410.

Yi, Y.J., Yang, Z.F., Zhang, S.H., 2011. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. Environ. Pollut. 159, 2575–2585.

Yin, S., Feng, C.H., Li, Y.Y., Yin, L.F., Shen, Z.Y., 2015. Heavy metal pollution in the surface water of the Yangtze estuary: a 5-year follow-up study. Chemosphere 138, 718–725.

Zhang, W.G., Feng, H., Chang, J.N., Qu, J.G., Xie, H.X., Yu, L.Z., 2009. Heavy metal contamination in surface sediments of Yangtze River intertidal zone: An assessment from different indexes. Environ. Pollut. 157, 1533–1543.

Zhang, X.Y., Lin, W.Z., Yu, R.Q., Sun, X., Ding, Y.L., Chen, H.L., Chen, X., Wu, Y.P., 2017. Tissue partition and risk assessments of trace elements in Indo-Pacific Finless Porpoises (Neophocaena phocaenoides) from the Pearl River Estuary coast, China. Chemosphere 185, 1197–1207.

Zhou, X.M., Guang, X.M., Sun, D., Xu, S.X., Li, M.Z., Seim, I., Jie, W.C., Yang, L.F., Zhu, Q.H., Xu, J.B., Gao, Q., Kaya, A., Dou, Q.H., Chen, B.Y., Ren, W.H., Li, S.C., Zhou, K.Y., Gladyshev, V.N., Nielsen, R., Fang, X.D., Yang, G., 2018. Population genomics of finless porpoises reveal an incipient cetacean species adapted to freshwater. Nat. Commun. 9.