LONG-TERM TEMPORAL TRENDS OF ROCKSHELL IMPOSEX AND BUTYLTIN COMPOUND RESIDUES IN MARINE ORGANISMS FROM KOREAN COASTAL WASTERS

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Introduction

Butyltin compounds (BTs) are globally ubiquitous contaminants in the marine environment because of their wide use as antifouling paint components on ship hulls, marine platforms, and fishing nets. BTs are of great concern in aquatic ecosystems due to their ecotoxicological effects. Sub-part-per-billion levels of TBT in seawater can cause reduced growth and viability of various larvae of mussels, and oyster shell anomalies and spat fall failure.[1] Many studies have reported that BTs can cause growth, developmental, and reproductive disorders in non-target marine species and are toxic to the immune systems of marine mammals.[2,3] The high level of BT contamination and their adverse health effects resulted in the restriction of the use of tributyltin (TBT) in antifouling paints. In October 2001, the International Maritime Organization (IMO) adopted the International Convention on the Control of Harmful Antifouling Systems on Ships. This Convention banned the application of TBT-based paint by January 2003 and the presence of those paints on vessels' hulls by January 2008. In Korea, restrictions on the use of TBT-based antifouling paints were partially introduced in 2000 for small boats, and their use was totally banned in 2003, and their residues on ships were banned in 2008. As a result, BT levels in coastal environments have been decreasing worldwide as well as in Korean coastal waters.[4,5] However, high levels of BTs are still detected in a variety of aquatic organisms and are found in water and sediments from estuaries and harbors.[3] Thus, the environmental occurrence and adverse health effects of BTs on marine organisms are still of concern. However, little is known about temporal trends of BTs in marine organisms including gastropods and cetaceans. In the present study, imposex and BT levels were monitored in rock shells, *Thais clavigera*, for the Korean coastal waters between 2004 and 2009, to assess the effectiveness of a legislative action on TBT-based antifouling paints. We also measured the concentrations and accumulation features of BTs in the livers of finless porpoises (*Neophocaena asiaeorientalis*) collected in 2003 and 2010 from Korean coastal waters. We also investigated temporal trends in BT levels to evaluate whether TBT regulation had a significant impact on BT accumulation in marine mammals from Korean coastal waters. The data set from this study can provide a useful baseline for long-term study of BT contamination in Asian coastal waters.

Materials and methods

Imposex levels were measured in the gastropod, *T. clavigera* (n=2165), collected from 26 stations along the Korean coast in spring (March–May) from 2004 to 2009. The sampling areas were eight sites near large ports (L) containing commercial and ferry services, i.e., Daecheon, Gwangyang, Yeosu, Ulsan, and Onsan, 15 sites near small fishing ports (S), and three sites as background areas (B) with no point sources such as shipping activities within several kilometers. The shortest distance from background sites to the fishing port was approximately 8 kilometers.

Identification of imposex in *T. clavigera* was performed following methods described in Horiguchi et al.[6] 10–20 specimens of *T. clavigera* from each station were used to measure occurrence and degree of imposex. 20–30 mm of *T. clavigera* was used to minimize effects of variation of animal's size on imposex parameters. Shell length was measured with vernier calipers. Shells were crushed using a small vice and the shells were removed. The sex determination was performed according to existence of female accessory sex organs, such as albumen, sperm-ingesting and capsule glands. Imposex was also evaluated in females, namely, percentage occurrence of imposex, and penis length, development of vas deferens and oviduct blockage (i.e., sterile individual). Male penis length was also measured. Imposex development indices applied were: (a) percentage occurrence of imposex females, and (b) relative penis length index (RPLI, %), calculated as the ratio of the mean penis length of imposex females to the mean penis length of males, at each station, multiplied by 100.

Thirty-three liver samples were collected from finless porpoises entangled in fishing nets along the South Sea (Busan coast) and Yellow Sea (Daecheon coast) during May–August in 2003 and 49 liver samples were collected along the Yellow Sea (Daecheon coast) during March–June in 2010 (Figure 1).

Concentrations of BTs, including TBT, dibutyltin (DBT) and monobutyltin (MBT), were determined. Detailed descriptions of the experimental procedures used to measure BTs have been reported previously.[4,5] In brief, tissue samples (1–2 g) were extracted twice by mechanical shaking for 1 h with 20 mL of 0.1% tropolone (Merck, Hohenbrum, Germany), methylene chloride (Merck, Darmstadt, Germany), and 10 mL of 50% HCl (Merck) in 50 mL Teflon tubes. The extract was hexylated with 2 mL of Grignard reagent, n-hexylmagnesium bromide (TCI, Tokyo, Japan), and then cleaned by passing through a florisil (Sigma-Aldrich, Milwaukee, WI, USA) column. A gas chromatograph with a mass spectrometer detector (GC–MSD; Agilent 6890, Wilmington, USA) was used to quantify the BTs.

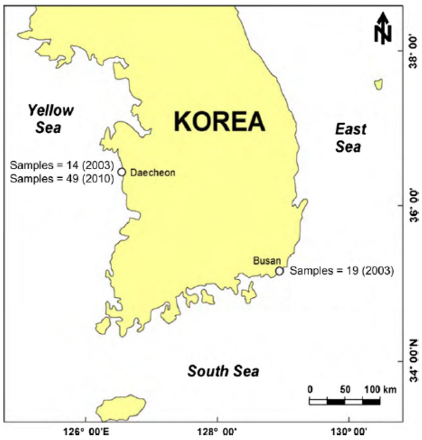

Figure 1. Sampling locations of finless porpoises (*Neophocaena asiaeorientalis*) collected from Korean coastal waters.

Results and discussion

Temporal variations in imposex frequency, RPLI, and TBT levels between 2004 and 2009 are shown in Figure 2. All the imposex indices decreased over the sampling year and their significant differences between sampling years were also found (Kruskal–Wallis test, $p < 0.05$). This indicates that imposex of *T. clavigera* in Korean coastal waters decreases over time. The mean imposex frequency showed values over 95% between 2004 and 2006, and started to decrease significantly from 2007. The mean of RPLI significantly decreased since 2006. This indicates effectiveness of the legislation action in Korean coastal waters.

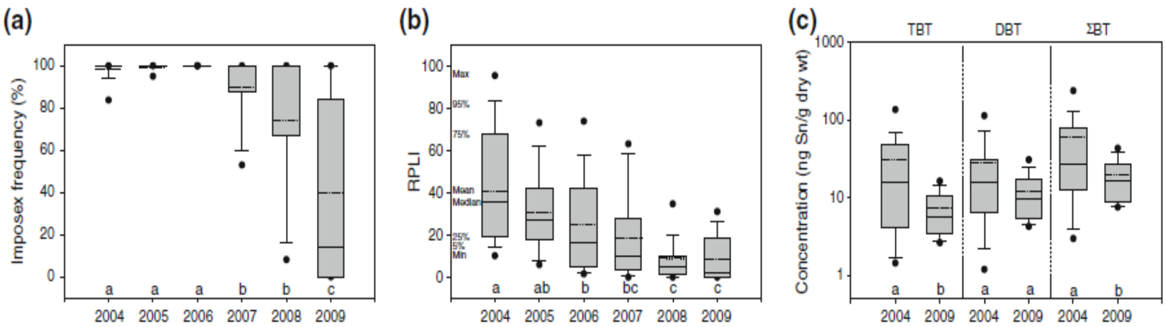

Figure 2. Temporal trends in (a) imposex frequency, (b) Relative Penis Length Index (RPLI), and (c) TBT, DBT, and BT tissue concentration from the Korean coast.

Apparent declines in imposex levels based on RPLI were observed at sites near large ports, small ports, and background areas (Figure 3). Despite the overall decreasing trend in imposex levels in *T. clavigera* along the Korean coast, all imposex indices were still higher in large ports than in small ports and background areas. In 2009 survey, the frequency of imposex females near large ports (mean ± SD, 77.1 ± 30.5%, n = 8) was significantly higher than those near small ports (21.4 ± 29.1%, n = 15; *t*-test, $p < 0.05$). There were also significant differences in RPLI between large and small ports (*t*-test, $p < 0.05$). In background areas, all imposex indices were zero. This indicates that the degree of imposex is increased with increased maritime activity. Based on these results, improvement and recovery of imposex caused by BT contamination will occur faster in areas with very low maritime activities than in those with high maritime activities.

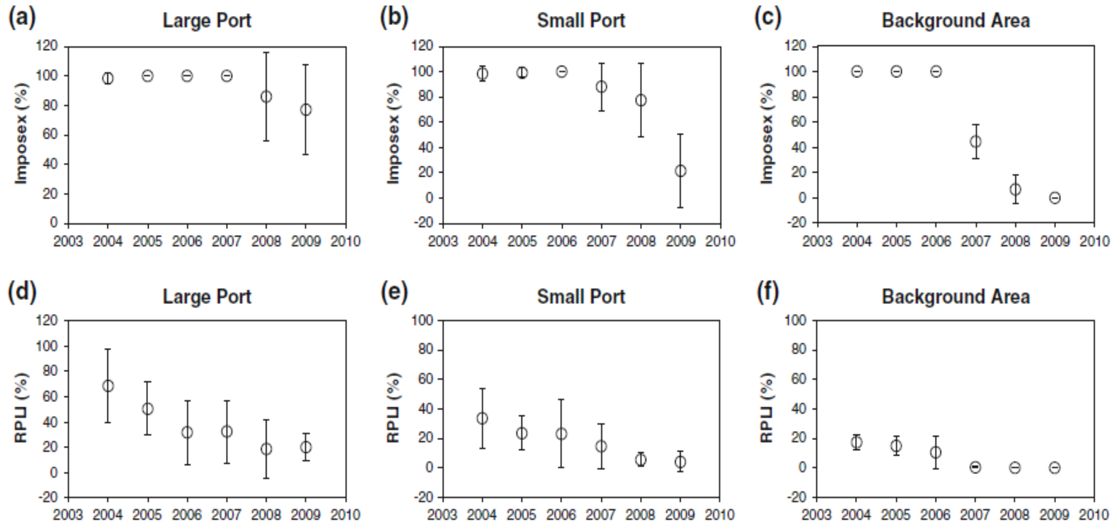

Figure 3. Temporal trends in imposex indices (imposex frequency, and Relative Penis Length Index [RPLI]) in large ports, small ports, and background areas on the Korean coast. Circles indicate means and bars indicate standard deviations. Circles without bars indicate standard deviation is zero.

For finless porpoises, the total concentrations of BTs were also significantly correlated with all the biometric parameters of body length, body weight, and age ($r = 0.503–0.610$, $p < 0.001$; Figure 4), indicating that BT accumulation in marine mammals is size- or age-dependent. Earlier studies have reported that higher BT levels occur in larger and older cetaceans.[7] The concentrations of MBT, DBT, TBT, and total BTs in finless porpoises were significantly different ($p < 0.001$) between the two sampling years of 2003 and 2010 (Figure 5), using Student's independence sample *t*-test (homogeneity test, $p < 0.05$). The total concentrations of BTs in finless porpoises collected from the South Sea (S03; 682 ± 291 ng/g wet weight) and Yellow Sea (Y03; 701 ± 371 ng/g wet weight) in 2003 were significantly higher than those measured in the Yellow Sea (Y10; 155 ± 58.4 ng/g wet weight) in 2010, indicating a clear decrease in BTs in marine mammals associated with TBT regulation in Korea. Earlier studies confirmed a clear decreasing trend in the concentrations of TBT in seawater and bivalves from Korean coastal waters.[4,5] We also calculated the half-life of BTs in finless porpoises collected from the Yellow Sea of Korea using first-order kinetics to estimate reduction rate of BTs.[4,5] In this study, the rate constants of MBT, DBT, TBT, and total BTs were –0.13, –0.26, –0.33, and –0.27 $year^{-1}$, and the calculated half-lives were 5.1, 2.6, 2.1, and 2.5 years, respectively. The half-lives of byproducts, MBT and DBT, were longer than that of TBT. A similar pattern in finless porpoises was reported by Nakayama et al.[8] It suggests that exposure of finless porpoises to TBT decreases faster than MBT and DBT. Therefore, TBT regulation has been effective at decreasing TBT concentrations in marine mammals as well as lower-trophic-level species such as mussels and oysters in the coastal environment of Korea. Our results emphasize the importance of legislative action against chemical contamination in coastal environment.

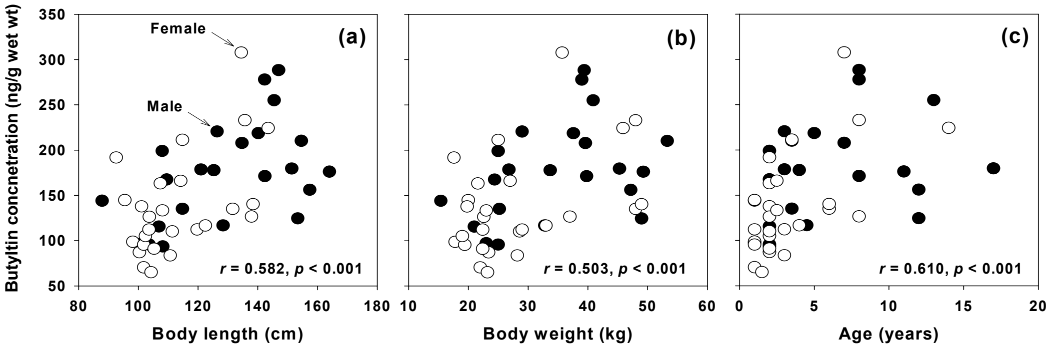

Figure 4. Correlations between total BT concentrations and (a) body length, (b) body weight and (c) estimated age of finless porpoises (*Neophocaena asiaeorientalis*) caught off the Yellow Sea of Korea in 2010.

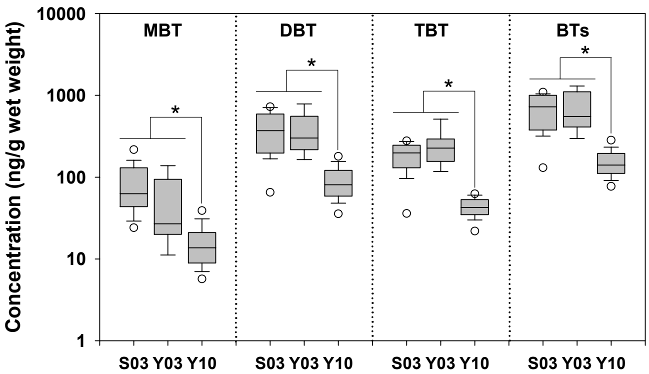

Figure 5. Comparison of concentrations of butyltin compounds in finless porpoises collected from the South Sea (S03) and Yellow Sea (Y03) in 2003, and the Yellow Sea in 2010 (Y10). * indicates significant differences at $p < 0.05$.

Acknowledgements
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