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Imposex levels and butyltin compounds (BTs) in *Hexaplex trunculus* (Linnaeus, 1758) from the northern Adriatic Sea (Italy): Ecological risk assessment before and after the ban

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ABSTRACT

The aim of this study was to compare imposex and butyltin compounds (BTs) data, collected before and after the organotin ban in 2008, in order to assess temporal and spatial variation of the phenomenon, the decline of BT contamination, and the effects on *Hexaplex trunculus* population in the coastal area of the northern Adriatic Sea, close to the Venice Lagoon. Both in marine and in lagoon sites, the results obtained in 2013–2015 showed a significant decline in the incidence of imposex in respect to those from the 2002 survey. In 2002, lagoon samples exhibited Relative Penis Size Index (RPSI) higher than marine samples, whereas no differences were detected in the recent survey, when all RPSI values were below 0.6%. Vas Deference Sequence Index (VDSI) mean values were over 4 before the ban introduction and below this value after that, indicating more critical conditions for gastropod population in 2002 rather than in 2013-15. Percentage of sterile females was up to 69% in 2002, whilst in the more recent survey no sterile female was found. Range of BT concentrations in gastropods decreased from 252 to 579 to 16–31 ngΣBT/g d.w. BT body burdens varied according to a gender dependant pattern, with higher concentrations observed in females than in males. A first attempt to propose a classification based on BT impact on *H. trunculus*, according to the Water Framework Directive, revealed that most sites were in Bad ecological status before the ban and attained a Poor/Moderate status after that.

1. Introduction

Imposex is a phenomenon described for the first time by Smith (1971), which consists of the superimposition of male sexual characters onto female gonochoristic gastropods. It is one of the most studied effect of tributyltin (TBT) which affected more than 260 species of gastropods, and it is used as TBT specific biomarker all over the world, mostly in those species which have shown a significant correlation between the degree of morphological alterations and the concentrations of the contaminant (Titley-O'Neal et al., 2011). In most of caenogastropoda species, imposex can lead to population decline as a consequence of sterility and reproduction failure (Bryan et al., 1986).

Among these species, *Hexaplex trunculus* (Linnaeus, 1758) has been used as a bioindicator of butyltin (BT) pollution in Mediterranean areas, such as along the coasts of Malta (Axiak et al., 1995, 2000, 2012), Tunisia (Lahbib et al., 2008, 2009, 2011; Anastasiou et al., 2016), Croatia (Garaventa et al., 2006, 2007; Carić et al., 2016; Erdelez et al., 2017) and Italy (Terlizzi et al., 1998, 1999, 2003; Chiavarini et al., 2003; Pellizzato et al., 2004; Garaventa et al., 2006, 2007; Anastasiou et al., 2016), both before and after the TBT global ban adopted in 2001 by the International Convention on the control of harmful antifouling systems on ships (AFS-Convention 2001). The global ban came into force in September 2008 (IMO, 2001), but in the European Union it was enacted by the EC Regulation 782/2003 which totally interdicted the application of organotin compounds on ships after 1st of July 2003 and forced the eradication of these compounds from ships from 1st January 2008 (EC, 2003). These regulations arose from evidences of organotin high toxicity to many aquatic organisms and significant long-term ecological impacts at very low concentrations on non-target marine species (Axiak et al., 2012). Due to their persistence, toxicity and

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bioaccumulation properties, TBT is among the priority hazardous substances according to the European Water Framework Directive 2000/60/CE (WFD) and its daughter Directive 2008/105/EC and Directive 2013/39/EU (EC, 2000, 2008a; EU, 2013). For transitional and coastal waters, Environmental Quality Standards (EQS) have been set at 0.2 ngTBT/l as annual average (AA)-EQS and at 1.5 ngTBT/l as Maximum Allowable Concentration (MAC)-EQS. Since these concentrations are lower than the BT levels that can be routinely measured, the worldwide use of gastropods for BT monitoring has been further promoted. Indeed, gastropods are very sensitive also to low levels of TBT, which are known to cause impairment to sexual development (Wilson et al., 2015). TBT fate in marine environments is also among the objectives which have to be considered for the achievement of the Good Environmental Status (GES) aimed by the Marine Strategy Framework Directive 2008/56/EC (MSFD; EC, 2008b) and, in this context, the measurement of imposex development in gastropods was proposed to be included within the MSFD monitoring programs (Noventa et al., 2014). Several biological TBT assessment criteria regarding the WFD were proposed using *Littorina littorea* (Oehlmann, 2002), *Nucella lapillus* (WFD-UKTAG, 2014), and a multispecies approach which considered together *N. lapillus, L. littorea* and *Tritia reticulata*, in order to monitor and compare larger geographical area, wider variety of water bodies and broader TBT pollution gradient (Laranjeiro et al., 2015). Following the approach described in the work of Laranjeiro et al. (2015), a first attempt using *H. trunculus* was very recently performed for the Croatian Adriatic coast (Erdelez et al., 2017).

In a recent study conducted in the Venice Lagoon, to propose imposex levels as an indicator of the impact of BTs within the WFD, classification class boundaries and Ecological Quality Ratio (EQR) were introduced for transitional waters by using *Nassarius nitidus,* a nassariid widespread in the lagoons of the Adriatic Sea. According to the WFD, the principle of the assessment criteria was to relate the degree of disruption at the population level, and the imposex intensity, as an indicator of the impact of BTs, by considering sterility, imposex incidence and the level of imposex (Cacciatore et al., 2016). The same approach could be easily adopt also for other gastropod species.

In many transitional waters, such as in the Venice Lagoon, *H. trunculus* and *N. nitidus* are sympatric gastropod species from different families, with the former larger in size and more sensitive to TBT than the latter (Pellizzato et al., 2004; Pavoni et al., 2007). Indeed, first signals of imposex are reported for *H. trunculus* at about 2.44 ngTBT/g d.w., whilst in *T. reticulata*, a species closely related to *N. nitidus*, the initiation of imposex was observed at 61.10 ngTBT/g d.w. (Axiak et al., 1995). As well as in transitional waters, *H. trunculus* is also widespread in coastal waters as defined by the WFD, and in marine waters as defined by the MSFD (EC, 2000, 2008b). Therefore, the use of *H. trunculus* could bridge the gap in monitoring programmes of wider areas, also under low TBT pollution.

Despite restrictions, TBT and its products of degradation (i.e., di- and mono-butyltin) are still present in marine and estuarine environments, probably due to TBT slow degradation rates in marine sediments, with a half-life ranging between 1 and 5 years. Furthermore, dibutyltin (DBT) and monobutyltin (MBT) are present in the marine environment not only due to the degradation of TBT, but also because of their industrial and agricultural use (Hoch, 2001). Although release from antifouling paints represented the main mode of introduction of TBT into aquatic environments, resuspension of contaminated sediments could currently lead to increased BT bioavailability for marine benthic organisms, even presently (Rüdel, 2003). In addition, it is to be noticed that illegal use of TBT-based antifouling paints can occur, as TBT is still produced as antifouling agent (Turner and Glegg, 2014).

The first studies of biological effects of TBT on *H. trunculus* population in the Venice Lagoon were conducted in 2002–2003 (Pellizzato et al., 2004; Garaventa et al., 2007) and they highlighted a positive correlation between occurrence of imposex and BT contamination. They also reported differences in contamination between marine and lagoon samples, with higher levels in the latter. However, since then some questions have remained, such as those concerning i) the occurrence of seasonal fluctuations in the values of measured imposex indices which may reflect changes in size and/or physiological condition of molluscs, ii) the different BT accumulation ability of males and females, and iii) the lack of imposex data related to organisms exposed at low organotin concentrations.

Previous studies reported the effect of male penis size related to reproductive cycle on imposex indices, especially those considering the penis length, in several gastropod species, such as *Trivia arctica, N. lapillus, Ocenebra erinaceus, T. reticulata, N. incrassatus* (Stroben et al., 1996), *Bolinus brandaris* (Ramon and Amor, 2002; Vasconcelos et al., 2010b) and *Thais clavigera* (Zhengyan, 2005). Lahbib et al. (2012) recently studied *H. trunculus* in Tunisia coastal lagoons, reporting the importance of male penis variation and specimen size in the Relative Penis Length Index (RPLI) calculation. However, no data are available for the gastropod populations of the Adriatic Sea where the environmental conditions are quite different and where the effects of the EU ban should be observed both on BT bioaccumulation and imposex indices.

In this context, the aim of this study was to compare imposex and BT body burden data obtained from animals collected before and after the ban, in order to assess temporal variation of the phenomenon, the decline of BT contamination and the condition of *H. trunculus* population in the coastal area of the northern Adriatic Sea, close to the Venice Lagoon. A spatial assessment was also approached by comparing lagoon and sea sites. In addition, in 2014–2015 one site was monitored monthly for one year to evaluate imposex levels in gastropods from an area with relatively low density of boat traffic, with a focus on seasonal variations and gastropod size differences, which may interact differently, affecting both BT bioaccumulation and imposex levels. Finally, an ~~first~~ attempt of ecological quality assessment using *H. trunculus* was proposed according to the WFD.

2. Materials and methods

2.1. Sample collection and preparation

Fig. 1 shows *H. trunculus* sampling sites, both inside and outside the Venice Lagoon. Samples were collected from February to August in 2002, in October 2013 and in June 2015. In detail, in 2002 gastropods were sampled at four sites inside the lagoon (site 1: Ca' Roman-South; site 2: San Pietro in Volta; site 3: Valleselle Sotto Vento; site 4: P.ta Sabbioni) and at two coastal sites outside the lagoon (site 5: Malamocco and site 6: Lido di Venezia). On the contrary, in the recent surveys, specimens were collected at two lagoon sites (site 7: Ca' Roman-North and site 8: S. Maria del Mare), one coastal site (site 9: Chioggia), and two off-shore sites (site 10: Caleri-Albarella, and site 11: Acqua Alta platform). Only from site 11, specimens were sampled monthly from July 2013 to June 2014. All lagoon sites were located next to marinas or dockyards, and near the main lagoon channels, the main routes toward ports for industrial or commercial cargo ships, cruise ships, ferryboats, tourist and pleasure vessels. Coastal sites (5, 6 and 9) were no more than 1 km from the coast, whilst the off-shore sites were located about 16 km (site 11) and about 6.5 km (site 10) from the coastline, respectively.

H. trunculus (>30 organisms per sample) were collected manually at lagoon sites at low tide and from a fishermen boat with a commercial dredge for clam fishing at marine sites. After collection, animals were immediately transported in cold and dark conditions to the laboratory, and maintained in separated aquaria with artificial marine seawater for 24 h without feeding. Before analyses, samples were stored at −20 °C.

2.2. Biological analyses

After thawing, the shell of each specimen was measured to the