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# Ecological quality status of the Adriatic coastal waters evaluated by the organotin pollution biomonitoring

A. Erdelez<sup>a</sup>, M. Furdek Turk<sup>b,\*</sup>, A. Štambuk<sup>c</sup>, I. Župan<sup>d</sup>, M. Peharda<sup>a</sup><sup>a</sup> Institute of Oceanography and Fisheries, Šetalište 1. Meštrovića 63, 21000 Split, Croatia<sup>b</sup> Department for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia<sup>c</sup> Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia<sup>d</sup> Department of Ecology, Agronomy and Aquaculture, University of Zadar, Trg kneza Višeslava 9, 23000 Zadar, Croatia

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## ABSTRACT

The aim of this study was to evaluate the post-legislation change in tributyltin (TBT) pollution at Croatian Adriatic coast. Gastropod *Hexaplex trunculus* and sediments were collected, nearly 10 years after TBT based antifouling paints were banned, at 12 locations along the coast where a previous study was conducted in 2005. The study showed a decline of TBT levels over the investigated period, although all gastropods populations were highly affected by imposex meaning that prohibition did not result in the recovery of populations. The further aim was to propose the Ecological Quality Ratio (EQR) boundaries for potential use of *H. trunculus* as a principal bioindicator in the assessment of the ecological status of the Mediterranean regarding TBT pollution, under the Water Framework Directive (WFD). According to the proposed EQR classes, the WFD target for achieving the Good ecological status of the marine environment by 2015 was not reached.

## 1. Introduction

Organotins, namely tributyltin (TBT), were introduced into the marine environment mainly through the application in antifouling coatings. TBT may cause various biological effects on different non-target organisms, but the main toxic effect caused by this compound in marine ecosystem is occurrence of imposex in prosobranch gastropods (Smith, 1981; Bryan et al., 1987; Graceli et al., 2013). Imposex is defined as superimposition of male sex characteristics in females (Smith, 1971). Previous studies demonstrated that some gastropod species develop imposex even at TBT concentrations in seawater as low as  $1 \text{ ng Sn l}^{-1}$  (Gibbs et al., 1988), and that imposex intensity increases as environmental TBT concentration increases and as TBT bioaccumulates (Bryan et al., 1987; Oehlmann et al., 1998a). At higher TBT concentrations advanced stages of imposex occur, severely affecting females' reproductive capabilities, population recruitment and structure (Gibbs et al., 1988).

Up to date > 200 species have been proposed to monitor TBT pollution worldwide, including *Hexaplex trunculus* (Horiguchi, 2017). Banded dye-murex *H. trunculus* is a common Mediterranean gastropod. This species inhabits littoral, is locally abundant, has limited mobility, does not have planktonic larval stage and develops imposex at TBT concentrations even lower than  $1 \text{ ng Sn g}^{-1}$  dry weight (d.w.) (Axiak

et al., 1995). All these traits make this species a reliable bioindicator of TBT pollution. During the last few decades, imposex in *H. trunculus* has been widely investigated in relation to TBT pollution in many Mediterranean countries, e.g. Malta (Axiak et al., 1995, 2003), Italy (Terlizzi et al., 1998, 1999, 2004; Chiavarini et al., 2003; Pelizzato et al., 2004; Garaventa et al., 2006, 2007), Israel (Rilov et al., 2000), Portugal (Vasconcelos et al., 2006a), Croatia (Prime et al., 2006; Garaventa et al., 2006, 2007; Stagličić et al., 2008) and Tunisia (Lahbib et al., 2007, 2009, 2010). The majority of these studies investigated spatial distribution of imposex occurrence and some have related it to TBT pollution.

Because the organotins exhibited negative effects on non-target organisms, primarily gastropods and bivalves, their use in antifouling coatings is nowadays banned in many countries worldwide, including all of Europe (Sonak et al., 2009). All antifouling coatings containing organotins are banned by the International Convention on the Control of Harmful Anti-fouling Systems on Ships (AFS Convention) since the year 2008. Croatia ratified AFS Convention in 2006, and in the same year TBT was included in the List of hazardous substances that are prohibited for use (Official gazette 17/2006), however ships containing organotins coatings have not been banned from entering Croatian ports until 2008. In EU, the prohibition of organotins on ships entered into force through Directive 2002/62/EC and Regulation EC/782/2003. In

\* Corresponding author.

E-mail address: [mfurdek@irb.hr](mailto:mfurdek@irb.hr) (M. Furdek Turk).

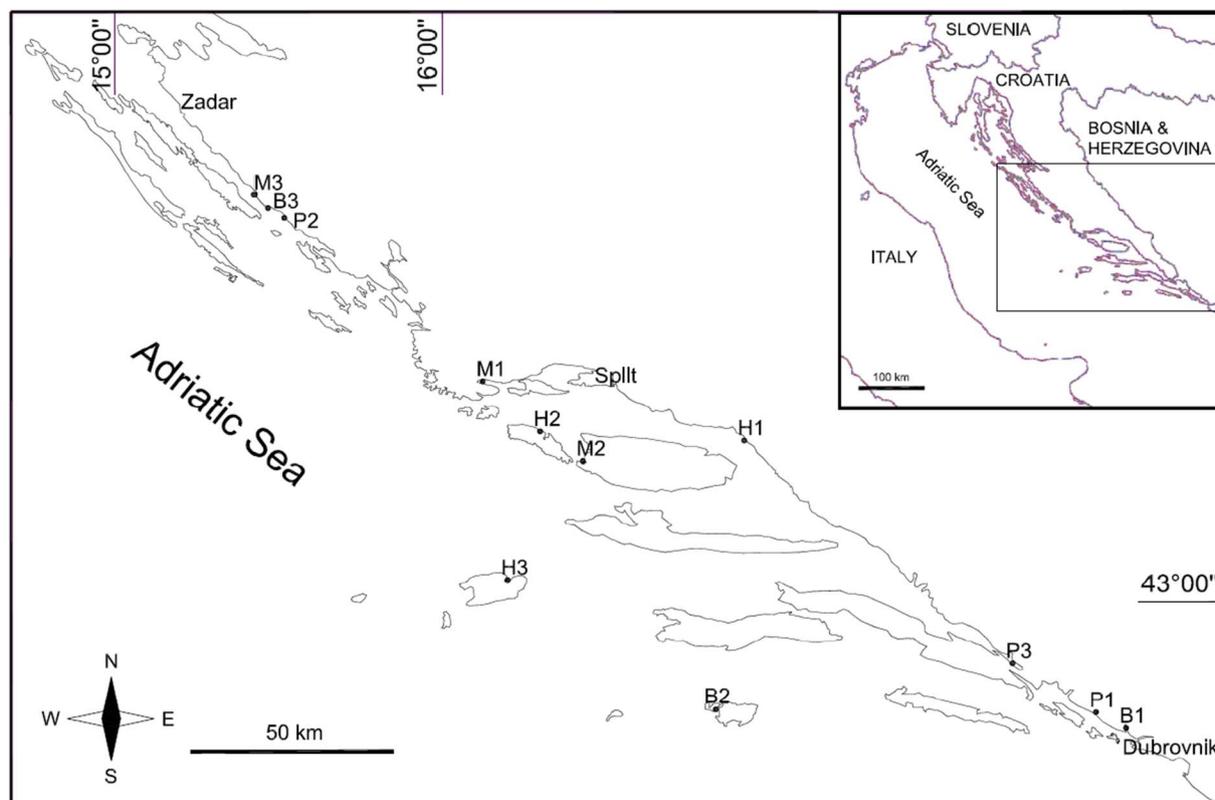


Fig. 1. Location of sites sampled for *Hexaplex trunculus* and sediments in 2015 (M<sub>1</sub> = Marina, M<sub>2</sub> = Milna, M<sub>3</sub> = Biograd, H<sub>1</sub> = Baška Voda, H<sub>2</sub> = Rogač, H<sub>3</sub> = Vis, B<sub>1</sub> = Zaton, B<sub>2</sub> = Lastovo, B<sub>3</sub> = Crvena Luka, P<sub>1</sub> = Brsečine, P<sub>2</sub> = Drage, P<sub>3</sub> = Mali Ston).

Annex VIII of the Water Framework Directive (WFD) 2000/60/EC organotin compounds are declared as one of the main pollutants, and TBT is enlisted as one of the priority substances in the field of water policy (2455/2001/EC) that should be monitored in order to evaluate the chemical status of a water body. The presence of TBT in the environment is regulated by the prescribed maximum allowed concentration in seawater. Considering that this concentration is extremely low,  $0.2 \text{ ng L}^{-1}$  ( $0.08 \text{ ng Sn L}^{-1}$ ) for all of Europe, chemical analysis of TBT at such low levels are often difficult to perform even with the most sensitive analytical methods. For this reason, the highly specific biological response to TBT, i.e. imposex in gastropods, is often used as a valuable tool in estimating the impact of TBT pollution in the marine environment. Therefore, imposex became a mandatory element under the OSPAR environmental monitoring programme (OSPAR, 2004). Furthermore, in the WFD monitoring programme it was proposed as one of the biological quality elements that could be used for evaluation and classification of ecological status of coastal waters (WFD-UKTAG, 2014). For this to be accomplished, classes of ecological status based on imposex levels should be defined as proposed by Laranjeiro et al. (2015).

Several recent studies reported the widespread appearance of imposex in the Mediterranean gastropod populations, such as in Sardinia and Tunisia (Anastasiou et al., 2015; Boulajfene et al., 2015; Abidli et al., 2013), however data on temporal trends of imposex status in the Mediterranean related to the TBT ban is limited (the only data is given by Lahbib et al. (2009) for the Tunisian coast). Also, no recent data on temporal trends of imposex occurrence in the Adriatic, the northernmost arm of the Mediterranean Sea, are available. However, measurements of TBT concentrations in seawater and mussels from Croatian coast for the time period 2009–2010 showed that eastern Adriatic was polluted with organotin compounds even after the ban (Furdek et al., 2012).

The first study of imposex occurrence in the Adriatic Sea was conducted by Terlizzi et al. (1998) in Brindisi area, Italy. Pelizzato et al.

(2004) and Garaventa et al. (2006, 2007) investigated imposex indices in *H. trunculus* collected in Venice area (Italy) in the time period 2002–2003, the former including also coastal area around western Istria peninsula that is part of Croatia. Furthermore, Stagličić et al. (2008) analysed banded dye-murex collected in 2004 and 2006 at Kaštela bay (Croatia), while Carić et al. (2016) presented data on imposex in *H. trunculus* collected from several sites in Dubrovnik area in 2006. In all of these studies the investigated populations were seriously affected by imposex (VDSI ranged from 2 to 5). The first observation of imposex in *H. trunculus* in the central Croatian Adriatic was conducted in 2005 (Prime et al., 2006) and represents the baseline data for the evaluation of temporal trend in imposex occurrence conducted in this study. It demonstrated high levels of imposex at 12 investigated locations categorized by boating activity, although three of them were categorized as “pristine” areas.

WFD set the year 2015 as the target deadline to achieve Good ecological and chemical status of the Mediterranean marine environment regarding TBT (Laranjeiro et al., 2015). The question is whether this has been accomplished? Based on the above presented review of literature data it can be stated that new data are necessary to evaluate the environmental impact of the TBT ban so this question could be answered.

The aims of this study were 1) to provide the first insight on tissue burden of butyltins (TBT and its degradation products, dibutyltin (DBT) and monobutyltin (MBT)) in populations of *H. trunculus* in central Croatian Adriatic, and to assess the relationship between butyltin concentrations in the tissue and sediment with the imposex level; 2) to evaluate the effect of enforced law restrictions on the use of organotins in antifouling paints over a 10-year period by analysis of changes in imposex occurrence in banded dye-murex populations at 12 location in the central Croatian Adriatic; 3) to propose Ecological Quality Ratio (EQR) boundaries for the potential use of imposex in *H. trunculus* as a biological quality element in the assessment of the ecological status of

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