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Imposex and organotin prevalence in a European post-legislative scenario: Temporal trends from 2003 to 2008

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ABSTRACT

Imposex levels and organotin tissue concentrations were assessed in *Nassarius reticulatus* females collected between June and August 2008 at 23 sites along the Portuguese coast. Females with imposex were present at all sampling sites with highest levels inside main harbors. Imposex parameters across sampling stations varied between 6.3% and 100% for %I, 0.2–4.4 for VDSI, 0.1–7.8 mm for FPL, 0.3–88.9% for RPLI and 0.0–1.1 for AOS. TBT levels varied between 3.5 and 380 ng Sn g^{-1} dw, representing an average proportion of 50.4% of total butyltins (∑BTs = MBT + DBT + TBT). Sterile females were detected at two locations. Highly significant correlations between imposex and TBT levels were found.

The efficacy of the EU legislation banning the use of TBT-based antifouling paints since 2003 (EC Regulation 782/2003) was evaluated by comparing the levels of imposex observed in 2008 with those reported for 2003. OTs tissue concentrations were also determined in preserved samples collected in 2003. There was a decrease in imposex and TBT tissue contamination between 2003 and 2008 indicating that a decline in TBT pollution has occurred in the Portuguese coast since the implementation of the legislation. Considering that the EC Regulation 782/2003 is an anticipation of the IMO AFS Convention, a global scale decrease in TBT pollution can be expected in the near future. Despite the rapid amelioration in the Portuguese coast, TBT pollution is still a problem as the Ecological Quality Objective (EcoQO) proposed by OSPAR Commission was not achieved in 91% of the surveyed sites.

1. Introduction

Tributyltin (TBT) is a biocide that enters the marine environment mainly through the leaching of antifouling (AF) paints from ship's hulls and other immersed structures. It is a well known endocrine disruptor capable of inducing sexual anomalies in marine gastropods as well as many other deleterious effects in wildlife and humans (for reviews see Bryan and Gibbs, 1991; Fent, 2006; Antizar-Ladislao, 2008). Imposex (defined as superimposition of male characters such as a penis and a *vas deferens* onto females gastropods) is a powerful biomarker of TBT pollution and has been used worldwide to map its distribution in the marine environment. Whilst initial reports focused mainly on spatial pollution gradients, in recent years, an evaluation of temporal pollution trends and population imposex recovery are the two principal monitoring targets in order to evaluate the effectiveness of the successive legislative measures. In Europe, the ban on the use of TBT AF paints on small boats (Directive 89/677/EEC) adopted in the late 1980s and early 1990s (in Portugal this directive was introduced in 1993) revealed itself ineffective in reducing TBT pollution (e.g. Barroso and Moreira, 2002; Santos et al., 2002). With the proven inefficacy of the partial restrictions, attention is now focused on the TBT global ban adopted by the International Maritime Organization (IMO) in 2001 that entered into force in September 2008. In the European Union (EU), the global ban was enacted by EC Regulation 782/2003 that adopted a total interdiction on the application of TBT-based formulations to vessel's hulls after 1st of July 2003 and the prohibition on the presence of those paints on vessel's hulls from 1st of January 2008. As this prohibition was extended to all vessels entering EU ports, a decline in TBT pollution in the EU is expected. It is now essential to monitor these changes in order to understand the degree of imposex recovery in gastropod indicator species, with 2003 and 2008 being benchmark years for this purpose.

The present work aims to create a base-line of imposex and organotin levels in *Nassarius reticulatus* (L.) for the Portuguese coast in 2008 and compare this baseline with that reported for 2003 (Sousa et al., 2005) in order to assess the effectiveness of EC Regulation 782/2003.

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2. Materials and methods

2.1. Study area

The Portuguese coastline extends for around 950 km and encompasses several estuarine areas where main harbors are located (Fig. 1). Those harbors include commercial and fishing ports, marinas and dockyards. Table 1 provides a summary of the evolution of commercial ship traffic in Portuguese harbors during 2003–2008. Recreational and fishing vessels also contribute to the naval traffic over the study area (for a more detailed description see Rato et al., 2009). Parallel to the coastline a maritime traffic line is positioned 40 km offshore. This is one of the main maritime traffic lines linking the Mediterranean, the north of Europe, Africa, and America, with an average of 100 ships crossing the line, per day (Delfaud et al., 2005). Several studies performed over the last two decades identified harbors as hotspots of TBT pollution in Portugal and also revealed that outside these areas the pollution is diffuse with lower levels along the entire coastline and in offshore waters (e.g. Santos et al., 2002; Sousa et al., 2005; Galante-Oliveira et al., 2006; Rato et al., 2006, 2008).

2.2. Sampling campaign

2.2.1. Sampling

Thirty-one locations spread along the Portuguese coast were surveyed between June and August 2008, but *N. reticulatus* was obtained only in 23 of those locations (Fig. 1; Table 2). The sampling campaign aimed to evaluate spatial and temporal evolution of imposex and organotin tissue content in the netted whelk *N. reticulatus* (L.) in the mainland Portuguese coast between 2003 and 2008; hence the sampling sites were the same as the ones previously selected (Sousa et al., 2005), with the exception of St. 19 that was deviated 250 m in the current campaign compared to the 2003 campaign. Whelks were captured by hand at the intertidal shore whereas at sublittoral sites baited hoop nets were used. The animals were brought to the laboratory and observed within two days. During that time they were maintained in permanently aerated seawater collected from respective sampling sites.

2.2.2. Imposex analysis

Wherever possible, 60 adults from each site were selected. Shell heights (distance from shell apex to lip of siphonal canal) were measured with vernier calipers to the nearest 0.1 mm. After narcotization (40 min; 7% $MgCl_2$ in distilled water), shells were cracked open with a bench vice and the animals gently removed, sexed and dissected under a stereo microscope. The following imposex parameters were assessed: percentage of imposex affected females (% I), *vas deferens* sequence index (VDSI), mean female penis length (FPL), mean male penis length (MPL), relative penis length index (RPLI = (FPL/MPL) $*$ 100), average oviduct stage (AOS) and percentage of sterile females (%STE). The penis length was measured (to the nearest 0.14 mm) with a stereo microscope graduated eyepiece. The VDSI was classified according to the scoring system proposed by Stroben et al. (1992a) except that the VDSI stage 4+ was converted to stage 5 in order to better describe spatial gradients, as proposed by Barroso et al. (2002a). Throughout the paper the VDSI values refers to this modified scale, however the values of $VDSI_{(4)}$ according to Stroben et al. (1992a) are given in Table 2 to allow comparisons with other works. The average oviduct stage (AOS) was ranked according to the 3-stage scale proposed by Barreiro et al. (2001). Parasitized animals were discarded from analysis.

2.3. Laboratory experiments

In order to understand the degradation of TBT in *N. reticulatus* over short time periods, animals collected from two locations with distinct pollution levels (St. 4 and St. 10) were allowed to depurate in the laboratory in artificial seawater (Crystal Sea® Bioassay Formulation, Marine Enterprises International) (salinity 30‰) for

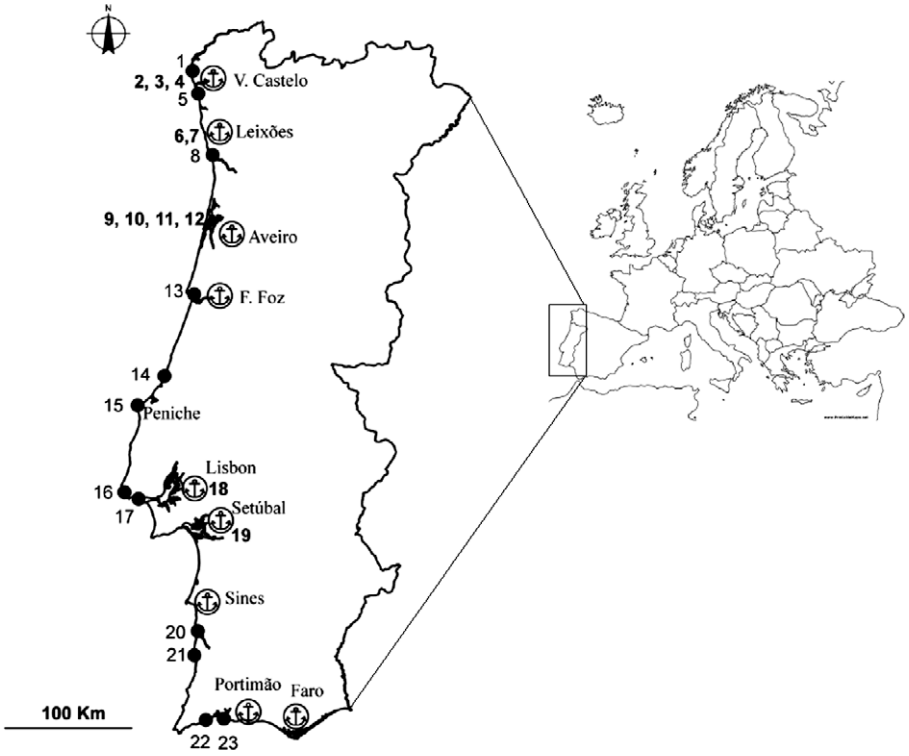

Fig. 1. Map of the Portuguese coast indicating the sampling stations and the main harbors ⚓.