



First assessment of the effectiveness of the international convention on the control of harmful anti-fouling systems on ships in Tunisia using imposex in *Hexaplex trunculus* as biomarker

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ABSTRACT

The global ban on the use of tributyltin (TBT) forced in September 2008 was ratified in Tunisia as late as June 2011. In this context, the present study aims to highlight the consequences of TBT contamination by monitoring 22 Tunisian sites before (2007) and after the ban (2012 and 2016) using as biomarker the occurrence of imposex in *Hexaplex trunculus*. From 2004 to 2016, complete recovery from imposex was reported in 11 sites. All average imposex indices calculated for each sampling year also decreased: imposex incidence from 61 to 27%, VDSI from 2 to 0.7, and RPLI from 15.7 to 1.6%. Ecological Quality Ratios (EQR) revealed that the sampling sites are in moderate to good ecological status. Overall, the present study confirms the effectiveness of the enacted legislation in reducing the impact of TBT pollution along the Tunisian coast.

1. Introduction

Tributyltin (TBT) is a concerning pollutant listed in the European Water Framework Directive that must be monitored in order to classify the environmental status of a water body (WFD Annex X, EC, 2013). Organotin, especially TBT, are well known for their potential biocidal properties that since the mid-1960's, prompted their use as fungicides, miticides, molluscicides, nematocides, ovidicides, rodent repellents, wood preservatives, and toxic additives in antifouling paints (Bennett, 1996). TBT is a matter of environmental concern due to its toxic effects, reported in a wide spectrum of organisms. Endocrine disruption is the best described consequence of TBT exposure, leading in gastropods to imposex, i.e. an irreversible superimposition of male-type genitalia in females (Heller, 2015). Thus, the occurrence of imposex is considered as an effective biomarker tool for monitoring exposure of marine gastropods to TBT leaching (Axiak et al., 2003).

Consequently, legislation on the use of TBT-based antifouling paints was issued in France in 1982, the U.K. in 1987, the U.S.A. in 1988, and Canada, Australia, and New Zealand in 1989 for vessels smaller than 25 m (excluding those made from aluminum) (Evans et al., 1994; Stewart, 1996). Vessels larger than 25 m were allowed to be treated with TBT-based antifouling paints as long as the maximum release rate

did not exceed $4 \mu\text{g}/\text{cm}^2/\text{day}$ (Stewart, 1996). This regulation revealed itself ineffective in reducing TBT pollution in many locations (e.g. Barroso and Moreira, 2002; Santos et al., 2002). For this reason, the International Convention on the Control of Harmful Anti-fouling Systems on Ships (AFS Convention) was adopted in October 2001. According to this convention, all ships shall not apply or re-apply organotin compounds that act as biocides in antifouling systems after 1 January 2003, and starting 1 January 2008 (effective date) all ships either (1) shall not bear such compounds on their hulls or external parts or surfaces; or (2) shall bear a coating that forms a barrier to such compounds leaching from the underlying non-compliant anti-fouling systems. The AFS Convention entered into force on 17 September 2008 and since then the efficacy of such legislation has been examined in many countries based on improvements of water quality, namely decreasing concentrations of TBT in water, sediment, and biota and recovery of the marine ecosystem (Sousa et al., 2009; Wilson et al., 2015). However, signs of contamination are still evident in ports, marinas and shipyards (Langston et al., 2015; Anastasiou et al., 2016).

Tunisia ratified the AFS Convention on 4 June 2011 through the Decree-Law No. 2011-49 published in the Official Journal of the Tunisian Republic. In the Tunisian market, TBT-based antifouling paints started being discarded in 2003 and replaced by new TBT-free

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products. This interested only one brand that has a partnership with an international company complying with the global ban adopted by the International Maritime Organization (IMO) in 2003. The composition of the remaining Tunisian paint brands is still unknown.

Several studies addressing TBT contamination along the Tunisian coast were published before the implementation of the AFS Convention (Lahbib et al., 2009, 2011a; Abidli et al., 2013). These studies showed slight improvements in TBT contamination only in sites with few boating traffic. However, following the implementation of the AFS Convention, it is expected to detect a significant and progressive recovery from previous TBT pollution levels. To verify this expectation, the temporal evolution of TBT contamination was monitored by quantifying the occurrence of imposex in the muricid gastropod *Hexaplex trunculus*. Monitoring surveys were carried out before (2007) and after (2012 and 2016) the ban on the use of TBT in Tunisia. For this purpose, each site considered in the 2004 study was revisited to ensure the reliability of this temporal comparison.

Overall, the present study provides data supporting the improvement of water quality after the IMO global ban in 2008, which should encourage other countries along the southern basin of the Mediterranean Sea, where no restrictions on the use of TBT-based paints have yet been implemented, to ratify the AFS convention.

2. Material and methods

2.1. Sampling

Between 25 and 50 adult *Hexaplex trunculus*, with sizes of 40–60 mm in shell length were collected between June and July 2007, 2012 and 2016 at 22 sites along the Tunisian coast (Fig. 1). Sampling was

performed by scuba diving or by gathering gastropods directly from fishing nets. The main features of the marine traffic in the sampling sites are compiled in Table 1. Most studied sites were located in the vicinity of a major source of TBT input into the marine environment, mainly commercial harbors (Bizerte, La Goulette, Sfax and Gabès). The main features of the marine traffic in the Tunisian commercial harbors are reported in Table 2. Tunisia receives marine traffic from 92 countries worldwide, most of them (61 countries) being signing parties of the AFS Convention. The number of non-signing countries is 31, accounting for 14.6% of the total shipping fleet (Table 2).

2.2. *Imposex* analyses

Immediately after collection, snails were taken to the laboratory and sacrificed. Specimens were measured for shell length (SL) to the nearest 0.1 mm using a vernier caliper and then deshelled using a bench vice. The soft body was extracted from the shell and the sex was determined based on the presence of a capsule gland in females and the presence of a large penis in males. The penis length was measured in both males and females (when ever present) under a dissecting microscope using a calibrated eyepiece with 0.1 mm precision. The Relative Penis Length Index (RPLI), defined as the mean length of the female penis (including healthy females) relative to male penis, was calculated using the following formula:

$$\text{RPLI} = \frac{\text{Mean female penis length}}{\text{Mean male penis length}} \times 100$$

The degree of imposex in each individual, expressed as the progression of the genitalia tract developmental stages (VDS), and the Vas Deferens Sequence Index (VDSI) (calculated as the mean of all individual

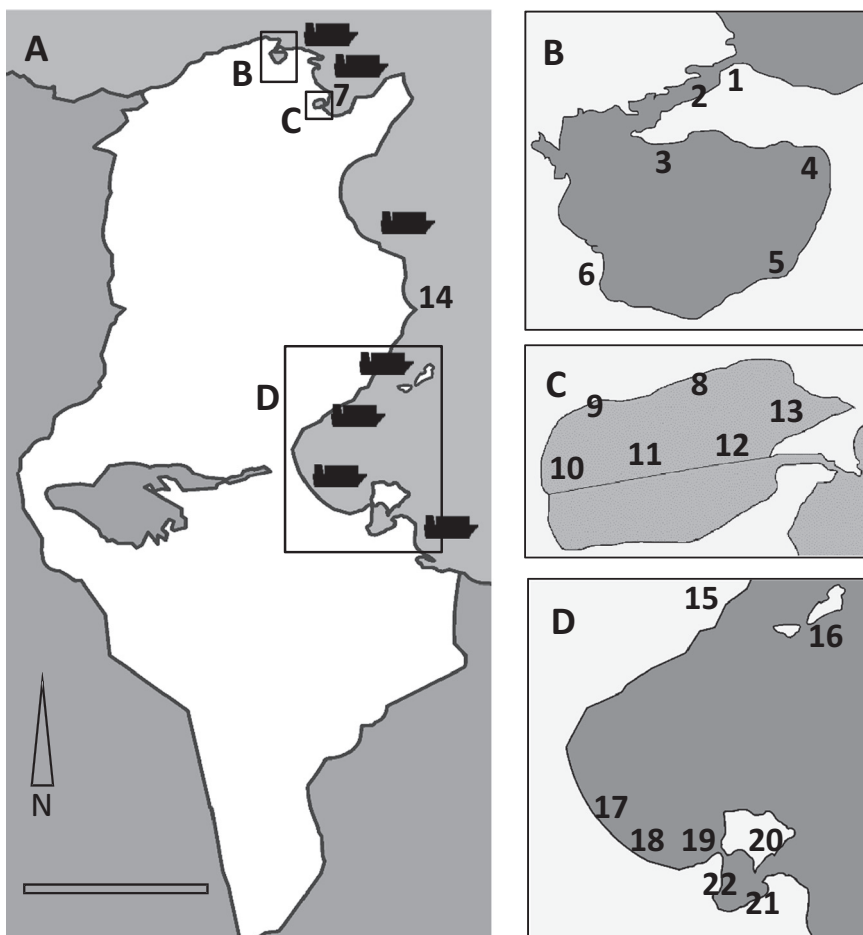


Fig. 1. Sampling stations of *Hexaplex trunculus* along the Tunisian coast. A: Tunisia map, B: Bizerte lagoon, C: North lake lagoon, D: Gulf of Gabes. 1: Zarzouna, 2: Baie des carrières, 3: Menzel Abderrahmen, 4: Menzel Jemil, 5: El Azib, 6: Menzel Bourguiba, 7: Punic port, 8–13: Tunis Northern Lagoon, 14: INSTM Monastir, 15: Fishing harbor of Sfax, 16: Kherkenah (El Attaya); 17: Gabès, 18: Zarat, 19: Adjim, 20: Gallala, 21–22: Boughrara. Scale bar = 200 km. The symbols of the boats indicate the location of the commercial harbors.

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