



Environmental status of TBT pollution in the Portuguese coastline: insights into persistence and regulatory implementation challenges

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ABSTRACT

This study constitutes the most comprehensive assessment of imposex along the Portuguese coast. It is based on a 22-year biomonitoring program using *Nucella lapillus* and *Tritia reticulata*, comparing new measurements from 2022 with historical data collected at the same locations. Since the EU ban on organotin (OT) antifouling (AF) systems in 2003, the intensity of imposex and OT tissue concentrations have declined in both species; however, in 2022, imposex remained widespread, with moderate to high levels still recorded in several areas. *N. lapillus* met the OSPAR Ecological Quality Objective (EcoQO) at all sampled sites, whereas *T. reticulata* failed to meet this target at 44% of the locations. This contrast reflects species distribution: *T. reticulata* inhabits a broader range of habitats, including more contaminated areas, making it a more effective bioindicator of OT pollution along the entire coast. Despite OT-based AF systems being banned on small vessels in 1993 by the Directive 89/677/EEC – nearly three decades before the current survey – and banned on all vessels in 2003 by the Regulation (EC) No. 782/2003, TBT continues to cause imposex, particularly in areas dominated by small-boat activity. These findings indicate spatially heterogeneous recovery patterns and suggest that vessel-size-dependent management and monitoring frameworks may influence post-ban environmental outcomes. Strengthened monitoring strategies targeting small-vessel-dominated areas may therefore support more effective long-term recovery.

1. Introduction

Between the 1960s and the 2000s, tributyltin (TBT) compounds were widely used as broad-spectrum biocides in antifouling (AF) paints to prevent biofouling (Bennett, 1996; Øystein Hjermann et al., 2022), contributing to one of the most severe global pollution events in history. According to Goldberg (1986) and Fent (1996), TBT ranks among the most toxic substances ever deliberately introduced into the marine environment by mankind. The extensive use and high toxicity of TBT compounds led to widespread adverse biological effects on non-target species (Sousa et al., 2014), prompting their progressive legal restriction. France was the first country to introduce legislation in 1982,

banning organotin (OT)-based AF paints on small boats (< 25 m), a measure soon extended across Europe – Portugal adopted the same ban in 1993 through Directive 89/677/EEC. As this proved insufficient to reduce TBT pollution (e.g., Barroso and Moreira, 2002; Minchin et al., 1997), the International Maritime Organization (IMO) was called upon to consider definitive, global-scale measures to restrict the use of OT compounds on all vessels (Champ, 2000). In this regard, in 2001, the IMO adopted the International Convention on the Control of Harmful Antifouling Systems on Ships (AFS Convention; IMO, 2001): a legally binding global ban on the application and reapplication of OT-based AF paints from January 2003, and their complete eradication from January 2008. However, the AFS Convention only entered into force in

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September 2008, 12 months after it was ratified by 25 countries representing at least 25% of the world's merchant shipping tonnage (IMO, 2001). Nevertheless, the European Union (EU) imposed these restrictions on Member States' fleets from July 2003, under Directive 2002/62/EC and Regulation (EC) No. 782/2003, which is why the application of OT-based AF paints on ships flying EU flags has been prohibited since that date, with a ban on vessels entering EU ports in place since January 2008.

Despite the need to assess the effectiveness of the EU and IMO bans in reducing TBT pollution, monitoring remains essential as TBT compounds are considered priority hazardous substances under EU legislation, namely within the Water Framework Directive 2000/60/EC and the Marine Strategy Framework Directive 2008/56/EC. These instruments establish frameworks for Member States to take the necessary measures to achieve and/or maintain Good Ecological Status, particularly within their coastal ecosystems. Therefore, monitoring these compounds is of great importance across the EU. Additionally, *imposex* – the superimposition of male sexual characteristics onto female neogastropods (Blaber, 1970; Smith, 1971, 1981) – has been used worldwide as a biomarker of TBT pollution. It is also subject to mandatory

monitoring under international legal agreements, such as the HELCOM Convention (HELCOM, 2023) and the OSPAR Convention (Øystein Hjermann et al., 2022; OSPAR, 2005). A decline in environmental OT concentrations and imposex incidence has been documented across Europe (e.g., Harrison et al., 2020; Ruiz et al., 2019; Cacciatore et al., 2018a, 2018b) and in other regions of the world (e.g., Jiang et al., 2025; França et al., 2021; Lahbib et al., 2018). Overall, these studies point to recovery trends in several gastropod populations, reflecting the impact of regulatory measures. However, contamination has not disappeared. Localised hotspots are still reported in coastal areas worldwide (e.g., Beyer et al., 2022; Uc-Peraza et al., 2022b; Gomes et al., 2021; Gessellen et al., 2018), particularly in harbour settings and sites with a long history of maritime activity, where elevated TBT concentrations and related biological effects continue to be observed.

To support the monitoring of the ecological status of the Portuguese mainland coast concerning TBT pollution, this study presents the most recent data on its spatial distribution in the country, following OSPAR guidelines. Imposex levels were assessed in two established bioindicator species – the dog whelk (*Nucella lapillus*) and the netted whelk (*Tritia reticulata*) – alongside the quantification of TBT and its degradation

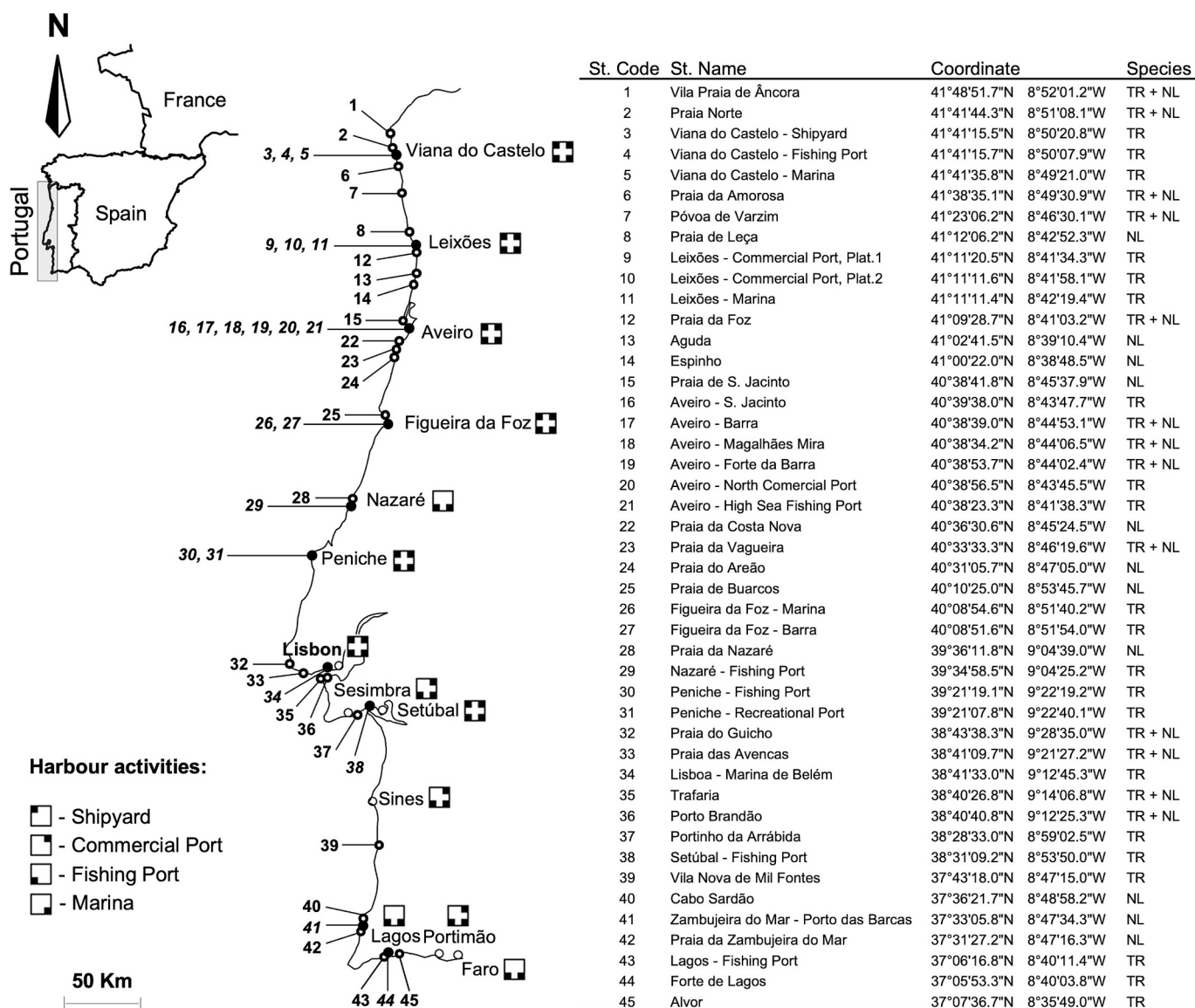


Fig. 1. Map of the Portuguese coast displaying sampling sites (St.1 to 45), their location details (geographic coordinates), and the species collected at each site (TR: *Tritia reticulata* and/or NL: *Nucella lapillus*). Sites within main harbours are indicated by black dots and numbered in *italics*, while square symbols represent vessel-related activities.

products, dibutyltin (DBT) and monobutyltin (MBT), in the whole soft tissues of females. Although less extensively used than TBT, triphenyltin (TPHT) – formerly applied as a co-biocide in AF systems and as an agricultural pesticide – is another OT known to induce imposex in gastropods (Barroso et al., 2002b; Laranjeiro et al., 2016). Previous studies have reported that environmental concentrations of TPHT along the Portuguese coast are markedly lower than those of TBT (Sousa et al., 2009; Barroso and Moreira, 2002; Barroso et al., 2002a). The present study further examines the occurrence of TPHT and its degradation products – diphenyltin (DPHT) and monophenyltin (MPHT) – in the same geographical area. To evaluate the temporal trends of imposex and gastropod OT contamination in the study area, the data obtained were compared with those from previous monitoring campaigns conducted over the past two decades, a period marked by the implementation of increasingly stringent regulations on OT use, both within the EU and globally. The overarching objective is to improve understanding of the long-term dynamics of these legacy contaminants to support the development of more effective environmental policies.

2. Materials and methods

2.1. Sampling and pre-treatment

Nucella lapillus and *Tritia reticulata* were collected from a total of 45 sampling sites along the Portuguese mainland coastline between April and September 2022 (see Fig. 1), in accordance with the Technical Annex 3 of the OSPAR JAMP Guidelines for Contaminant-Specific Biological Effects (OSPAR, 2008). These sites had previously been established by Barroso and Moreira (2002) and by Barroso et al. (2002a) to determine the levels of imposex in *N. lapillus* and *T. reticulata*, respectively. Specimens were sampled by hand at the intertidal zone during low tides; baited hoop nets were also used to collect netted whelks in sublittoral areas. The animals were transported to the laboratory and maintained in artificial seawater (salinity of 35 and temperature of 18 ± 1 °C) with continuous aeration for up to 5 days to allow for digestive tract depuration. Only sexually mature specimens (adults) were selected for imposex analysis: dog whelks with a shell height (SH) ≥ 17.5 mm (after Galante-Oliveira et al., 2010) and netted whelks displaying a complete white columellar callus and teeth on the outer lip of the shell. Before imposex assessment, *T. reticulata* specimens were narcotised for 60 min in 7% MgCl₂ in distilled water, while *N. lapillus* was observed without narcotisation (OSPAR, 2005, 2008). The SH was measured using Vernier callipers to the nearest 0.1 mm. Specimens were then removed from their shells, sexed, and dissected for imposex analysis under a stereo microscope (equipped with a graduated eyepiece for measuring penis length “PL” to the nearest 0.14 mm). After characterising imposex, a pooled sample of whole soft tissues from 10 to 15 females per sampling site was preserved at -20 °C for organotin analysis (as described below).

2.2. Imposex analysis

The indices used to characterise imposex expression included: for *N. lapillus*, after Gibbs et al. (1987), the percentage of imposex-affected females (%I), the mean female penis length (FPL), the mean male penis length (MPL), the relative penis size index ($RPSI = FPL^3/MPL^3 \times 100$), the vas deferens sequence index (VDSI), and the percentage of sterile females (%S); for *T. reticulata*, following Stroben et al. (1992a), the %I, FPL, MPL, the relative penis length index ($RPLI = FPL/MPL \times 100$), the VDSI, and %S. The vas deferens sequence (VDS) in each female was classified using specific categorisation schemes, and the average VDS stages observed in a sample determine the VDSI $[(\sum_{i=1}^n VDS_i)/n]$, indicating the intensity of imposex within the sampled population. In *T. reticulata*, VDS stages were classified using the scoring system developed by Stroben et al. (1992a), using a numerical value of 4 for VDS 4+

(hereafter referred to as VDSI₄). An adaptation proposed by Barroso et al. (2002a) was also applied to assign a numerical value of 5 for VDS stage 4+ (hereafter referred to as VDSI₅), providing improved discrimination of imposex levels between sites. For comparative purposes, both measures are reported in Table 1. The occurrence of aphaallic females was treated differently for each species: in the case of *T. reticulata*, these instances were classified as b-type VDS development, according to Stroben et al. (1992a), whereas aphaallic dog whelk females (a rare condition along the Portuguese coast) were excluded from the analysis to ensure comparability with other studies. Additionally, parasitised animals were excluded from the dataset for both species. VDSI was used to classify the ecological status of the sampling sites (OSPAR, 2005, 2011), ranging from best – A – to worst – E. For *N. lapillus*, the classes are defined as follows: (A) VDSI < 0.3 ; (B) $0.3 \leq VDSI < 2.0$; (C) $2.0 \leq VDSI < 4.0$; (D) $4.0 \leq VDSI \leq 5.0$; (E) VDSI > 5.0 . For *T. reticulata*, the classes are: (A, B) VDSI₄ < 0.3 ; (C) $0.3 \leq VDSI_4 < 2.0$; (D) $2.0 \leq VDSI_4 \leq 3.5$; (E) VDSI₄ > 3.5 . The Ecological Quality Objective (EcoQO) is considered achieved when assessment Class A or Class B is attained, with the threshold value set at the boundary between classes B and C.

2.3. Organotin analysis

To correlate organotin (OT) exposure with imposex expression and assess whether fresh inputs of OTs were occurring in the study area in 2022, TBT and ThPT (and their degradation metabolites: DBT and MBT; DPHT and MPHT) were quantified in the tissues of *N. lapillus* and *T. reticulata* from selected sites (2 and 14 sites, respectively). Chemical analyses were prioritised at stations showing elevated imposex levels and/or located within harbour areas considered potential contamination hotspots. Each pooled sample of whole soft tissues from the females analysed for imposex was freeze-dried, ground to a powder in a mortar, and then analysed following the procedures described by Uc-Peraza et al. (2022b). Briefly, 0.5 g of freeze-dried gastropod tissue was accurately weighed, spiked with 100 ng of a surrogate standard (tripropyltin – TPrT) and extracted with 9 mL of tropolone in methanol (0.03%, w/v) and 1 mL of glacial acetic acid on a vortex mixer for 1 min. Extracts were pre-cleaned with 100 mg of PSA Silica. Then, 5 mL of supernatant was derivatised with sodium tetraethylborate at pH = 4.5. Analytes were recovered by a liquid-liquid extraction with hexane (5 mL). The supernatants were cleaned up in an activated silica column, and analytes were eluted with hexane and hexane/toluene. Finally, extracts were evaporated, fortified with the internal standard (100 ng of tetrabutyltin – TeBT), and analysed by GC–MS using selected ion monitoring (SIM) mode.

Quality assurance and quality control were based on regular analyses of blanks, spiked matrices and certified reference material for mussel tissue (ERM-477CE/European Commission). Recoveries for the surrogate standards varied between 70 and 122%, while RSD was below 20%. Limits of detection (LODs) and limits of quantification (LOQs) for OTs were ≤ 0.3 and ≤ 1.0 ng Sn g⁻¹, respectively. All concentrations are reported as ng Sn g⁻¹ (dry weight).

To better characterise the temporal onset of the OTs quantified in the study area, the butyltin degradation index ($BDI = ([MBT] + [DBT])/[TBT]$) was then calculated, as proposed by Díez et al. (2002) and applied according to Laranjeiro et al. (2018). Understood as an interpretive interval rather than a strict cut-off value, BDI < 1 indicates the occurrence of fresh inputs of TBT.

2.4. Data analysis

Sampling metadata, including exact collection dates, are archived by the research team and will be reported to the International Council for the Exploration of the Sea (ICES) after publication through the Portuguese competent authority, the Directorate-General for Natural Resources, Safety and Maritime Services (DGRM). Reporting will take place within the framework of the OSPAR Coordinated Environmental

Table 1

Levels of imposex in *Nucella lapillus* and *Tritia reticulata* recorded along the Portuguese coast in 2022. The number of males and females analysed (N♂ and N♀, respectively), mean male and female shell height (SH♂ and SH♀, respectively; expressed in mm), mean male and female penis length (MPL and FPL, respectively; expressed in mm), relative penis size index (RPSI; expressed in percentage), relative penis length index (RPLI; expressed in percentage), vas deferens sequence index (VDSI), and percentage of imposex-affected females (%) are indicated. Mean values of shell height and penis length are presented together with their standard deviations (SD), rounded to the nearest unit, in the format “mean^(SD)”. Alongside VDSI values, the symbol “+” indicates the presence of aphaallic females (i.e., *N. lapillus* females with VDS ≥ 3 but lacking a penis, and *T. reticulata* females with b-type VDS). For sites where *T. reticulata* females exhibited VDS stage 4+ [assigned a numerical value of 4 for the calculation of VDSI₄ and 5 for VDSI₅ (following Barroso et al., 2002a, as detailed in the Materials and Methods)] both VDSI values are presented in the format “VDSI₄ | VDSI₅”. n.a.: not applicable.

<i>Nucella lapillus</i>									
St. code	n♂	SH♂	MPL	n♀	SH♀	FPL	RPSI	VDSI	%I
1	25	25.1 ⁽²⁾	4.80 ⁽¹⁾	35	25.4 ⁽²⁾	0	0	0.2	20
2	14	18.4 ⁽¹⁾	2.77 ⁽¹⁾	18	19.2 ⁽¹⁾	0	0	0.3	28
6	12	21.5 ⁽¹⁾	2.47 ⁽⁰⁾	18	20.9 ⁽²⁾	0.26 ⁽⁰⁾	1.19	+1.6	100
7	28	23.3 ⁽²⁾	4.12 ⁽¹⁾	32	24.0 ⁽¹⁾	0	0	0.2	19
8	27	19.9 ⁽²⁾	3.72 ⁽¹⁾	32	20.9 ⁽²⁾	0	0	0.3	31
12	32	21.7 ⁽²⁾	3.33 ⁽¹⁾	28	22.2 ⁽²⁾	0	0	0.6	57
13	26	22.7 ⁽³⁾	4.07 ⁽¹⁾	34	23.6 ⁽²⁾	0	0	0.2	18
14	31	23.0 ⁽²⁾	4.48 ⁽⁰⁾	30	23.7 ⁽³⁾	0	0	0.2	23
15	16	19.6 ⁽²⁾	3.94 ⁽¹⁾	43	21.2 ⁽²⁾	0	0	0.4	37
17	20	30.8 ⁽²⁾	5.17 ⁽¹⁾	37	31.3 ⁽²⁾	0	0	0.2	24
18	11	24.4 ⁽⁴⁾	3.91 ⁽⁰⁾	19	25.8 ⁽⁴⁾	0	0	0.3	26
19	28	23.3 ⁽²⁾	4.12 ⁽¹⁾	31	27.8 ⁽³⁾	0	0	0.2	19
22	17	22.2 ⁽²⁾	4.22 ⁽¹⁾	43	22.5 ⁽²⁾	0	0	0.3	26
23	24	22.4 ⁽²⁾	4.36 ⁽¹⁾	34	22.7 ⁽¹⁾	0.03 ⁽⁰⁾	0.00	0.4	38
24	17	22.6 ⁽²⁾	4.60 ⁽¹⁾	42	22.9 ⁽²⁾	0	0	0.2	21
25	29	19.9 ⁽²⁾	3.48 ⁽¹⁾	31	20.1 ⁽²⁾	0	0	0.2	19
28	20	18.5 ⁽¹⁾	3.48 ⁽¹⁾	39	19.2 ⁽²⁾	0	0	0.2	23
32	10	21.3 ⁽²⁾	3.99 ⁽⁰⁾	27	21.5 ⁽²⁾	0.02 ⁽⁰⁾	0.00	0.7	63
33	3	18.9 ⁽²⁾	3.17 ⁽⁰⁾	17	19.5 ⁽¹⁾	0	0	+0.4	41
35	20	22.3 ⁽²⁾	3.84 ⁽¹⁾	15	23.2 ⁽²⁾	0	0	0.4	40
36	32	22.0 ⁽²⁾	3.47 ⁽¹⁾	29	23.6 ⁽²⁾	0	0	0.5	45
40	13	21.0 ⁽²⁾	3.23 ⁽¹⁾	13	22.1 ⁽²⁾	0	0	0.3	31
41	9	19.3 ⁽¹⁾	3.40 ⁽⁰⁾	18	20.0 ⁽²⁾	0.21 ⁽⁰⁾	0.02	+1.1	61
42	23	20.4 ⁽²⁾	4.01 ⁽¹⁾	33	21.4 ⁽²⁾	0	0	0.2	24
<i>Tritia reticulata</i>									
St. code	n♂	SH♂	MPL	n♀	SH♀	FPL	RPLI	VDSI	%I
1	11	25.7 ⁽¹⁾	9.72 ⁽²⁾	34	28.3 ⁽²⁾	0	0	0.1	9
2	15	20.4 ⁽²⁾	9.81 ⁽¹⁾	30	23.3 ⁽³⁾	0.21 ⁽⁰⁾	2.10	+0.1	10
3	9	24.0 ⁽¹⁾	10.13 ⁽¹⁾	30	26.7 ⁽²⁾	4.56 ⁽³⁾	45.0	+3.1	97
4	24	22.9 ⁽³⁾	10.93 ⁽¹⁾	18	24.3 ⁽³⁾	6.42 ⁽²⁾	58.7	+3.7 4.0	100
5	23	28.4 ⁽²⁾	12.88 ⁽²⁾	26	29.7 ⁽²⁾	1.81 ⁽²⁾	14.1	0.9	38
6	30	20.8 ⁽²⁾	9.78 ⁽¹⁾	29	24.0 ⁽²⁾	0.34 ⁽⁰⁾	3.50	+0.1	14
7	6	21.2 ⁽²⁾	12.23 ⁽¹⁾	15	23.2 ⁽²⁾	0	0	+0.3	33
9	29	21.7 ⁽²⁾	10.95 ⁽¹⁾	15	25.0 ⁽²⁾	0.45 ⁽⁰⁾	2.10	+0.4	27
10	14	23.1 ⁽¹⁾	11.48 ⁽¹⁾	46	25.7 ⁽²⁾	0.25 ⁽⁰⁾	2.10	0.0	2
11	16	21.5 ⁽²⁾	10.72 ⁽²⁾	34	22.6 ⁽²⁾	4.69 ⁽²⁾	43.80	3.4 3.5	100
12	30	21.1 ⁽¹⁾	9.88 ⁽²⁾	33	23.1 ⁽¹⁾	0	0	+0.1	9
16	22	21.6 ⁽²⁾	10.83 ⁽²⁾	36	24.3 ⁽²⁾	0	0	0.0	0
17	32	26.4 ⁽²⁾	11.60 ⁽¹⁾	28	28.3 ⁽²⁾	1.30 ⁽⁰⁾	11.20	0.1	11
18	23	26.4 ⁽²⁾	11.70 ⁽¹⁾	34	27.6 ⁽²⁾	0.40 ⁽⁰⁾	3.40	0.1	6
19	20	22.7 ⁽²⁾	11.06 ⁽²⁾	40	24.6 ⁽²⁾	0.43 ⁽⁰⁾	3.90	0.0	3
20	8	23.2 ⁽¹⁾	10.54 ⁽¹⁾	30	24.8 ⁽²⁾	0.25 ⁽⁰⁾	2.30	0.0	3
21	17	24.1 ⁽²⁾	12.68 ⁽³⁾	39	26.3 ⁽²⁾	0.20 ⁽⁰⁾	1.60	0.1	5
23	2	23.6 ⁽⁰⁾	12.07 ⁽⁰⁾	7	27.9 ⁽²⁾	0	0	0.0	0
26	17	25.5 ⁽²⁾	11.50 ⁽³⁾	44	27.0 ⁽²⁾	0	0	0.0	0
27	19	24.2 ⁽²⁾	11.13 ⁽³⁾	49	27.0 ⁽²⁾	0	0	0.0	0
29	11	20.5 ⁽²⁾	9.13 ⁽³⁾	14	21.1 ⁽²⁾	0.49 ⁽⁰⁾	5.40	+2.6	100
30	11	19.3 ⁽¹⁾	10.08 ⁽²⁾	18	20.3 ⁽¹⁾	0.28 ⁽²⁾	2.80	+2.4	94
31	9	19.4 ⁽¹⁾	9.07 ⁽⁴⁾	28	20.8 ⁽²⁾	0.42 ⁽²⁾	4.60	+2.3	93
32	9	24.5 ⁽¹⁾	10.87 ⁽³⁾	14	26.0 ⁽²⁾	0.04 ⁽⁰⁾	0.30	+0.7	57
33	9	19.5 ⁽¹⁾	8.68 ⁽¹⁾	36	21.2 ⁽²⁾	0	0	+0.1	11
34	2	25.4 ⁽³⁾	5.70 ⁽⁰⁾	2	23.8 ⁽⁴⁾	0.42	7.32	3.0	100
35	14	23.9 ⁽²⁾	9.80 ⁽³⁾	27	25.1 ⁽²⁾	0.89 ⁽⁰⁾	9.10	0.8	30
36	0	n.a.	n.a.	29	23.2 ⁽¹⁾	0.58 ⁽⁰⁾	n.a.	+0.3	21
37	14	21.8 ⁽²⁾	13.19 ⁽³⁾	45	23.1 ⁽²⁾	0.08 ⁽⁰⁾	0.60	+0.1	9
38 ₂₀₂₂	4	20.2 ⁽²⁾	11.55 ⁽⁴⁾	10	20.3 ⁽²⁾	8.13 ⁽¹⁾	70.40	3.6	100
38 ₂₀₂₃	40	21.2 ⁽²⁾	10.30 ⁽²⁾	25	21.9 ⁽²⁾	8.63 ⁽¹⁾	83.80	3.8 4.5	100
39	24	19.6 ⁽²⁾	12.46 ⁽²⁾	31	20.4 ⁽¹⁾	0.02 ⁽¹⁾	0.20	+0.1	3
43	23	20.7 ⁽¹⁾	7.42 ⁽²⁾	22	21.1 ⁽²⁾	0.74 ⁽⁰⁾	10.00	+0.9	45
44	14	22.0 ⁽²⁾	6.81 ⁽¹⁾	20	23.6 ⁽²⁾	0	0	0.0	0
45	15	23.1 ⁽²⁾	8.47 ⁽²⁾	30	23.8 ⁽²⁾	0	0	0.0	0

Monitoring Programme (CEMP), using the OSPAR Hazardous Substances Assessment Tool (OHAT; <https://dome.ices.dk/ohat/>).

The temporal evolution of imposex levels along the Portuguese mainland coast was assessed by comparing the data obtained in 2022 with that from previous monitoring campaigns, specifically: i) those using *N. lapillus* as a bioindicator, conducted in 2000 (Barroso and Moreira, 2002), 2003 (Galante-Oliveira et al., 2006), 2006 and 2008 (Galante-Oliveira et al., 2011), 2011 (Oliveira, 2017), and 2014 (Laranjeiro et al., 2018); and ii) those using *T. reticulata*, conducted in 2000 (Barroso et al., 2002a), 2003 (Sousa et al., 2005), 2006 (Rato et al., 2009), 2008 (Sousa et al., 2009), and 2011 and 2014 (Laranjeiro et al., 2018).

Trends in imposex parameters from 2000 to 2022 were assessed using the non-parametric Mann–Kendall correlation test (τ). Where significant temporal trends were identified, their magnitudes were estimated using the non-parametric Sen's slope estimator (Q). This analysis was conducted separately for each species, initially considering all sites included in each monitoring campaign, and subsequently restricted to sampling stations revisited in all seven campaigns under study. The approach was also used to test the temporal trend in the TBT body burden of *T. reticulata* females. To statistically evaluate the overall evolution of imposex intensity (i.e., VDSI) and incidence (i.e., %I) along the Portuguese coast, the Friedman test (s) was applied to the subset of sites revisited between 2000 and 2022. Other correlations presented in the present study refer to non-parametric Spearman rank correlations. The Mann–Kendall trend test and Sen's slope estimator were computed using the add-on for Microsoft Excel XLSTAT (version 2025.1, Lumivero, Denver, USA). All other statistical analyses were performed using GraphPad Prism (version 10.2.3, GraphPad Software, San Diego, USA).

3. Results

3.1. Imposex levels in 2022

Due to natural variations in habitat and distribution, *Nucella lapillus* was collected at 24 of the 45 sites surveyed in 2022, and *Tritia reticulata* at 34, with both species co-occurring at 13 locations (Table 1). In this monitoring campaign, the incidence of imposex (%I) in *N. lapillus* ranged

from 18 to 100%, the Vas Deferens Sequence Index (VDSI) from 0.2 to 1.6, and the Relative Penis Size Index (RPSI) from 0 to 1.19%. No sterile females were recorded, and only four aphyllid females were observed, occurring at three sites: one at St. 6 (representing 5.6% of the females analysed at this station), one at St. 33 (5.9%), and two at St. 41 (11.1%).

In *T. reticulata*, the %I ranged from 0 to 100%, the VDSI from 0 to 3.7 (VDSI₄ / 4.0 (VDSI₅), and the Relative Penis Length Index (RPLI) from 0 to 70.4%. No sterile females were recorded. Female aphyllid (i.e., b-type VDS) remained widespread in this species across the study area in 2022, being detected at 47% of the sampled sites, ranging from 0 to 100% across stations. At St. 38 (VDSI₄ = 3.6; Table 1), the small sample size collected in 2022 prompted resampling in 2023, which confirmed the very high imposex intensity (VDSI₄ = 3.8 | VDSI₅ = 4.5; Table 1).

3.2. Organotin levels in 2022

The body burdens of butyltins (BTs: tributyltin, dibutyltin, and monobutyltin – TBT, DBT, and MBT, respectively) and phenyltins (PhTs: triphenyltin, diphenyltin, and monophenyltin – TPhT, DPhT, and MPhT, respectively) in females are presented in Table 2. Except for a single value of 11.8 ng Sn g⁻¹ dw of MPhT in the tissues of *T. reticulata* collected in 2023 at St. 38, the PhTs were consistently below the limit of detection (LOD: 0.3 ng Sn g⁻¹ dw) in both species, as were the BTs in the soft tissues of *N. lapillus* females. In *T. reticulata*, BT concentrations ranged from < LOD to 86.2 ng Sn g⁻¹ dw for MBT, from < LOD to 167 ng Sn g⁻¹ dw for DBT, and from < LOD to 287 ng Sn g⁻¹ dw for TBT. Resampling of *T. reticulata* at St. 38 in 2023 confirmed high levels of OTs (MBT = 686.9, DBT = 205.4 and TBT = 241.0 ng Sn g⁻¹ dw; Table 2).

Tributyltin was the predominant BT in the soft tissues of *T. reticulata* females, accounting on average for 40% of the total BTs ($\sum BTs = MBT + DBT + TBT$). The BDI provided evidence of recent contamination at St. 38 in 2022 (0.9) and also at St. 3 (1.0), (Table 2).

Fig. 2 illustrates the relationship between imposex intensity and TBT body burden in *T. reticulata* females. A statistically significant positive correlation was observed between VDSI and the natural logarithm–transformed TBT concentrations (Spearman's rank correlation coefficient, $r = 0.7198$, $p = 0.0051$).

Table 2

Butyltin and phenyltin concentrations (expressed as ng Sn g⁻¹ dry wt) in whole soft tissues of some female *Nucella lapillus* and *Tritia reticulata* specimens collected along the Portuguese coast in 2022 and analysed for imposex. BDI: Butyltin Degradation Index; CRM: Certified Reference Material; DBT: Dibutyltin; DPhT: Diphenyltin; LOD: Limit of Detection; LOQ: Limit of Quantification; MBT: Monobutyltin; MPhT: Monophenyltin; TPhT: Triphenyltin; n.a.: not applicable.

<i>Nucella lapillus</i>								
St. Code	TBT	DBT	MBT	TPhT	DPhT	MPhT	%Rec TPrT	BDI
6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	76	n.a.
41	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	60	n.a.
<i>Tritia reticulata</i>								
St. Code	TBT	DBT	MBT	TPhT	DPhT	MPhT	%Rec TPrT	BDI
2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	89	n.a.
3	146.0	88.6	56.7	< LOD	< LOD	< LOD	71	1.0
4	91.2	72.3	58.5	< LOD	< LOD	< LOD	99	1.4
5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	96	n.a.
9	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	77	n.a.
11	48.1	38.1	41.2	< LOD	< LOD	< LOD	72	1.6
29	18.1	22.3	17.4	< LOD	< LOD	< LOD	110	2.2
30	12.1	10.7	14.2	< LOD	< LOD	< LOD	111	2.1
31	15.1	14.0	12.0	< LOD	< LOD	< LOD	120	1.7
34	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	98	n.a.
35	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	115	n.a.
36	46.1	30.2	40.3	< LOD	< LOD	< LOD	109	1.5
38 ₂₀₂₂	287.0	167.0	86.2	< LOD	< LOD	< LOD	71	0.9
38 ₂₀₂₃	241.0	205.4	686.9	< LOD	< LOD	11.8	85	3.7
43	12.1	8.6	11.4	< LOD	< LOD	< LOD	89	1.7
% Rec CRM	91.5%	98.1%	93.6%	n.a.	n.a.	n.a.		
LOD / LOQ	0.3 / 1.0	0.3 / 1.0	0.3 / 1.0	0.3 / 1.0	0.3 / 1.0	0.3 / 1.0		

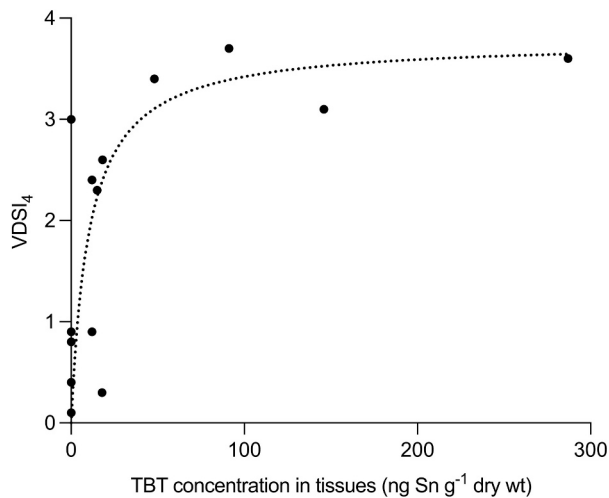


Fig. 2. Relationship between VDSI₄ and TBT concentration in *Tritia reticulata* female tissues (ng Sn g⁻¹ dry wt) sampled along the Portuguese coast in 2022. For visualisation purposes, values below the limit of detection (< LOD) were treated as near zero, and the trend line was fitted manually.

3.3. Spatio-temporal evolution of TBT pollution in the study area between 2000 and 2022

The evolution of imposex in *N. lapillus* and *T. reticulata* over the 22-year study period is presented in Fig. 3. Trend analysis across all sampled sites showed a significant decrease, as demonstrated by the negative correlation between VDSI and time along the Portuguese coast in both species: *N. lapillus* ($\tau = -0.632$; $p < 0.0001$; Fig. 3a); *T. reticulata* ($\tau = -0.409$; $p < 0.0001$; Fig. 3b). The decline was statistically less pronounced in *T. reticulata*, as indicated by the lower absolute value of Sen's slope ($Q = -0.014$), compared with the steeper slope observed in *N. lapillus* ($Q = -0.129$).

To identify a more realistic temporal trend over the years, the analysis was restricted to sites common to the seven sampling campaigns: 10 stations for the dog whelk and 12 for the netted whelk (Fig. 4). The overall evolution of the imposex condition in both species was assessed by applying the Friedman test: the highly significant differences observed in both the intensity and the incidence of imposex in *N. lapillus* (VDSI: $s = 57.67$, $p < 0.0001$, Fig. 4a; %I: $s = 47.13$, $p < 0.0001$, Fig. 4b) and *T. reticulata* (VDSI₅: $s = 66.00$, $p < 0.0001$, Fig. 4c; %I: $s = 60.42$, $p < 0.0001$, Fig. 4d) support the graphical representation of the decreasing trend of pollution throughout the time series under study (2000–2022). Mann-Kendall correlations corroborate, once again, these highly significant decreasing trends in both VDSI and %I for both species during the assessment period: VDSI showed a significant decline in both *N. lapillus* ($\tau = -0.753$; $p < 0.0001$; Fig. 4a) and *T. reticulata* ($\tau = -0.496$; $p < 0.0001$; Fig. 4c). In terms of magnitude, the absolute value of Sen's slope was greater in *N. lapillus* ($Q = -0.161$), suggesting a faster reduction in imposex intensity compared to *T. reticulata* ($Q = -0.157$). The %I also declined significantly over the 22 years in *N. lapillus* ($\tau = -0.649$; $p < 0.0001$; Fig. 4b) and *T. reticulata* ($\tau = -0.545$; $p < 0.0001$; Fig. 4d); however, the decline was steeper in *T. reticulata* ($Q = -3.915$), suggesting a faster reduction in imposex incidence than in *N. lapillus* ($Q = -2.667$).

The imposex data for *T. reticulata* show higher dispersion compared to the dog whelk. This outcome is influenced by the wider distribution of this species, which extends from the relatively clean open coastal shore to the more sheltered harbours, where the main historical sources of TBT pollution are located. Indeed, while the common sites where *N. lapillus* was sampled from 2000 to 2022 are situated along the shoreline, in *T. reticulata* they are also distributed within estuaries, which include historical hotspots of TBT pollution associated with naval activities such as shipyards, commercial and fishing ports, and marinas. Therefore, VDSI₅ values for *T. reticulata*, which provide better discrimination of imposex levels between sites (Barroso et al., 2002a), were further analysed after pooling the sampling sites according to the predominant maritime activity at each location, namely: “shipyard” (St. 3), “coastal shore” (diffuse sources; St.s 2, 6, 12, 17, 33 and 39), “fishing ports and

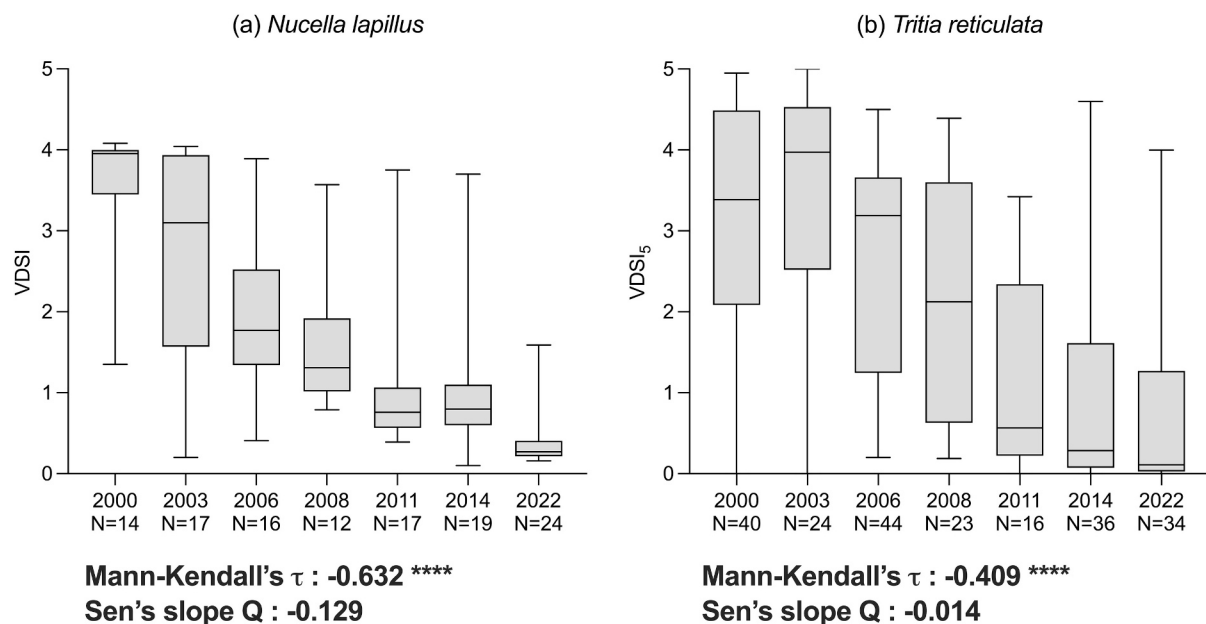


Fig. 3. Temporal evolution of VDSI in (a) *Nucella lapillus* and (b) *Tritia reticulata*, from 2000 to 2022, based on the complete set of monitoring sites for each surveyed year (the number of sites – N – is indicated below the x-axis). Data from 2000 to 2014 were previously published. For *N. lapillus*, data correspond to 2000 (Barroso and Moreira, 2002), 2003 (Galante-Oliveira et al., 2006), 2006 and 2008 (Galante-Oliveira et al., 2011), 2011 (Oliveira, 2017), and 2014 (Laranjeiro et al., 2018). For *T. reticulata*, data correspond to 2000 (Barroso et al., 2002a), 2003 (Sousa et al., 2005), 2006 (Rato et al., 2009), 2008 (Sousa et al., 2009), and 2011 and 2014 (Laranjeiro et al., 2018). Box plots represent the median (horizontal line), the interquartile range (25th–75th percentiles; grey box), and the minimum and maximum values observed (whiskers). Mann-Kendall's and Sen's slope results are indicated below the respective plot. ****: $p < 0.0001$.

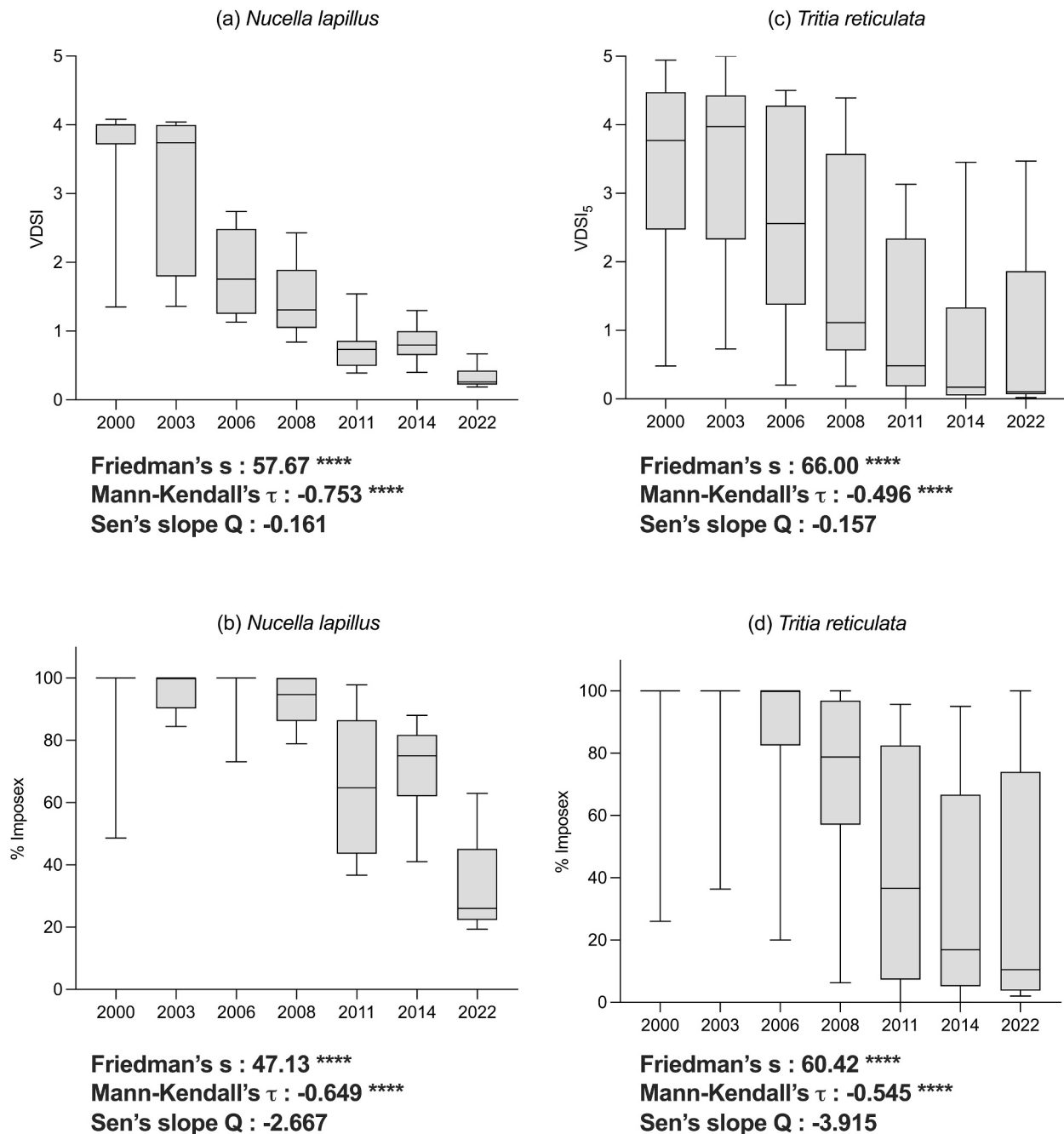


Fig. 4. Temporal evolution of VDSI and %I in *Nucella lapillus* [(a) and (b), respectively] and *Tritia reticulata* [(c) and (d), respectively], from 2000 to 2022, based solely on sampling sites common to all monitoring campaigns. Data from 2000 to 2014 were previously published. For *N. lapillus*, data correspond to 2000 (Barroso and Moreira, 2002), 2003 (Galante-Oliveira et al., 2006), 2006 and 2008 (Galante-Oliveira et al., 2011), 2011 (Oliveira, 2017), and 2014 (Laranjeiro et al., 2018). For *T. reticulata*, data correspond to 2000 (Barroso et al., 2002a), 2003 (Sousa et al., 2005), 2006 (Rato et al., 2009), 2008 (Sousa et al., 2009), and 2011 and 2014 (Laranjeiro et al., 2018). Box plots represent the median (horizontal line), the interquartile range (25th–75th percentiles; grey box), and the minimum and maximum values observed (whiskers). Friedman's, Mann-Kendall's, and Sen's slope results are indicated below the respective plot. ****: $p < 0.0001$.

marinas" (small boats: St.s 11, 18 and 30), and "commercial ports" (large commercial vessels: St.s 10 and 20). At St. 3 (the Viana do Castelo shipyard), imposex levels remained elevated over the two decades, showing only a slight decline from their maximum scores to 3.1 (Table 1), indicating only modest recovery. Regarding the remaining groups of stations (Fig. 5a–c), a statistically significant decreasing trend in VDSI₅ was detected, although marked differences were evident. Along the coastal shore, the decline was gradual ($\tau = -0.637$; $p < 0.0001$; $Q = -0.126$) and recovery was nearly complete (Fig. 5a). In fishing ports and marinas, although imposex decreased over the 22 years ($\tau = -0.552$; $p < 0.0001$; $Q = -0.136$; Fig. 5b), recovery remained only partial, as

moderate to high levels were still observed in 2022. Conversely, at commercial ports, VDSI₅ levels exhibited the steepest overall decline ($\tau = -0.802$; $p < 0.0001$; $Q = -0.266$; Fig. 5c), reaching almost full recovery. Notably, this downward trend in VDSI₅ levels of *T. reticulata* sampled at commercial ports was not accompanied by a corresponding decrease in the maritime traffic of large vessels in the main ports along the Portuguese continental coast during the period under analysis (Fig. 6).

The decreasing trend in imposex levels, as described here, is corroborated by a decline in TBT concentrations in *T. reticulata* tissues between 2000 and 2022 (Fig. 7), based on previously published data for

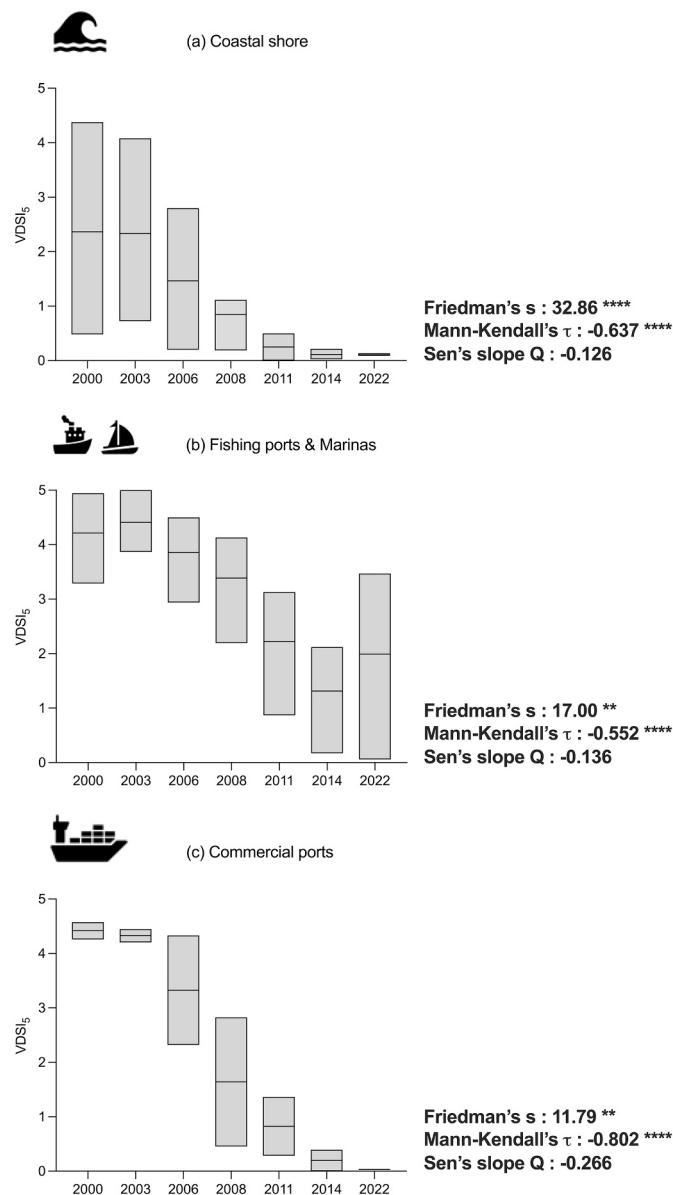


Fig. 5. Temporal evolution of VDSI in *Trinia reticulata* collected at sites located in (a) 'Coastal shore' areas, (b) 'Fishing ports & Marinas', and (c) 'Commercial ports'. Data from 2000 to 2014 were previously published: 2000 (Barroso et al., 2002a), 2003 (Sousa et al., 2005, 2009), 2006 (Rato et al., 2009), 2008 (Sousa et al., 2009), and in 2011 and 2014 (Laranjeiro et al., 2018). Box plots show the mean (horizontal line) and the minimum and maximum values observed (the lower and upper limits of the grey box, respectively). Friedman's, Mann-Kendall's, and Sen's slope results are indicated below the respective plot. **: $p < 0.01$; ****: $p < 0.0001$.

2000 (Barroso et al., 2002a), 2003 (Sousa et al., 2005, 2009), 2008 (Sousa et al., 2009), and the present study (2022). A highly significant negative correlation was also observed between TBT body burden in netted whelk females and the elapsed time ($\tau = -0.386$; $p < 0.0001$).

4. Discussion and conclusions

Imposex represents one of the most well-documented examples of specific, dose-dependent biological responses to environmental contaminants, and is arguably among the most effective biomarkers ever applied in pollution monitoring. Although certain already-banned organotins (e.g., tripropyltin and tricyclohexyltin) can elicit weak imposex responses under laboratory conditions (Bryan et al., 1988;

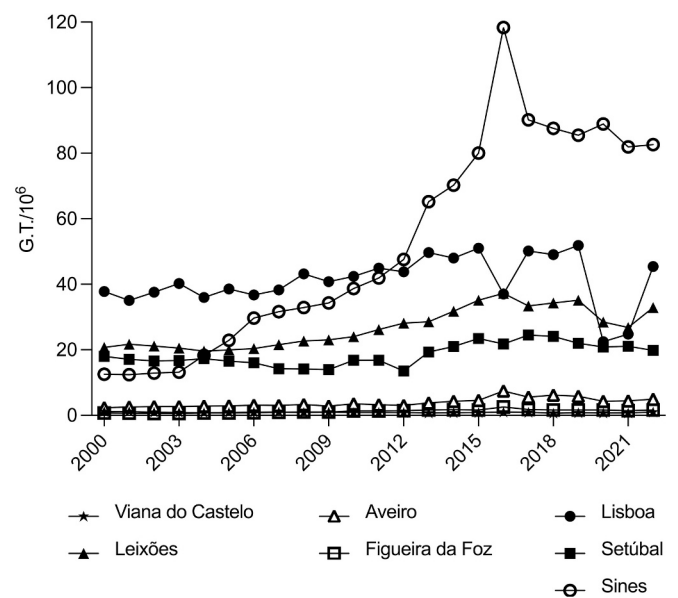


Fig. 6. Data on commercial ship traffic at the main Portuguese ports from 2000 to 2022 (INE, 2023). G.T. – Gross Tonnage.

Horiguchi et al., 2000), only TBT and TPhT have been unequivocally identified as environmentally relevant contaminants capable of inducing imposex in wild populations (Horiguchi et al., 2002, 2001, 2000, 1997, 1995; Schulte-Oehlmann et al., 2000) – including *N. lapillus* (Bryan et al., 1988; Laranjeiro et al., 2016) and *T. reticulata* (Barroso et al., 2002b). Therefore, analysis of imposex trends can reveal the temporal and spatial dynamics of TBT and TPhT pollution along the Portuguese coast. By integrating multi-decadal observations with a wide spatial coverage, the present study provides an updated perspective on the status of organotin (OT) pollution in Portugal and the effectiveness of legislation in addressing this problem.

4.1. Imposex trends: declining, yet lingering

The 22-year comparison presented in Figs. 3 and 4 demonstrates a clear reduction in both the incidence and severity of imposex in *N. lapillus* and *T. reticulata*. The ecological impacts have also diminished, as evidenced by the absence of sterile females in 2022, in contrast to past observations: for example, in the 2000 survey, during the pre-ban period, Barroso and Moreira (2002) reported sterility in *N. lapillus* females at St.s 2 and 28, while Barroso et al. (2002a) documented the same phenomenon in *T. reticulata* at St.s 4, 5, 9, 11, 29, 30, 35, and 36. This downward trend indicates that international and national regulatory measures – such as IMO's AFS Convention, EU legislation and the complementary Rotterdam Convention (www.pic.int) – have been effective in reducing OT pollution along the Portuguese coast. When viewed in a broader international context, the reduction observed along the Portuguese coast mirrors trends reported in other European regions where the same bioindicator species have been monitored under regional sea conventions. In North Atlantic waters, long-term monitoring of *N. lapillus* within the OSPAR framework indicates a sustained decline in imposex intensity, with most areas now showing low VDSI values and only occasional elevations in historically impacted sites (Øystein Hjermmann et al., 2022; OSPAR Hazardous Substances Assessment Tool, <https://dome.ices.dk/ohat/>). Comparable assessments in parts of the Baltic Sea under HELCOM, where *N. lapillus* is also used as an indicator species, report similar improving trends, albeit with some residual localised impacts (HELCOM, 2023). The VDSI range recorded in the present study for *N. lapillus* (0.2–1.6), together with the absence of sterile females and organotin concentrations below detection limits, falls within this post-ban recovery spectrum. For *T. reticulata*, declining but spatially

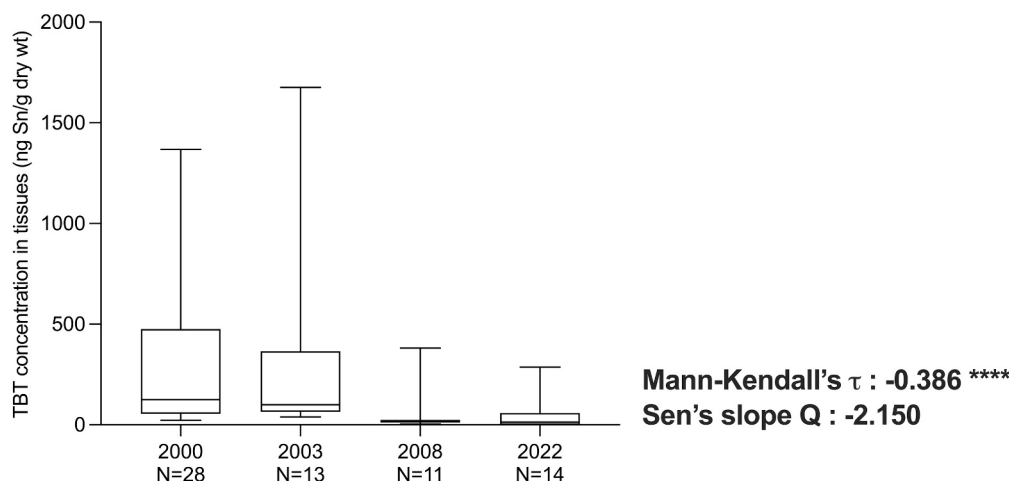


Fig. 7. Box plot showing TBT concentration in the tissues of *Tritia reticulata* females collected in 2000 (Barroso et al., 2002a), 2003 (Sousa et al., 2005, 2009), 2008 (Sousa et al., 2009), and 2022 (current study) along the Portuguese coast, considering the sites revisited in 2022. The number of samples analysed each year (N) is indicated below the x-axis. Mann-Kendall's and Sen's slope results are indicated. ****: $p < 0.0001$.

heterogeneous imposex patterns have likewise been documented along North Atlantic coasts under the OSPAR monitoring framework (Øystein Hjermann et al., 2022; OSPAR Hazardous Substances Assessment Tool, <https://dome.ices.dk/ohat/>). The VDSIs values observed here (0–4.0), in the absence of sterile females, indicate ongoing recovery, although measurable biological responses persist at some sites. Maximum tissue TBT concentrations reached $287 \text{ ng Sn g}^{-1} \text{ dw}$, pointing to post-ban exposure in biota. Tissue concentrations of a similar order of magnitude have been reported for *T. reticulata* in post-ban surveys along the Iberian Atlantic coast (Ruiz et al., 2015), supporting the interpretation that the levels measured in the present study are consistent with moderate to high post-ban exposure in recovering coastal systems. Comparable recovery trajectories have also been described in other muricid and nassarid species in the Adriatic Sea (Cacciatore et al., 2018a, 2018b; Erdelez et al., 2017) and across several coastal regions worldwide (e.g., Jiang et al., 2025; França et al., 2021; Lahbib et al., 2018). Nevertheless, persistent contamination hotspots continue to be documented, particularly in enclosed harbour environments and historically impacted coastal areas (Uc-Peraza et al., 2022b; Gomes et al., 2021; Gessellen et al., 2018).

In fact, despite the improvements, imposex remains widespread along the Portuguese mainland coast nearly three decades after the partial ban of OT-based AF systems on small vessels and almost two decades after the full ban on all vessels, with many locations still exhibiting moderate to high levels. This persistence indicates that the legislative measures have been only partially successful. In fact, both species continue to exhibit imposex at most surveyed sites: *N. lapillus* at all 24, and *T. reticulata* at 29 out of 34 sites. *N. lapillus* met the OSPAR EcoQO (VDSI <2) at all sampling stations, with 10 sites classified as Class A and 14 as Class B. In contrast, *T. reticulata* exceeded the EcoQO threshold (VDSI₄ of 0.3) at 44% of the surveyed locations, reaching OSPAR Class C at 7 sites, Class D at 6 sites, and Class E at 2 sites. These divergent outcomes between the two bioindicators can be attributed to differences in habitat distribution and sampling coverage. *T. reticulata* has a broader distribution, extending from the open coast to sheltered, fine-sediment habitats near ports, marinas, and shipyards, due to both its wide habitat tolerance and lower sensitivity to OT compounds (Bryan et al., 1993). In contrast, *N. lapillus* is largely restricted to rocky open-coast sites, which typically exhibit comparatively lower contamination levels. Consequently, *T. reticulata* provides more extensive coverage and represents a more effective bioindicator for assessing the spatial gradients of OT pollution.

Understanding the temporal dynamics of imposex recovery requires consideration of the age of the animals used in monitoring surveys. At

Aveiro (Portugal), both species reproduce annually: *T. reticulata* spawns from February to July (Barroso and Moreira, 1998), whereas *N. lapillus* reproduces year-round, with a peak in winter-spring (Galante-Oliveira et al., 2010). Because imposex is irreversible (Stroben et al., 1992a; Gibbs et al., 1987), reductions in imposex metrics over time occur primarily through cohort turnover as unaffected juveniles replace contaminated adults. Along the Portuguese coast, *T. reticulata* is estimated to live up to 11 years (although these old specimens are rare in natural populations), with females reaching sexual maturity at 4–5 years of age; imposex becomes apparent at approximately 2 years old and reaches maximum development from the 4th or 5th year of life (Barroso et al., 2005). For biomonitoring surveys, only adult specimens – typically 4–5 years old – were selected, with age inferred from shell morphology. In *N. lapillus*, longevity ranges from 5 to 6 years (Feare, 1970), and sexual maturity is attained at 2–3 years old (Gibbs, 1999). Over the 22-year interval between the earliest and most recent biomonitoring surveys, multiple generations have been produced. Therefore, the persistence of imposex here reported indicates that OT compounds remain bioavailable and are still being accumulated by new whelk generations in recent years. Overall, these findings underscore a key paradox: although legislative bans on OT-based AF systems have markedly reduced imposex levels over time, complete recovery has not been achieved. This shortfall is particularly evident in areas dominated by small vessels, where the pace of recovery has been very slow (Fig. 5b).

4.2. Persisting Organotin tissue contamination

A comparison of OT body burdens in both bioindicators between the pre-ban (2000) and post-ban (2022) periods reveals a substantial decrease in contamination (Table 2 and Fig. 7). In 2000, TBT and TPhT were detected in *N. lapillus* females at 92% and 50% of surveyed sites, with concentrations ranging from 30 to $147 \text{ ng Sn g}^{-1} \text{ dw}$ and 12 to $21 \text{ ng Sn g}^{-1} \text{ dw}$, respectively. In 2022, neither compound was detected in the dog whelk tissues at the 2 sites with the highest imposex scores (Table 2). For the netted whelk, at the 14 sites common to both survey periods, contamination also decreased markedly: in 2000, female TBT concentrations ranged from 22 to $1368 \text{ ng Sn g}^{-1} \text{ dw}$, falling to values ranging from $< \text{LOD}$ ($< 0.3 \text{ ng Sn g}^{-1} \text{ dw}$) to $287 \text{ ng Sn g}^{-1} \text{ dw}$ in 2022; TPhT concentrations in 2000 ranged from < 10 to $256 \text{ ng Sn g}^{-1} \text{ dw}$, while in 2022 they were consistently below the detection limit of $0.3 \text{ ng Sn g}^{-1} \text{ dw}$.

Unsurprisingly, the reduction in OT concentrations in the female tissues of both bioindicators along the Portuguese coast has mirrored the

decline in imposex in these species, because there is a well-established dose-response relationship of this biomarker, consistent with what is illustrated in Fig. 2. Data from the 2022 survey confirmed the significant correlation between *T. reticulata* imposex severity and TBT tissue concentration. Fig. 2 shows that, after a steep initial increase of VDSI₄ up to approximately 50 ng Sn g⁻¹ dw of TBT, VDSI₄ stabilised at around 3.5 for the highest TBT concentrations in gastropod tissues, which did not exceed 287 ng Sn g⁻¹ dw. A similar pattern was observed in 2000; however, VDSI₄ reached a higher plateau of around 4 within the range of 300–1400 ng Sn g⁻¹ dw (Barroso et al., 2002a). Therefore, beyond a threshold of around 50 ng Sn g⁻¹ dw, further increases in TBT produce little additional VDSI elevation as the vas deferens reaches its maximum development. These results demonstrate that the concentrations detected in *T. reticulata* in recent years remain within the range capable of rapidly inducing imposex and promoting marked development of the vas deferens and penis. This further explains why imposex levels remain

high in 2022, despite the reduction of OT body burden over time.

The BT contamination detected in *T. reticulata* tissues cannot be attributed to historical exposures. As noted above, the specimens analysed in 2022 were approximately 4–5 years old and, therefore, must have accumulated TBT in recent years. Moreover, the biological half-life of TBT in gastropod tissues is on the order of a few months. Laboratory injection experiments estimated biological half-lives of 19.1 days in *T. reticulata* and 32 days in *N. lapillus*, while depuration under TBT-free conditions resulted in longer half-lives of 65 days in the nassariid females (nevertheless, trace body burdens were still detectable after 14 months) (Stroben et al., 1992b). Data from our research team (Sousa et al., 2009) indicated TBT half-lives in *T. reticulata* female tissues of 25 and 34 days at St. 3 (Viana do Castelo – shipyard) and St. 18 (Aveiro – Magalhães Mira), respectively. Other studies reported half-lives of 50 to over 100 days in *N. lapillus* under environmental exposure (Bryan et al., 1987). Hence, TBT detected in *T. reticulata* tissues in 2022 is likely to

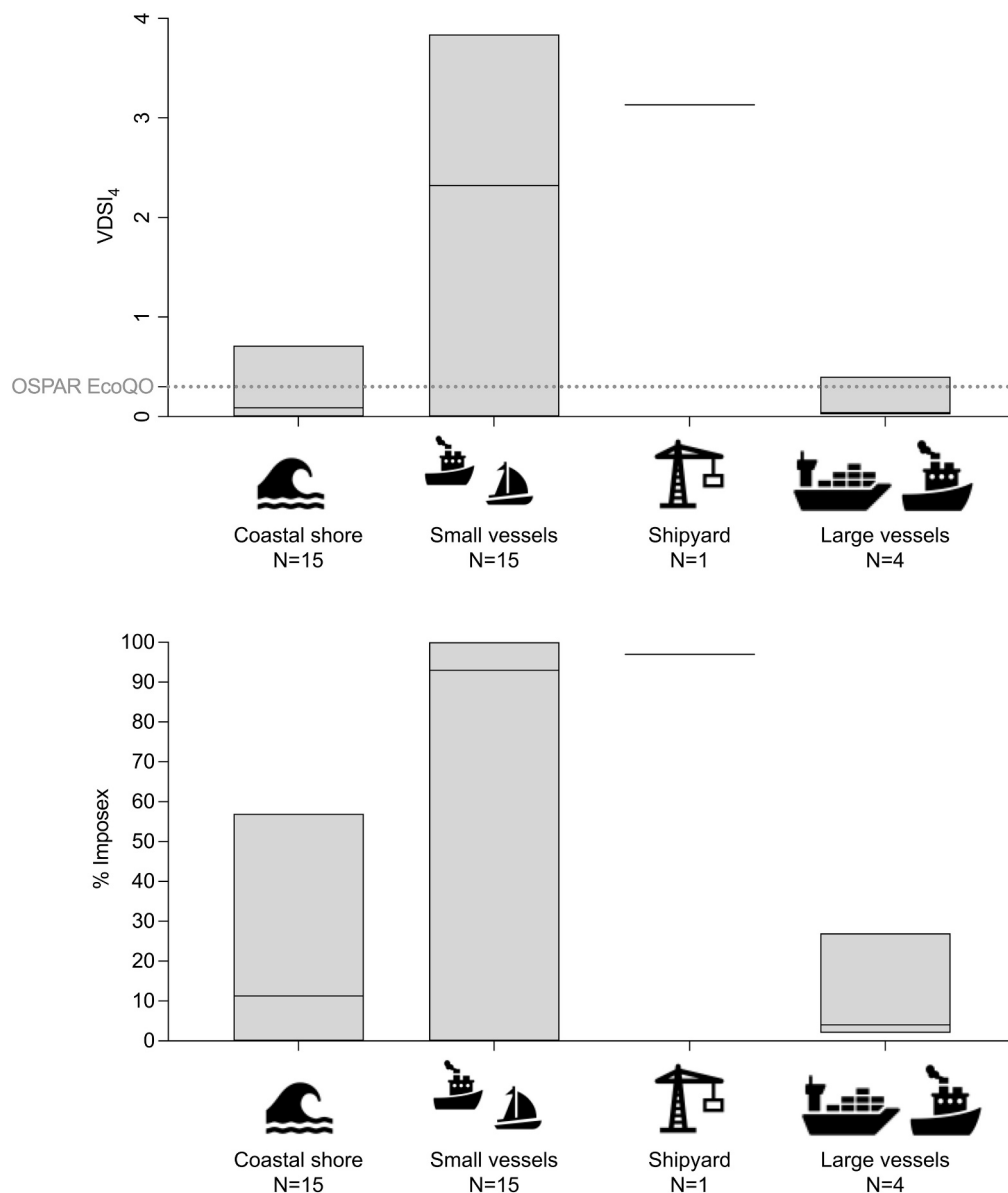


Fig. 8. Values of VDSI₄ and %I in *Tritia reticulata* recorded in the present survey (2022), averaged by sampling station according to the associated type of naval activity and vessel size: 'Coastal shore' (no naval facilities); 'Small vessels' (marinas and local/coastal fishing ports – vessels typically <25 m in length); 'Shipyard' (facility for large vessels); and 'Large vessels' (commercial ports and the high-sea fishing port – vessels >25 m in length). Box plots show the mean (horizontal line) and the minimum and maximum values observed (lower and upper edges of the grey box, respectively). The number of samples per box (N) is shown below the x-axis. In the upper panel (VDSI₄), the OSPAR EcoQO (VDSI <0.3) is shown as a dashed line for reference.

reflect recent rather than historical exposure during the animals' lifespan. Finally, BDI values for *T. reticulata*, although higher than the pre-ban period, remained relatively low in 2022, ranging from 0.9 to 2.2. Notably, two sites in northern and central Portugal showed evidence of recent TBT contamination: St. 38 – Setúbal Fishing Port exhibited a BDI of 0.9, while St. 3 – Viana do Castelo shipyard recorded 1.0. These findings provide compelling evidence that, even decades after its prohibition, TBT persists in the environment, remains bioavailable, and continues to be bioaccumulated by gastropods, thereby inducing imposex.

4.3. Ongoing sources of organotin pollution

Small-boat areas, together with shipyards, have become the major pollution hotspots along the Portuguese coast, as recovery in these sites has been markedly slower. As shown in Fig. 5, the decline of imposex in *T. reticulata* progressed much faster at large vessel ports – harbouring international commercial fleets (Fig. 5c) – than in small vessel areas – marinas and small-scale fishing ports (Fig. 5b). For example, commercial harbours such as Leixões (St.s 9 and 10) and Aveiro (St. 20), as well as the High Sea Fishing Port of Aveiro (St. 21; included in the 'Large vessels' category in Fig. 8, as it accommodates fishing vessels well over 25 m in length, in some cases extending to around 80 m – which differ markedly from the typical local/coastal fishing boats classified under the 'Small vessels' category), experienced a drastic reduction in pollution since the pre-ban period, despite increased naval traffic (Fig. 6). In 2000, *T. reticulata* displayed VDSI₄ values of 3.9, 3.3, and 3.7 at St.s 9, 10, and 20, respectively (Barroso et al., 2002a), while a VDSI₄ of 4.0 was reported at St. 21 in 1998 (Barroso et al., 2000). By 2022, these values had declined to nearly zero: 0.4, 0.0, 0.0, and 0.1, respectively (Fig. 8). In contrast, reductions in small boat areas were only moderate over the same timeframe (Fig. 5b), with both imposex intensity and incidence remaining very high in 2022 (Fig. 8).

Several factors may explain the pattern described above. One concerns legislation: although OT-based AF systems are prohibited on all vessels, only ships ≥ 400 gross tonnage (GT) are subject to mandatory surveys. Consequently, small boats are not routinely inspected, increasing the risk of inadequate maintenance of AF coatings. In principle, such inspections should be unnecessary, given that the use of OT-based AF paints on vessels < 25 m in length had already been banned in several European countries by the late 1980s or early 1990s (Directive 89/677/EEC) – Portugal adopted this Directive in 1993. However, Eklund and Watermann (2018) and Ytreberg et al. (2016) showed that tin (used as a proxy for the presence of OTs on boat hulls), with mean values exceeding $50 \mu\text{g Sn cm}^{-2}$, was detected in the mid-2010s on 42%, 24%, 23%, and 37% of leisure boats in Denmark, Finland, Germany, and Sweden, respectively. One important source of TBT to the environment regards residues from maintenance work on small boat hulls containing old coatings (Eklund et al., 2014; Eklund and Eklund, 2014). Eklund et al. (2008) showed that substantial TBT contamination persisted in Swedish marinas many years after the above-referred ban, as the compound continues to leach from older paint layers on pleasure craft hulls. These residues are exposed during mechanical cleaning and high-pressure washing. Experiments show that a significant release of TBT occurs under high-pressure hull washing even if the TBT coating is beneath copper paints (Ytreberg, 2012). Additionally, detached anti-fouling paint particles (APPs) from washing often reach the aquatic environment via drainage (Eklund et al., 2008). The absence of systematic inspections on small boats also facilitates the illegal use of OT-based AF systems, as Uc-Peraza et al. (2022a) reported that TBT-based coatings are still produced and marketed worldwide; although no direct evidence of this illegal practice exists in Portugal, Laranjeiro et al. (2018) provided observations suggesting that it cannot be excluded.

Another potential factor concerns sediment management in harbours. Sediments are recognised as long-term reservoirs of TBT, where this OT can persist for years, or even decades (Dowson et al., 1996;

Langston and Pope, 1995). Over time, sediments transitioned from a sink to a source through diffusion of TBT back into the water column or via the remobilisation of APPs historically accumulated in this compartment (e.g., Abreu et al., 2020; Ruiz et al., 2015). Sediment dredging required to maintain navigability – more frequent at large vessel harbours – removes substantial volumes of contaminated material, thereby reducing local TBT loads. Hence, different dredging practices at each location may lead to distinct collateral OT remediation outcomes.

4.4. Ecological impacts

The worldwide use of OT-based AF paints has established TBT and TPhT as global pollutants. These compounds are highly persistent, bio-accumulative, and toxic, being classified as persistent, bioaccumulative, and toxic (PBT) and very persistent and very bioaccumulative (vPvB) substances under EU regulations (SCHER, 2006). Even at extraordinarily low concentrations, TBT exerts severe toxic effects on aquatic organisms. Virtually undetectable levels in seawater can induce imposex in gastropods: in *N. lapillus*, exposure below 0.5 ng Sn L^{-1} triggers imposex, levels above 2 ng Sn L^{-1} cause female sterility, and concentrations of $3\text{--}5 \text{ ng Sn L}^{-1}$ lead to near-complete sterilisation and population extinction (Gibbs, 1999). Not surprisingly, imposex has emerged as a widespread global phenomenon, reported in nearly 270 gastropod species (Titley-O'Neal et al., 2011). TBT has also severely impacted oyster aquaculture worldwide. In the Pacific oyster *Magallana gigas*, abnormal shell growth occurs at concentrations above 0.7 ng Sn L^{-1} , while embryogenesis and larval survival are impaired at concentrations exceeding 7 ng Sn L^{-1} (Alzieu, 1996, 2000). Some authors argue that TBT is the most toxic chemical for bivalve embryos and larvae ever evaluated in this context (Gosling, 2003), with adverse effects occurring at concentrations as low as 3 ng Sn L^{-1} . Molluscs are among the most sensitive taxa, but TBT toxicity extends far beyond this group: concentrations $> 0.5 \text{ ng Sn L}^{-1}$ can inhibit cell division in diatoms and impair reproduction and development in microcrustaceans (Maguire, 2000; Alzieu and Michel, 1998). Similarly, exposure to extremely low levels ($\geq 0.1 \text{ ng Sn L}^{-1}$) of TBT can compromise fish reproduction by reducing sperm viability and skewing sex ratios toward males (McGinnis and Crivello, 2011; Santos et al., 2006; McAllister and Kime, 2003). Its demonstrated ability to act as an agonist of nuclear receptors (e.g., RXR and PPAR γ) has raised additional concerns regarding its toxicological significance across animal taxa, including humans (Fonseca et al., 2020; Urushitani et al., 2018; Nishikawa et al., 2004).

It is possible to infer the levels of TBT in water using imposex as a proxy (Gibbs, 1999). This method provides only approximate estimates but allows a general assessment of TBT aqueous concentration (OSPAR, 2011): Class C ($0.1\text{--}2 \text{ ng Sn L}^{-1}$); Class D ($2\text{--}3 \text{ ng Sn L}^{-1}$); Class E ($3\text{--}15 \text{ ng Sn L}^{-1}$). In the present study, 7 sampling sites fell into OSPAR Class C (St.s 5, 7, 9, 32, 35, 36, 43), 6 into Class D (St.s 3, 11, 29, 30, 31, 34), and 2 into Class E (St.s 4, 38). Thus, in at least 8 sites (classes D and E), TBT levels are sufficient to induce sterility in *N. lapillus*, if this species occurred there. At all these 15 sites, TBT may produce deleterious effects in other taxa besides gastropods, according to the thresholds mentioned above. These findings highlight that, despite the long-standing ban on OT-based AF systems, the ecological impacts of TBT remain ongoing and may persist silently on the Portuguese coast.

4.5. Lessons to be learned

Environmental monitoring plays a crucial role in continuously evaluating the effectiveness of legislation and in guiding the adjustment of regulatory frameworks over time. The long-term monitoring presented in this study demonstrates that current legislative measures have not fully succeeded in controlling OT pollution on the Portuguese coast. Indeed, it is striking that, despite OT-based AF systems being banned on small vessels in 1993 – almost three decades before the current survey –

extending to all vessels in 2003, TBT continues to cause imposex, particularly in areas dominated by small-boat activity. Although the precise flaws remain uncertain, there is evidence that several legislative adjustments could have provided greater efficacy, offering valuable lessons for the future. First, mandatory inspections of AF systems on small vessels (leisure craft and inshore fishing boats) might have reduced the re-exposure of legacy TBT-based coatings on boat hulls. Such inspections would also have discouraged any ongoing illegal use of OT-based AF paints. Second, a stricter application of rules regarding the containment of paint debris during hull cleaning of small vessels could have reduced local inputs from these sources. For large vessels, potential improvements could have included stronger control of effluent discharges and the release of APPs from shipyards. Finally, regarding sediments, enhanced in situ management of highly contaminated areas might have been considered. For instance, integrating navigational dredging with remediation objectives, while ensuring control of contaminant release during dredging, could have reduced the persistence of TBT at hotspots. By focusing on these persistent sources – as supported by multi-decadal data – future policies can accelerate ecosystem recovery and move more quickly toward the EU and OSPAR target of zero pollution.

CRediT authorship contribution statement

Susana Galante-Oliveira: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Susana Coelho:** Writing – original draft, Investigation, Formal analysis. **Daniel Pereira:** Investigation. **Isabel B. Oliveira:** Investigation. **Filipe Laranjeiro:** Investigation. **Ana C.A. Sousa:** Writing – review & editing, Supervision, Methodology, Investigation. **Mariana da Luz Potes:** Investigation. **Rodrigo Moço Batista:** Investigation. **Gilberto Fillmann:** Writing – review & editing, Writing – original draft, Validation, Resources, Investigation, Formal analysis. **Carlos M. Barroso:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI, 2025) and Grammarly (Grammarly Inc., 2025) to improve the readability and language of the manuscript. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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