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Imposex, female sterility and organotin contamination of the prosobranch *Nassarius reticulatus* from the Portuguese coast

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ABSTRACT: Samples of *Nassarius reticulatus* (L.) were obtained from May to July 2000 on the W Portuguese coast. *N. reticulatus* imposex (superimposition of male characters onto female prosobranchs) increased with the proximity of harbours. The percentage of imposex-affected females (%I) varied between 0 and 100%. The penis length index (PLI) and the relative penis length index (RPLI) were between 0 and 10.7 mm and 0.0 and 92.1%, respectively. The vas deferens sequence index (VDSI) ranged from 0 to 5. The average oviduct stage (AOS) varied between 0.0 and 1.3. Sterile females (22 specimens) were found at 8 of the 40 sampling stations with frequencies between 5 and 50% of the total females sampled at each site. Sterility occurred only in females highly affected with imposex. Tributyltin (TBT) and triphenyltin (TPT) female body burden varied from <20 to 1368 and from <10 to 256 ng Sn g^{-1} dry weight (dry wt), respectively. The TPT residue was, on average, 18% of the TBT residue, and there was a highly significant correlation between TBT and lnTPT ($r = 0.88$, $p < 0.001$). Dibutyltin (DBT) and monobutyltin (MBT) female body burden varied from <34 to 721 and from <24 to 703 ng Sn g^{-1} dry wt, respectively. TBT represented the major fraction (34 to 60%) of the total butyltins, followed by DBT (24 to 40%) and MBT (10 to 31%). Highly significant correlations ($p < 0.001$) were established between the lnTBT female residue and the indices RPLI ($r = 0.88$), $VDSI_{(5)}$ ($r = 0.81$) and AOS ($r = 0.72$), as well as between TBT and percentage female sterility (%STER) ($r = 0.61$).

KEY WORDS: *Nassarius reticulatus* · Imposex · Portugal · Tributyltin · Triphenyltin · Sterility

INTRODUCTION

Ships' antifouling paints containing triorganotin compounds — tributyltin (TBT) and, to a lesser extent, triphenyltin (TPT) — were introduced in the 1960s (Bennett 1996). Imposex, the superimposition of male characters onto female prosobranchs (Smith 1971), has been reported for over 118 species worldwide (Bettin et al. 1996). It is now well established that imposex is caused by organotin pollution. The ecological impact of this phenomenon may be severe since imposex may cause female sterility in some species. This impact is particularly strong in those species that lack a planktonic larval stage, such as *Nucella lapillus* (L.), which are vulnerable to local extinction (Bryan et al. 1986). Legislation to ban the use of TBT paints on small boats (<25 m) was introduced in France in 1982, mainly motivated by the negative impact of TBT pollution on oyster farming. Later, similar legislation was applied in most European (EC directive 89/677/EEC) and other countries (Mora 1996, Stewart 1996). Portugal adopted the above EC directive in 1993 and the Portuguese Navy imposed a total ban on their ships in 1992. Although TBT contamination and imposex declined in many coastal areas (Gibbs & Bryan 1994), the problem is still acute in others where large vessels predominate. In face of that, the International Maritime Organization

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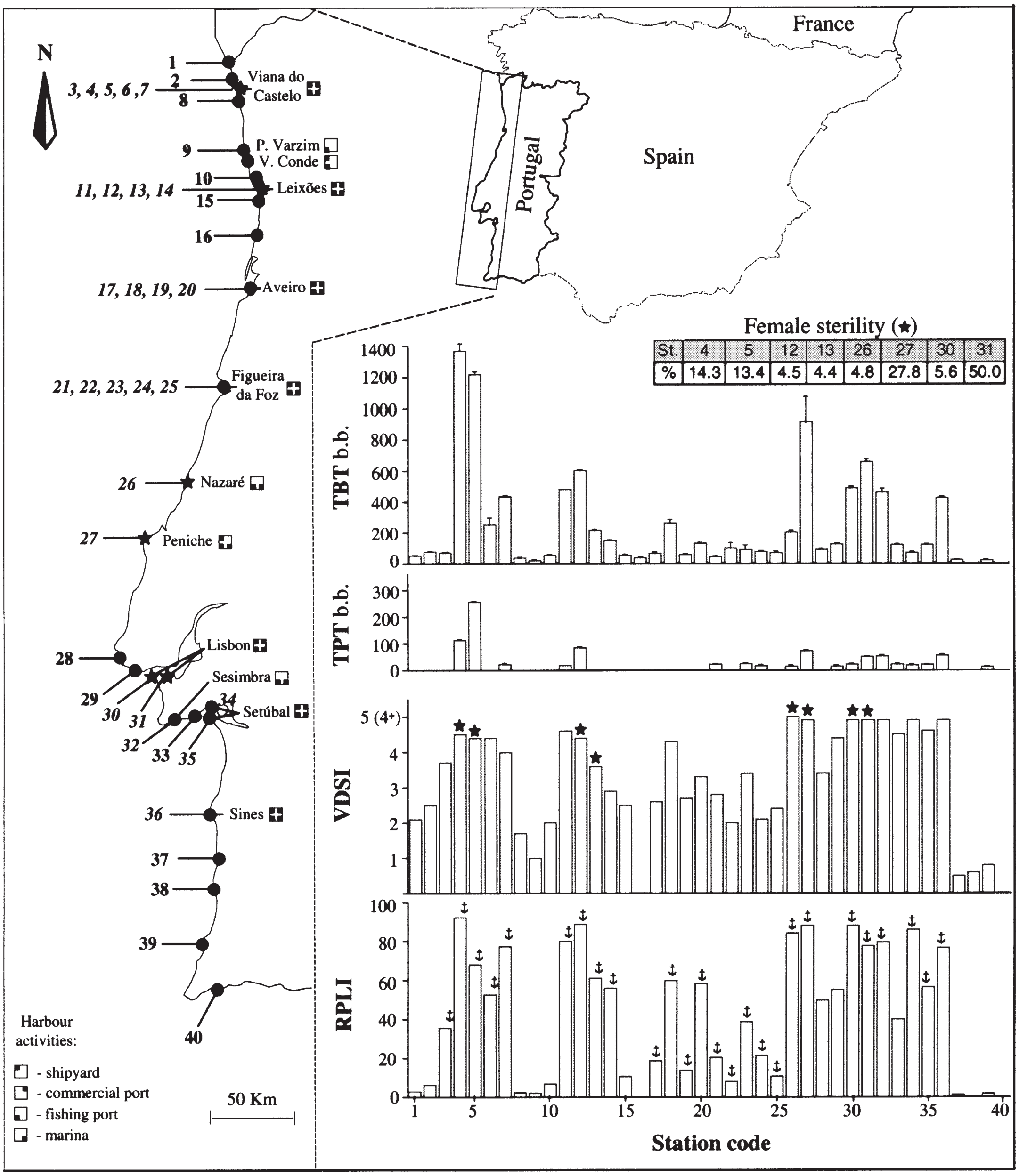

Fig. 1. *Nassarius reticulatus*. Map of the Portuguese coast indicating the sites (1 to 40) where specimens were collected and the location of the main harbours. Italic code numbers represent sampling stations located inside harbours. The graphic bars represent values of relative penis length index (RPLI), vas deferens sequence index (VDSI), and the mean tributyltin (TBT) and triphenyltin (TPT) whole female body burden (b.b.) (ng Sn g^{-1} dry weight [dry wt]), for each sampling station. Error bars correspond to 1 SD. ⚓: sampling stations located inside harbours; ★: occurrence of female sterility. For additional data compare Table 1

(IMO) has recently approved a convention for a global ban on the use of organotin antifouling paints by 1 January 2003, considering 1 January 2008 to be the last date for having these paints on a vessel (Champ 2000).

The response of prosobranchs to TPT pollution is poorly understood. This compound was found to induce imposex in *Thais clavigera* (Küster) (Horiguchi et al. 1997) but not in *Nucella lapillus* (L.) (Bryan et al. 1988). TPT derivatives are used in agrochemical applications (Bennett 1996, Mensink et al. 1996) and are still added in low proportions to some antifouling paints (Stäb et al. 1995, 1996). Japan prohibited the domestic market of TPT products in 1989 (Horiguchi et al. 1994). However, there is still a low world concern about the use of this compound, mainly due to the scarce knowledge of its biological effects.

Nassarius reticulatus is a ubiquitous species along the European coast (Graham 1988) that was proposed as a bioindicator of TBT pollution by Stroben et al. (1992a). The aims of the present work were (1) to evaluate the impact of organotin pollution on the populations of *N. reticulatus* on the coast of Portugal; (2) to survey the occurrence of female sterility in this area; and (3) to provide additional validation of the use of this species as a TBT bioindicator at the southern range of its distribution.

STUDY AREA

The surveyed area was the Portuguese coast between Vila Praia de Âncora (northern limit) and Praia da Luz (southern limit) (Fig. 1, Table 1), where the main Portuguese harbours are situated. These harbours are generally located inside natural embayments, such as estuarine systems, and may include commercial and fishing ports, marinas and shipyard facilities (Fig. 1), which in most cases are close to each other. Table 1 summarises relevant information regarding boat traffic and shipyard activity along the W Portuguese coast. Lisbon harbour is the main national commercial port, followed by Leixões, Setúbal, Aveiro, Sines, Figueira da Foz and Viana do Castelo, a rank that has been maintained since 1993. The Portuguese fishing fleet in 1998 was composed, in terms of gross tonnage stood (GTs), of 12.7% local small boats (<9 m length) (~9580 boats), 51.0% offshore boats (many with more than 25 m length) (1543 boats) and 36.3% long-distance vessels (>25 m length) (63 vessels) (information given by National Fishery Data Bank [DGPA]). Fishing boat GTs and the total fish download at each harbour (Table 1) provide an estimate of the relative distribution of the fleet in the W coast. In this part of the Portuguese coast, there are 17 main marinas with an average berthing capacity (YBC) of 260 yachts, and only 2 of them are located outside the above mentioned harbours: Oporto-Douro (250 YBC) and Cascais (638 YBC) (Anonymous 2000b). There are also many small boat landings spread along the coast. The main shipyards in W Portugal are located in the harbours of Viana do Castelo (V. Castelo), Vila do Conde (V. Conde), Aveiro, Figueira da Foz (F. Foz), Peniche, Lisbon and Setúbal (Table 1).

Table 1. Characterisation of boat traffic and shipyard activity in the Portuguese coast: total number of commercial ships called at each port during 1999 and respective total gross tonnage stood (GTs) (T: tons) (Anonymous 2000a); fishing boat GTs registered in 1999 and average fish download (Fish dow.) in the period 1998 to 1999 (information obtained from the National Fishery Data Bank), as a parameter to estimate the relative importance of fishing boat traffic between harbours; local leisure boat traffic is classified according to the yacht number berthing capacity (YBC) of all marinas at each site (Anonymous 2000b). P: presence of shipyards in the harbours

Harbours	Commercial shipping No.	Commercial shipping GTs ($\times10^6$ T)	Fishing boats GTs ($\times10^3$ T)	Fishing boats Fish dow. ($\times10^3$ T)	Marina YBC	Shipyards
V. Castelo	302	1.1	8.6	2.0	300	P
P. Varzim	–	–	2.8	3.6	241	
V. Conde	–	–	3.3	–	–	P
Leixões	3037	20.7	5.4	30.4	240	
Aveiro	1211	2.7	32.5	20.1	270	P
F. Foz	316	0.6	2.9	16.0	233	P
Nazaré	–	–	1.1	4.6	70	
Peniche	–	–	8.2	23.5	150	P
Lisbon	3597	37.8	14.2	175.5	1887	P
Sesimbra	–	–	4.4	16.8	130	
Setúbal	1706	17.3	2.2	3.5	155	P
Sines	773	13.4	0.8	10.3	230	

MATERIALS AND METHODS

Sampling and pre-treatment. *Nassarius reticulatus* was sampled from May to July 2000. Sampling locations were selected in order to provide an extensive coverage of the W coast, including the main national harbours (Fig. 1, Table 1). Geographical co-ordinates were determined with a mobile global positioning system (GPS) at each sampling site. The specimens were collected by hand at the intertidal shore and with baited hoop nets at sub-litoral sites. The animals were maintained in aquaria for about 3 to 4 d. About 40 animals were analysed for each station. Only adult animals (i.e. those presenting white columellar callus and teeth on the outer lip) were selected. They were narcotised using 7% $MgCl_2$ in distilled water. The shell height (distance from shell apex to lip of siphonal canal) was measured with vernier callipers to the nearest 0.1 mm. The shells were cracked open with a bench vice, and the individuals were sexed and dissected under a stereo microscope. Parasitised specimens were discarded from the analysis.

Imposex analysis. The following imposex parameters were determined for each station: mean female penis length index (PLI), relative penis length index (RPLI = mean female penis length × 100/mean male penis length), vas deferens sequence index (VDSI), average oviduct stage (AOS) and percentage of females affected by imposex (%I). The penis length was measured using 1 mm graduated graph paper under a stereo microscope. The VDSI was classified

Table 2. Data on *Nassarius reticulatus* collected along the Portuguese coast: mean ($\bar{x}$) shell heights of males (♂) and females (♀) (mm), percentage of imposex-affected females (%I), mean female penis length index (mm) (PLI), average oviduct stage (AOS), vas deferens sequence index ($VDSI_4$) and mean dibutyltin (DBT) and monobutyltin (MBT) whole female body burden (b.b.) at each sampling station. Standard deviations are given as a percentage of the mean: (a) 0 to 5%; (b) 5 to 10%; (c) 10 to 15%; (d) 15 to 20%; (e) 20 to 25%; and (f) 25 to 30%. DW: dry weight; nq: not quantifiable; *1 replicate. For additional data compare Fig. 1

Station code and name	Coordinates (EUR50)	♂ (N)	♂ shell height ($\bar{x}$)	♀ (N)	♀ shell height ($\bar{x}$)	%I	PLI ($\bar{x}$)	AOS	$VDSI_{(4)}$	Tissue b.b. ng Sn g^{-1} DW DBT	MBT
1. Vila Praia de Âncora	41° 48.93 N–8° 51.94 W	21	21.9[b]	22	22.1[b]	91	0.4	0.0	2.1	57[a]	31[b]
2. Praia Norte	41° 41.85 N–8° 51.13 W	21	21.1[b]	26	22.5[b]	100	0.8	0.0	2.5	63[a]	nq
3. V. Castelo – Marégrafo	41° 41.43 N–8° 49.71 W	15	22.6[b]	22	23.8[c]	100	4.5	0.1	3.5	97[b]	nq
4. V. Castelo – Estaleiro	41° 41.34 N–8° 50.26 W	15	22.9[b]	28	25.3[b]	100	11.5	1.0	3.9	654[a]	463[c]
5. V. Castelo – Cais	41° 41.38 N–8° 50.01 W	15	22.1[b]	15	22.9[b]	100	7.7	0.5	3.6	561[d]	462[b]
6. V. Castelo – Marina	41° 41.70 N–8° 49.20 W	15	22.0[b]	21	22.6[b]	100	6.3	0.3	3.9	155[f]	nq
7. V. Castelo – Barra	41° 41.06 N–8° 50.24 W	14	23.2[b]	26	25.9[b]	100	9.8	1.0	3.6	195[a]	101[c]
8. Praia da Amorosa	41° 38.72 N–8° 49.31 W	17	22.3[b]	37	23.7[b]	81	0.3	0.0	1.7	37[e]	nq
9. Póvoa do Varzim	41° 23.18 N–8° 46.40 W	17	24.2[b]	26	24.9[b]	69	0.3	0.0	1.0	nq	nq
10. Praia de Leça	41° 12.21 N–8° 42.82 W	15	23.5[c]	26	24.4[b]	85	0.9	0.0	2.0	34[b]	24[a]
11. Porto de Leixões – Plat. 2	41° 11.42 N–8° 41.43 W	15	24.1[b]	26	24.7[b]	100	10.4	1.2	3.9	205*	158*
12. Porto de Leixões – Marina	41° 11.30 N–8° 42.24 W	20	22.5[b]	22	24.3[b]	100	10.7	0.7	3.9	354[a]	184[b]
13. Porto de Leixões – Plat. 1	41° 11.26 N–8° 41.89 W	15	26.6[b]	23	25.9[b]	100	7.4	0.7	3.3	194[a]	103[b]
14. Porto de Leixões – Barra	41° 10.75 N–8° 41.43 W	15	23.3[c]	24	24.3[b]	100	6.3	0.0	2.9	78[a]	33[b]
15. Praia da Foz	41° 09.78 N–8° 41.10 W	15	25.0[b]	27	25.1[b]	100	1.3	0.0	2.5	44[b]	31[d]
16. Espinho	41° 00.44 N–8° 38.71 W	15	25.4[b]	25	26.9[b]	0	0.0	0.0	0.0	nq	nq
17. Aveiro – S. Jacinto	40° 39.84 N–8° 43.56 W	14	21.8[c]	17	24.0[c]	94	2.6	0.0	2.6	49[d]	nq
18. Aveiro – Porto Com. Norte	40° 39.06 N–8° 43.76 W	15	22.7[b]	27	23.6[b]	100	7.2	0.1	3.7	131[c]	45[d]
19. Aveiro – Barra	40° 38.71 N–8° 44.82 W	15	23.6[b]	27	24.3[b]	100	1.8	0.0	2.7	62[c]	nq
20. Aveiro – Magalhães Mira	40° 38.65 N–8° 44.06 W	15	25.0[b]	21	25.5[c]	100	7.0	0.0	3.2	81[a]	30[b]
21. F. Foz – Marina	40° 08.91 N–8° 51.67 W	10	25.8[b]	17	27.1[a]	100	2.2	0.0	2.8	40[a]	nq
22. F. Foz – Barra	40° 08.86 N–8° 51.90 W	15	24.5[b]	26	26.6[b]	85	1.1	0.1	2.0	37[c]	nq
23. F. Foz – Estaleiro	40° 08.60 N–8° 51.55W	14	23.7[b]	15	24.8[b]	100	5.5	0.1	3.4	55[e]	44[c]
24. F. Foz – Porto de Pesca	40° 08.52 N–8° 51.43 W	14	28.7[b]	15	28.4[b]	87	3.0	0.0	2.1	59[a]	nq
25. F. Foz – Cais Comercial	40° 08.90 N–8° 51.60 W	15	25.4[b]	15	25.7[b]	100	1.4	0.0	2.4	nq	49[a]
26. Nazaré – Porto de Pesca	39° 35.04 N–9° 04.39 W	18	22.6[b]	21	22.5[b]	100	8.7	1.1	4.0	177[a]	116[b]
27. Peniche – Porto de Pesca	39° 21.15 N–9° 22.52 W	19	19.2[d]	18	19.4[b]	100	8.5	0.3	4.0	671[a]	703[b]
28. Praia do Guincho	38° 43.74 N–9° 28.46 W	22	22.6[b]	25	23.0[c]	100	7.3	0.3	3.3	103[a]	62[e]
29. Praia das Avencas	38° 41.21 N–9° 21.27 W	15	24.1[b]	28	24.4[b]	100	7.6	0.1	4.0	121[a]	74[a]
30. Lisboa – Trafaria	38° 40.55 N–9° 14.09 W	20	23.3[b]	18	22.1[c]	100	7.7	1.3	4.0	275[a]	145[c]
31. Lisboa – Porto Brandão	38° 40.77 N–9° 12.29 W	17	20.2[b]	14	19.8[c]	100	9.0	0.8	4.0	721[a]	558[a]
32. Sesimbra – Porto de Pesca	38° 26.25 N–9° 06.76 W	15	23.5[b]	26	23.7[b]	100	10.1	0.8	4.0	413[a]	327[c]
33. Portinho da Arrábida	38° 28.58 N–8° 58.97 W	16	22.4[b]	20	22.9[b]	100	4.3	0.0	3.8	79[a]	49[a]
34. Setúbal – Porto de Pesca	38° 31.17 N–8° 52.58 W	25	21.1[b]	20	22.1[c]	100	7.9	0.8	4.0	63[b]	36[a]
35. Setúbal – Tróia	38° 26.25 N–9° 06.76 W	15	21.3[b]	23	21.7[b]	100	5.9	0.3	4.0	90[a]	48[b]
36. Sines – Porto de Pesca	37° 57.28 N–8° 52.21 W	16	22.1[b]	24	22.1[b]	100	8.7	0.9	4.0	239[a]	108[a]
37. Vila Nova de Mil Fontes	37° 43.30 N–8° 47.25 W	16	23.1[b]	27	23.4[b]	26	0.1	0.0	0.5	nq	nq
38. Zambujeira do Mar	37° 33.20 N–8° 47.44 W	15	22.6[b]	20	23.7[b]	58	0.01	0.0	0.6	nq	nq
39. Praia da Arrifana	37° 17.82 N–8° 52.11 W	15	20.1[d]	20	20.4[b]	44	0.2	0.0	0.8	nq	nq
40. Praia da Luz	37° 05.21N–8° 43.64 W	15	19.8[a]	32	20.8[b]	0	0.0	0.0	0.0	nq	nq

according to the scoring system proposed by Stroben et al. (1992a). The degree of oviduct convolution, assessed by AOS, was ranked according to the 3-stage scale of Barreiro et al. (2001). The percentage of sterile females (%STER) per sample was also determined.

Organotin analysis. TBT, dibutyltin (DBT), monobutyltin (MBT) and TPT were measured in the whole tissues of pooled females for each station. The analysis was performed by the Servicios Xerais de Apoio á Investigación (Universidade da Coruña). The procedures used are described by Quintela et al. (2000) and are largely based on the methods of Szpunar et al. (1996). The analysis was performed for 2 separate replicates of each sample, and the results are given as mean values with the respective standard deviation. The detection limit of the method was 20 ng Sn g^{-1} dry weight (dry wt) for butyltins (BT) and 10 ng Sn g^{-1} dry wt for TPT.

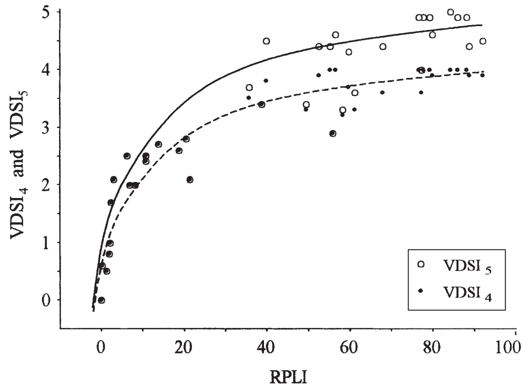

Fig. 2. *Nassarius reticulatus*. Relationship between RPLI and VDSI, which may be computed converting VDS stage 4+ to either 4 ($VDSI_{(4)}$) or 5 ($VDSI_{(5)}$). Lines fitted by eye

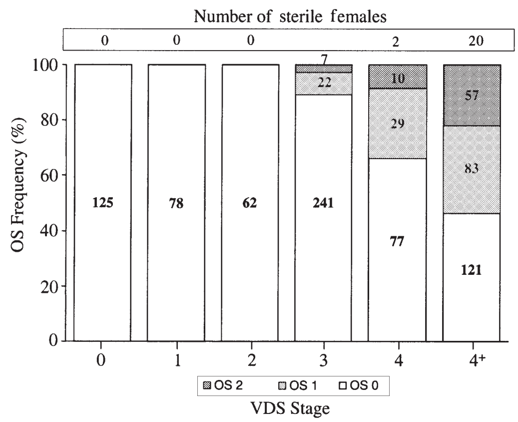

Fig. 3. *Nassarius reticulatus*. Relative and absolute frequencies of oviduct stages (OS) observed for each VDS stage in females. Number of sterile females presenting each VDS stage

RESULTS

Imposex indices

The imposex levels of *Nassarius reticulatus* at the different stations are summarised in Fig. 1 and Table 2. %I varied between 0 and 100. PLI and RPLI varied, respectively, between 0 and 10.7 mm and 0.0 and 92.1% (the mean male penis length was 12.1 mm). VDSI ranged from 0 to the maximum possible value of 5 (computing VDS stage 4+ as 5; see below). The VDS classification system developed by Stroben et al. (1992a), with stages 0 to 4+, provided a satisfactory description of the imposex in the observed females. Nevertheless, some differences were found: in 85.7% of the females showing stage 4 the vas deferens did not join the vulva, whereas in 33.9% of the females presenting stage 4+ the vas deferens ran parallel to the capsule gland instead of joining this gland. Most females (97.6%) presented a-type VDS stages, i.e. with simultaneous penis development (Stroben et al. 1992a). The b-type females occurred at Stns 1, 8, 9, 24 and 38, where VDSI was low (0.8 to 2.3). Regarding the computation of VDSI, Stroben et al. (1992a) proposed the use of the same numerical value of 4 to represent both VDS stages 4 and 4+ (here designated as $VDSI_{(4)}$). However, when stage 4+ is computed with a numerical value of 5 ($VDSI_{(5)}$) the correlation between VDSI and RPLI across samples is improved ($r_{(VDSI(5))} = 0.90$; $r_{(VDSI(4))} = 0.85$), resulting in an increased power of discrimination between stations (Fig. 2). This aspect was relevant in this study since the total number of females in VDS stage 4+ was more than twice the number of females in stage 4 (Fig. 3). Nevertheless, $VDSI_{(4)}$ values are given in Table 2 to allow comparisons with other works.

The AOS index varied between 0.0 and 1.3 and was significantly correlated with VDSI across stations ($r = 0.68$; $p < 0.001$). Oviduct convolution occurred only in females presenting a VDS stage higher than 2. OS stage 1 occurred in 8, 25 and 32% of the females showing VDS stages 3, 4 and 4+, respectively. OS stage 2 was observed in 3, 9 and 22% of corresponding females. Half of the females that exhibited VDS stage 4+ had a convoluted oviduct (Fig. 3).

Female sterility

Females carrying aborted egg capsules inside the capsule gland were found at 8 stations in the ports of Viana do Castelo, Leixões, Nazaré, Peniche and Lisbon. These females were considered to be sterile since they were incapable of laying egg capsules. In total 22 sterile females were found, and their frequencies varied from 5 to 50% of the females per site (Fig. 1). Sterility occurred only on females that were highly affected with imposex: 20 sterile females showed VDS 4+ and the remaining VDS 4 (Fig. 3).

In the sterile females the aborted egg capsules formed a compact brown mass that, in some cases, gave to the capsule gland a darker and enlarged external appearance, reminiscent of VDS stage 6 of *Nucella lapillus* (Gibbs & Bryan 1986), except that there was no external sign of vulva blockage by vas deferens proliferation. Nevertheless, in 5 females the vulva was externally obstructed: in 3 it was covered with a smooth tissue excrescence; in 1 there was a free solid cylindrical excrescence, 4 mm in length, growing over the vulva; in the other the vulva was very small and appeared to be constricted by the capsule gland wall

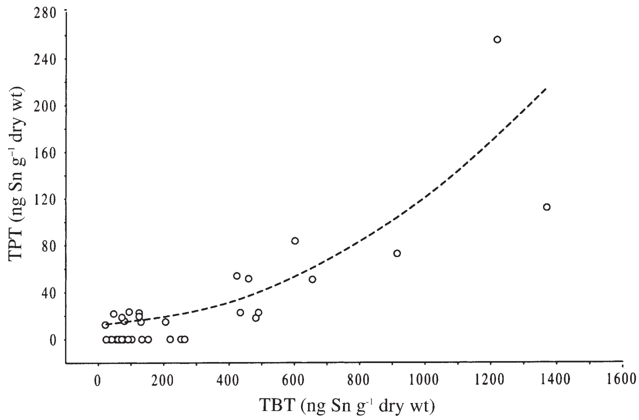

Fig. 4. *Nassarius reticulatus*. Relationship between TBT and TPT female b.b. across sampling stations. Null values of TPT b.b. represent levels below the analytical detection limit of 10 ng Sn g^{-1} dry wt and were excluded from line fitting (by eye)

Organotin tissue contamination

Nassarius reticulatus organotin body burden at the different stations is summarised in Fig. 1 and Table 2. TBT female body burden varied between <20 and 1368 ng Sn g^{-1} dry wt, whereas TPT ranged from <10 to 256 ng Sn g^{-1} dry wt. There was an evident relationship between triorganotin tissue contamination levels and the proximity of harbours enclosing ports, marinas or shipyards. Among the 13 stations located outside harbours, the TBT body burden did not exceed 128 ng Sn g^{-1} dry wt, and the TPT was only detected in 2 stations with values not higher than 15 ng Sn g^{-1} dry wt. Where both compounds were quantifiable, the TPT residue was, on average, 18% of the TBT residue. The TPT concentration increased exponentially with that of TBT (Fig. 4), and a highly significant correlation was established between TBT and lnTPT ($r = 0.88$, $p < 0.001$). DBT and MBT female body burden varied from <34 to 721 ng Sn g^{-1} dry wt and from <24 to 703 ng Sn g^{-1} dry wt, respectively. At the stations where all BT were quantifiable, TBT represented the major fraction (34 to 60%) of the total BT, followed by DBT (24 to 40%) and MBT (10 to 31%).

RPLI, VDSI, AOS and %STER versus TBT contamination

Highly significant correlations ($p < 0.001$) were established between lnTBT and the indices RPLI ($r = 0.88$), $VDSI_{(5)}$ ($r = 0.81$) and AOS ($r = 0.72$), as well as between TBT and %STER ($r = 0.61$). Clear relationship patterns were found between the intensity of imposex and the level of TBT tissue concentration across stations (Figs. 5 & 6). The RPLI and $VDSI_{(5)}$ rose rapidly up to values around 60 to 80% and 4.3 to 4.5, respectively, for TBT tissue concentrations of about 200 ng Sn g^{-1} dry wt. Then both indices tended to a plateau of approximately 85% for RPLI and 4.6 for $VDSI_{(5)}$, at 700 and 400 ng TBT-Sn g^{-1} dry wt, respectively (Figs. 5 & 6). The AOS index showed a similar trend, reaching a plateau of about 1.0 at a TBT tissue concentration of 400 ng Sn g^{-1} dry wt (Fig. 6). Female sterility was only observed at stations where TBT body burden exceeded 200 ng Sn g^{-1} dry wt (Fig. 5).

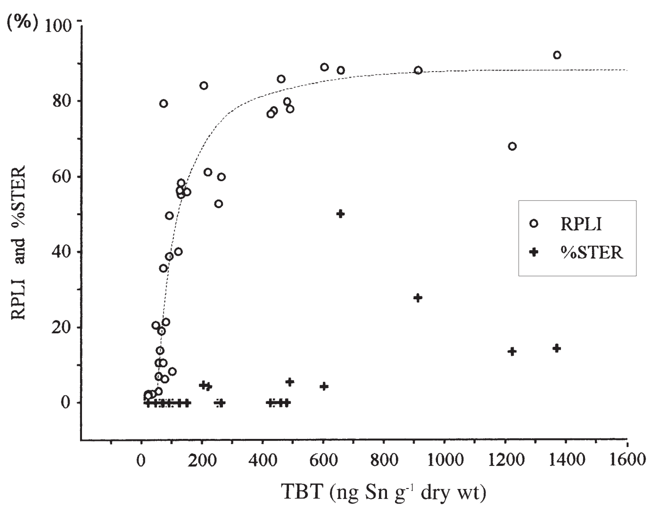

Fig. 5. *Nassarius reticulatus*. Relationship between the female TBT b.b. and the RPLI and percentage of female sterility (%STER). Line fitted by eye

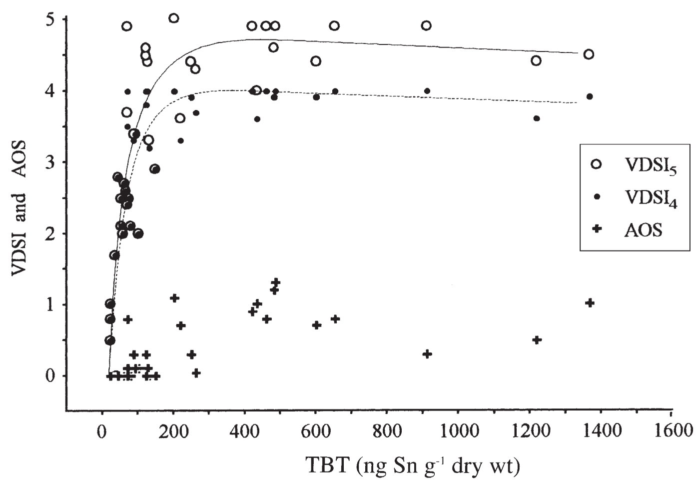

Fig. 6. *Nassarius reticulatus*. Relationship between the TBT female b.b. and the VDSI and average oviduct stage (AOS) index. Lines fitted by eye

DISCUSSION

Biomonitoring organotin pollution

Nassarius reticulatus occurs along the entire Portuguese mainland coast. In the present survey, it was commonly found at sandy sheltered places of rocky intertidal areas along the seashore, but it was particularly abundant in sandy or muddy sediments inside estuarine systems, where it may withstand a reduction of salinity to about 16 psu (Fretter & Graham 1985). Nevertheless, the species was very scarce or absent from extremely exposed shores and from several visited sites, namely along the margins of the Tagus (Lisbon) and the Sado (Setúbal) estuaries. Yet the distribution of the species provided a fairly good monitoring coverage of the coast, including the harbours located inside estuarine systems (Viana do Castelo, Aveiro, Figueira da Foz, Lisbon and Setúbal).

Nassarius reticulatus imposex levels were highly correlated with TBT tissue concentrations. RPLI and VDSI depicted the gradient profiles of organotin pollution in the coast around the harbours (Fig. 1). For instance, at Viana do Castelo, the large range of RPLI and $VDSI_{(5)}$ values observed at Stns 2 to 8 clearly show an increasing TBT gradient from the adjacent coast to the harbour. Identical patterns can be observed at Stns 10 to 15 (around Leixões) and at Stns 28 to 31 (around Lisbon) (Fig. 1). At Aveiro (Stns 17 to 20) and Figueira da Foz (Stns 21 to 25) increasing gradients are also well marked from the mouths towards the organotin sources inside the estuarine systems (Fig. 1).

The $VDSI_{(5)}$ renders a better assessment of imposex intensity and a better power of discrimination between highly contaminated stations than $VDSI_{(4)}$. This is to be expected since the transition from VDS stage 4 to 4^+ corresponds to a substantial development of the vas deferens and penis. The adoption of $VDSI_{(5)}$ would also allow for a more gradual transition to a possible VDS stage 6, corresponding to the abortion of egg capsules. Even so, the discrimination between severely contaminated sites always remains difficult since both VDSI and RPLI reach a plateau for high TBT body burdens (in our study >400 and >700 ng Sn g^{-1} dry wt, respectively) (Figs. 5 & 6). A similar trend was also observed by other authors (Bryan et al. 1993, Barreiro et al. 2001). In view of that, Barreiro et al. (2001) proposed the use of the AOS index for discerning among highly polluted sites, as they found increasing values of AOS for a range of TBT residues where RPLI and VDSI had already reached a plateau. In the present study, although AOS was highly correlated to organotin contamination, it did not offer advantages in this respect.

Nassarius reticulatus can be recommended as a key species for organotin biological effects monitoring in the maritime area covered by the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic. In fact, this is a common species in this area that may depict organotin gradients along the coastline in relation to the proximity of harbours, even when they are located inside estuaries. Moreover, this is a moderately sensitive species, when compared to the highly sensitive *Nucella lapillus* or to the poorly sensitive *Littorina littorea* (L.), and may therefore provide a better description of organotin gradients around harbours, as was shown at Aveiro by Barroso et al. (2000). On the other hand, the nassariid may be present in hotspots of organotin contamination where *N. lapillus* may already be extinct.

Imposex versus TBT contamination

The present study and other published works provide useful information regarding the imposex-TBT body burden relationship in *Nassarius reticulatus* for the NE Atlantic coast, between SW England and SW Portugal, which represents a considerable portion of the OSPAR maritime coastal area. Bryan et al. (1993) published data for SW England between 1985 and 1993 and found differences in the imposex-TBT body burden relationships between pre- and post-TBT ban periods. In either case, there was a sharp increase in RPLI up to 70 % (referred to non-narcotised animals) for TBT residues less than 200 ng Sn g^{-1} dry wt, as observed in the present study. For specimens collected in NW Spain during 1998 to 1999 the RPLI reached a plateau of about 90 % at 700 ng TBT-Sn g^{-1} dry wt, resembling the trend described here for Portugal, although the increase of RPLI at less contaminated levels was less pronounced in NW Spain (Barreiro et al. 2001). However, the relationship described by Stroben et al. (1992a) for the coasts of Brittany and Normandy in 1988 to 1991 is considerably different from that obtained in the present study. For example, females with 200 ng TBT-Sn g^{-1} dry wt exhibited about maximum $VDSI_{(4)}$ and RPLI = 70 % in Portugal but only about $VDSI_{(4)}$ = 2 and RPLI = 3 % in Brittany and Normandy, despite the fact that the mean male penis length was about the same in the 2 regions (12 to 13 mm). Although this may partly be attributed to different analytical techniques used — since unspecific methods may overestimate TBT concentrations when other triorganotin residues (such as TPT) are abundantly present — other hypothesis may help to explain the observed discrepancies. One relates to the fact that TBT body burden indicates a short-term bioaccumulation, whilst imposex, being irreversible, is a long-term indicator of the presence of organotin compounds (Bryan et al. 1993). This latter aspect is important considering the life span of the species, which may reach more than 10 yr (Tallmark

1980, Bryan et al. 1993, C.M.B. unpubl.). Nevertheless, and despite the fact that restrictions on the use of TBT paints were introduced at different dates in Europe (1982 in France; 1987 in England; 1990 in Spain; 1993 in Portugal), the above imposex-TBT body burden relationships were established after similar periods following the TBT ban in each country. Another hypothesis could be related to geographical variability of the species response to organotin.

TBT versus TPT contamination

In the present survey, TPT was <10 ng Sn g^{-1} dry wt in about half of the sampled stations, whereas TBT in the same places ranged from 22 to 262 ng Sn g^{-1} dry wt with a mean value of 97 ng Sn g^{-1} dry wt. In the remaining stations, TPT varied between 4 and 62 % (average 18 %) of the TBT residue. As for its sources, TPT is a widely used fungicide in agriculture (potato, rice, sugar beet and celery cropping) and is applied in antifouling paints as a small percentage (approximately 8 %) together with TBT (Stäb et al. 1996). Considering the high correlation between the 2 organotins in the tissues of *Nassarius reticulatus* in Portugal, TPT pollution presumably originated from its use in antifouling paints. According to Barreiro et al. (2001), in NW Spain the body burden fraction of TPT in this species is lower (maximum of 14.4 % of TBT), and it is not correlated with that of TBT. Nevertheless, in NW Spain the TPT residue fraction is higher in the vicinity of marinas than it is close to commercial ports. Tolosa et al. (1996) also reported higher concentrations of TPT in the sub-surface waters close to marinas at the Côte d'Azur. In Portugal TPT body burden was not particularly high near the marinas at Stns 6, 12 and 21 (Fig. 1). Comparing *N. reticulatus* with other species, Ruiz et al. (1998) reported similar low fractions of TPT (about 10 to 50 % of TBT) in the tissues of *Nucella lapillus* from NW Spain, but this contrasts with *Buccinum undatum* (L.) from Eastern Scheldt (The Netherlands) (Mensink et al. 1996) as well as with *Thais clavigera* (Küster) and *T. bronni* (Dunker) from Japan (Horiguchi et al. 1994), which presented higher levels of TPT than of TBT residues.

Impact of organotin pollution

The imposex levels of *Nassarius reticulatus* in the Portuguese harbours are comparable to those reported for the major contaminated harbours of NW Spain (e.g. Coruña, Ferrol and Vigo) (Barreiro et al. 2001) and France (e.g. Roscoff harbour) (Oehlmann et al. 1993), and for the most contaminated sites in SW England before TBT restrictions (e.g. Dart Estuary and Plymouth Sound) (Bryan et al. 1993). The development of the female penis and sperm duct (vas deferens) has been consistently correlated with organotin pollution in this and other field studies (Stroben et al. 1992a,b, Bryan et al. 1993, Oehlmann et al. 1993, Huet et al. 1995, Barroso et al. 2000, Barreiro et al. 2001), and it has also been shown, under laboratory conditions, that these male characters are induced by exposure to TBT (Stroben et al. 1992b, Bettin et al. 1996). *N. reticulatus* females from Portugal also presented some degree of oviduct convolution. This was previously described by Stroben et al. (1992a) in France, although only afflicting 2 % of the analysed females, and by Barreiro et al. (2001) in NW Spain, to an extent comparable to that found in the present study. Smith (1980) reported the same phenomenon for *Ilyanassa* (= *Nassarius*) *obsoleta* (Say) and considered that it was a sign of masculinisation because it resembles the sinuous seminal vesicle of the males. This syndrome was highly correlated with organotin body burden in the present survey and in NW Spain (Barreiro et al. 2001), but there is still the need to establish a causal relationship using experimental designs. The present work also reveals that female sterility is highly correlated with organotin contamination but, again, there is no experimental support showing that sterility results from organotin exposure. The first report on *N. reticulatus* sterility came from the west of France (Huet et al. 1995), where 2 sterile females with aborted egg capsules were found at a highly TBT polluted site. Recently, Barreiro et al. (2001) found 40 sterile females, also with aborted egg capsules, at highly polluted places in NW Spain, affecting up to 26 % females per station. In the present survey, 22 similar sterile females were found at the most polluted sites of Portugal (Fig. 1), afflicting up to 50 % of the females per station. Hence, sterilisation of *N. reticulatus* females appears to be a broad phenomenon consistently associated with high organotin pollution levels and maximum VDS scores (4^+) (Fig. 3), which deserves further investigation.

The present work has shown that organotin pollution remains a cause for concern on the Portuguese coast, especially in the estuarine systems where most harbours are located. This may indicate that the implementation in 1993 of restrictions on the use of TBT antifouling paints was not effective and that vessels larger than 25 m and shipyards probably still are the main sources of organotin contamination, which reinforces the need for further restrictions.

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