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Imposex and organotin body burden in the dog-whelk (*Nucella lapillus* L.) along the Portuguese coast

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***Nucella lapillus* imposex—superimposition of male characters onto prosobranch (a subclass of gastropod molluscs) females—and organotin female body burden were surveyed on the Portuguese coast, from Vila Praia de Âncora (northern limit) to Praia da Luz (southern limit), at 17 sampling stations, between May and August 2003. The vas deferens sequence index (VDSI), the relative penis size index (RPSI), the percentage of females affected with imposex (%*I*) and the percentage of sterile females (%*S*) were used to assess the level of imposex at each site. VDSI, RPSI and %*I* were 0.20–4.04, 0.0–42.2% and 16.7–100.0%, respectively. Sterile females were found at stations 2 (6.2%), 5 (4.0%) and 7 (5.0%). Tributyltin (TBT) and dibutyltin (DBT) female body burdens were 23–138 and <10–62 ng Sn/g dry weight, respectively. TBT female body burden was significantly correlated with RPSI and VDSI [Spearman rank order linear correlation: RPSI vs TBT body burden (b.b.) $r = 0.71$, $p < 0.01$; VDSI vs logTBT body burden $r = 0.71$, $p < 0.01$]. Imposex and TBT b.b. were highest at sites located in the proximity of harbours, where TBT leaching from antifouling paints is more intense owing to the high concentration of ships and dockyard activities.**

KEYWORDS: organotin; TBT; DBT; imposex; sterility; *Nucella lapillus*; Portuguese coast

Tributyltin (TBT) compounds have been extensively used as biocide agents in ship anti-fouling paints since the mid 1960s.[1] Their deleterious effects on non-target organisms became apparent in the 1970s with the upsurge of prosobranch gastropod females with male characteristics, which was termed 'imposex' by Smith.[2] After the mid 1980s many studies described TBT toxicity on organisms over a broad taxonomic spectrum, from bacteria to vertebrates, and its severe negative impacts on ecosystems. Legislation to ban the use of organotin antifouling paints on small boats (<25 m) was introduced for the first time in France in 1982, mainly motivated by the negative impact of TBT pollution on oyster farming. Latterly, similar legislation was applied throughout Europe; Portugal adopted this ban in 1993 but it was insufficient to reduce TBT pollution. Barroso and Moreira showed that TBT pollution increased in the Portuguese coast from 1987 to 2000 and suggested that this could be linked to the increase of large ship traffic during that period.[3] The International Marine Organization (IMO) adopted the International Convention on the Control of Harmful Antifouling Systems on Ships, according to which organotin antifouling systems cannot be applied or re-applied on any ship after 1 January 2003 and ships shall not bear such compounds after 1 January 2008. The present work aims to assess the most recent evolution of TBT pollution on the Portuguese coast and to create a baseline for the IMO ban to allow future evaluation of its effectiveness. The dogwhelk, *Nucella lapillus* (L.), was used as an indicator of the level of TBT pollution, as recommended by the OSPAR Joint Assessment and Monitoring Program (JAMP) guidelines.[4] *Nucella lapillus* is a common gastropod species of the Atlantic rocky shores, distributed in Europe from the north of Russia to the south of Portugal.[5] This species has a limited dispersion—a life cycle without a planktonic phase and with weak adult mobility—and develops imposex at very low levels of TBT in water (<0.5 ng Sn/L).[6] In advanced

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states of imposex the females may become sterilized owing to the overgrowth of the vas deferens, which blocks the vulva and prevents the egg capsules being released, which has caused population extinctions at severely polluted sites throughout Europe.[7,8] For this reason the actual extent of female sterility was also surveyed in *N. lapillus* populations along the Portuguese coast.

About 45–60 adult *Nucella lapillus* were collected by hand at the intertidal rocky shore from May to August 2003 at stations 1–17 along the Portuguese coast (Fig. 1; Table 1). The shell height (apex to siphonal canal length) was measured with vernier callipers to the nearest 0.1 mm. After shell removal, the animals were sexed and analysed for imposex without narcotization. The penis length was measured using a stereo microscope with a graduated eyepiece to the nearest 0.14 mm. The relative penis size index [RPSI = mean female penis length $(FPL)^3 \times 100$/mean male penis length $(MPL)^3$], the vas deferens sequence index (VDSI), the percentage of females affected with imposex (%*I*) and the percentage of sterile females (%*S*) were determined for each station, according to Gibbs *et al*.[9] Parasitized specimens were discarded from the analysis. TBT and dibutyltin (DBT) were measured by atomic absorption spectroscopy in homogenized whole tissues of 10–15 females from each station. The analytical procedures were largely based on the methods of Ward *et al*.[10] and are fully described by Bryan *et al*.[7] Recoveries of TBT and DBT were 100 and 92%, respectively, and were corrected by the use of standard additions in all samples. Detection limits for TBT and DBT were 10 ng Sn/g dry weight.[7]

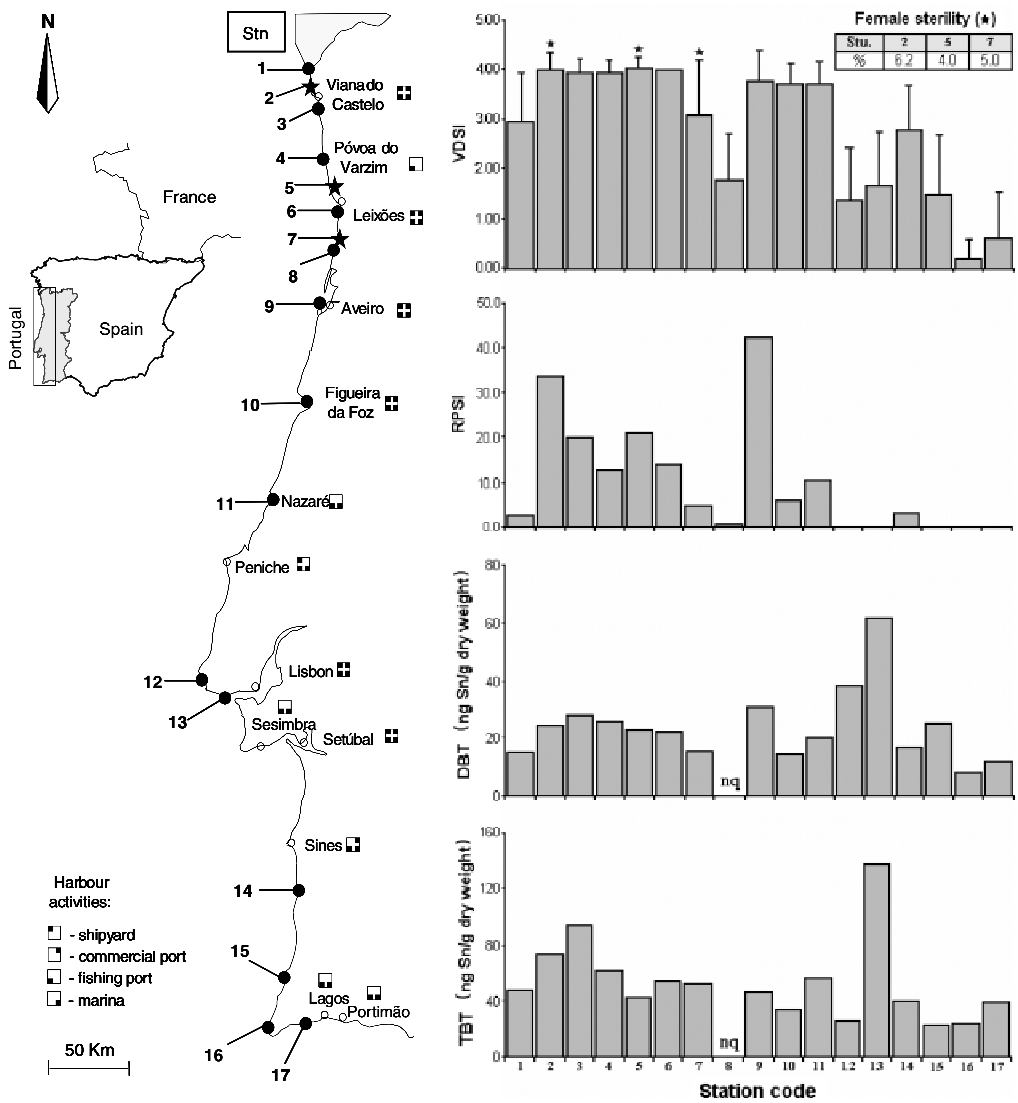

Figure 1. *Nucella lapillus*. Map of the Portuguese coast indicating sampling sites (1–17) and main harbour activities. The histograms represent values for vas deferens sequence index (VDSI), relative penis size index (RPSI), tributyltin (TBT) and dibutyltin (DBT) whole female body burden (ng Sn/g dry weight). nq, Not quantified. The asterisks indicate occurrence of female sterility.

Table 1. *Nucella lapillus*. Number of analysed specimens (*N*) at each site, males and females, with the indication of mean ($\bar{x}$) shell heights (mm) and the percentage of females affected with imposex (%*I*). Standard deviations (SD) are rounded off to unity and given next to the mean value in the format 'mean$^{(SD)}$'. For additional data compare with Fig. 1. Time comparisons of *Nucella lapillus* imposex indices and organotin female body burdens (ng Sn/g dry weight), between 2000 (Barroso and Moreira, 2002) and 2003 (current study), at common sites along the Portuguese coast

Station code and name	Coordinates (EUR 50)	♂ *N*	$\bar{x}$ ♂ Shell height (mm)	♀ *N*	$\bar{x}$ ♀ Shell height (mm)	RPSI		VDSI		*U*	*p*	%*I*		TBT (ng Sn/g dry weight)		DBT (ng Sn/g dry weight)	
						2000	2003	2000	2003			2000	2003	2000	2003	2000	2003
1. Vila Praia de Âncora	41°48.93N–8°51.94W	22	$21.0^{(2)}$	28	$22.3^{(3)}$	11.70	2.70	3.81	2.96	44.000	***	100.0	100.0	70	48	50	15
2. Praia Norte	41°41.85N–8°41.13W	20	$19.2^{(1)}$	32	$20.9^{(2)}$	21.70	33.80	4.04	4.00	401.00	NS	100.0	100.0	90	74	51	24
3. Praia da Amorosa	41°38.72N–8°49.31W	12	$20.5^{(2)}$	14	$20.2^{(2)}$	33.20	19.80	3.95	3.93	150.00	NS	100.0	100.0	135	94	99	28
4. Póvoa do Varzim	41°23.18N–8°46.40W	19	$22.5^{(1)}$	31	$23.1^{(2)}$	8.70	12.70	3.96	3.94	378.00	NS	100.0	100.0	63	62	38	26
5. Praia de Leça	41°12.21N–8°42.82W	31	$22.4^{(2)}$	25	$23.2^{(2)}$	14.70	21.00	4.00	4.04	240.00	NS	100.0	100.0	77	42	66	23
6. Praia da Foz	41°09.78N–8°41.10W	25	$20.9^{(2)}$	25	$21.1^{(2)}$	28.40	14.10	4.00	4.00	250.00	NS	100.0	100.0	117	55	112	22
7. Aguda	41°03.09N–8°39.18W	37	$21.2^{(2)}$	20	$21.0^{(2)}$	—	4.80	—	3.10	—	—	—	100.0	—	53	—	16
8. Espinho	41°00.44N–8°38.71W	20	$21.0^{(2)}$	25	$21.9^{(3)}$	4.30	0.60	3.77	1.84	17.500	***	100.0	92.0	37	—	34	—
9. Aveiro	40°38.71N–8°44.82W	25	$23.0^{(4)}$	25	$23.6^{(4)}$	32.90	42.20	4.00	3.80	230.00	NS	100.0	100.0	95	47	98	31
10. Fig. Foz	40°10.18N–8°53.26W	20	$19.4^{(2)}$	31	$19.6^{(1)}$	4.40	5.80	3.55	3.74	410.00	NS	100.0	100.0	—	34	—	14
11. Nazaré	39°36.26N–9°04.49W	20	$18.2^{(1)}$	31	$19.1^{(2)}$	18.70	10.70	4.08	3.74	264.50	**	100.0	100.0	—	57	—	20
12. Praia do Guincho	38°43.74N–9°28.46W	20	$19.2^{(2)}$	33	$20.1^{(1)}$	0.10	0.10	1.35	1.36	506.00	NS	48.6	85.0	30	26	120	38
13. Praia das Avencas	38°41.21N–9°21.27W	23	$20.5^{(3)}$	32	$21.9^{(2)}$	30.70	0.04	4.00	1.66	22.000	***	100.0	84.4	147	138	180	62
14. Vila nova de Mil Fontes	37°43.30N–8°47.25W	20	$21.1^{(1)}$	25	$22.4^{(2)}$	8.40	3.00	3.15	2.80	206.00	NS	95.5	100.0	77	40	48	17
15. Zambujeira do Mar	37°33.20N–8°47.44W	20	$18.3^{(1)}$	31	$19.5^{(2)}$	—	0.00	—	1.48	—	—	—	83.9	—	23	—	25
16. Praia do Amado	37°15.22N–8°38.45W	17	$21.6^{(2)}$	18	$22.9^{(3)}$	—	0.00	—	0.20	—	—	—	16.7	—	24	—	<10
17. Praia da Luz	37°05.21N–8°43.64W	20	$20.8^{(2)}$	31	$22.3^{(2)}$	0.00	0.00	1.43	0.61	383.50	NS	38.5	41.9	nd	39	nd	12

U, Mann–Whitney *U*-test result; ** $p < 0.01$; *** $p < 0.001$; ND, not detectable; —, not analysed; NS, not significant.

Nucella lapillus imposex and butyltin contamination was ubiquitous on the Portuguese coast (Fig. 1; Table 1). RPSI, VDSI and %*I* were 0.0–42.2%, 0.20–4.04 and 16.7–100.0%, respectively. Sterile females were found at stations 2 (6.2%), 5 (4.0%) and 7 (5.0%), but these low levels presumably pose a low risk of population extinction at these sites. TBT and DBT female body burdens were 23–138 and <10–62 ng Sn/g dry weight, respectively. RPSI and VDSI were significantly correlated with TBT female body burdens (Spearman rank order linear correlation: RPSI vs TBT body burden $r = 0.71$, $p < 0.01$; VDSI vs logTBT body burden $r = 0.71$, $p < 0.01$). Station 13 was excluded from the correlation analysis since the population at this site is affected by the Dumpton syndrome. This syndrome was first described and coined by Gibbs for a dog-whelk population in southeast England and consists of a genetic deficiency that causes the underdevelopment of the genital system, leading to a lack of penis or undersized penis and incompletely developed gonoducts in males.[11] The syndrome seems to be advantageous to the populations living at highly TBT polluted sites since females carrying the deficiency may not become sterilized.[11]

Nucella lapillus was only collected from sites on the open coast, some of them very close (less than 1 mile) to main harbours (stations 2, 3, 5, 6, 9, 10, 11 and 13), others close to small boat anchorage places (stations 1, 4, 7, 8 and 14) and others located at pristine areas (stations 12, 15, 16 and 17). As a consequence, imposex and butyltin body burden levels were higher in the two former groups of stations (Fig. 1), which points out the link between TBT pollution and the proximity of ships or boats, most of which are known to still bear TBT-based antifouling coatings.

Nucella lapillus was found at all sites already sampled in 2000 by Barroso and Moreira.[3] Considering that female maturation occurs at about 2–3 years, the three-year period elapsing between 2000 and 2003 surveys is large enough to depict temporal trends in TBT pollution. There was a significant decrease in the VDSI between 2000 and 2003 at stations 1, 8, 11 and 13, but no significant changes were observed in the remaining sites (Mann–Whitney *U*-test, see Table 1). The nature of the data regarding the RPSI and TBT body burden does not allow a meaningful statistical comparison between the two surveys. Nevertheless, the simple analysis of the data suggests that the RPSI has a less consistent change, increasing at some sites and decreasing at others, while the TBT female body burden has apparently decreased at all sampling sites, despite different analytical methods being employed in the two surveys (Table 1). Although inconclusive, there are signs of a possible slight slowing down tendency of imposex and TBT pollution that has to be confirmed by further studies. Hopefully, after the IMO ban there will be a consistent global decrease in TBT that will be easily detected in future research using similar methods.

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