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Imposex of the netted whelk *Nassarius reticulatus* (Prosobranchia) in Brittany along a transect from a point source

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Abstract: Imposex, the development of additional male sex organs in female prosobranch snails, is caused by endocrine disruptors with androgenic potential, such as tributyltin (TBT), a biocide and a component of antifouling paints. In this paper, imposex intensities in *Nassarius reticulatus*, collected from 2004 to 2006 at 7 different sites in the coastal area of Roscoff, were investigated. In all years, the vas deferens sequence index (VDSI) showed a decrease along a transect with distance from the harbour, the correlation being significant. In addition to VDSI values, the penis length of males and females was measured. The median female penis length decreased with increasing distance from the harbour. This correlation was significant in all sampling years. Neither the VDSI nor the female penis length showed significant differences in all sampling years for a given site. A comparison of pooled VDSI data in *N. reticulatus* with the amount of TBT-Sn in water and snail tissue resulted in a highly positive relation. Harbours can be contaminated with TBT for several years, therefore our observations indicate still high TBT concentrations in the sediments. This conclusion is supported by the results of a temporal trend monitoring of imposex intensities in *N. reticulatus* at four stations in and around Roscoff over a period of 17 years (1989 to 2006). While there is only little evidence for a recovery from imposex intensities at two stations in or directly influenced by a harbour, VDSI values dropped at two reference stations.

Résumé : *Imposex chez la nasse réticulée* Nassarius reticulatus *(Prosobranchia) en Bretagne le long d'un transect partant d'un point source.* Imposex, le développement d'organes sexuels mâles surnuméraires chez la femelle de gastéropodes prosobranches est provoqué par des pertrubateurs endocrines au potentiel androgénique, tel que le tributyl-étain (TBT), un pesticide et un composant des peintures antifouling. Dans ce papier est étudiée l'intensité de l'imposex chez *Nassarius reticulatus*, récoltée entre 2004 et 2006 à sept stations de la zône côtière de Roscoff. Chaque année, l'indice du vas deferens (VDSI) diminue le long d'un transect partant du port, la corrélation étant significative. En complément des valeurs de VDSI, la longueur du pénis des mâles et des femelles a été mesurée. La médiane de la longueur du pénis des femelles diminue avec l'éloignement du port. Cette corrélation est significative pour chacune des années étudiées. Ni le VDSI, ni la longueur du pénis des femelles ne montrent de différences significatives par site entre les différentes années étudiées. Une comparaison des données regroupées de VDSI chez *N. reticulatus* avec la concentration de TBT-Sn dans l'eau et dans les tissus de l'espèce met en évidence une forte corrélation positivie. Les ports peuvent être contaminés par le TBT depuis des

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années, par conséquent nos observations indiquent des concentrations de TBT encore élevées dans les sédiments. Cette conclusion est confortée par les résultats de l'évolution temporelle de l'intensité d'imposex chez *N. reticulatus* à quatre stations et autour de Roscoff sur une période de 17 ans (1989 à 2006). Bien qu'il y ait peu de signes d'une restauration du taux d'imposex aux deux stations situées dans le port ou sous son influence directe, les valeurs de VDSI ont diminué aux deux stations de référence.

Keywords: Molluscs • Endocrine disruption • Biomonitoring • Tributyltin • Temporal trend

Introduction

The phenomenon of penis-bearing females in dioecious gastropods was first described by Blaber (1970). Under the influence of endocrine disruptors with an androgenic potential, such as tributyltin (TBT), female prosobranch snails develop additional male sex organs (vas deferens and/or penis). This phenomenon is called imposex (Smith, 1971) or pseudohermaphroditism (Jenner, 1979). Tributyltin has been used as a biocide in antifouling paints and wood preservatives and as a slimicide for breweries, cooling towers as well as textile, paper and pulp mills (Rexrode, 1987). France was the first country to draw up legislative restrictions for TBT in antifouling paints which were triggered by observed shell malformations in cultivated oysters (*Crassostrea gigas*, Thunberg, 1793; Oehlmann, 2004). Since the late 1970s prosobranch populations became extinct in areas suffering from high TBT exposure like in the vicinity of harbours, marinas and along great shipping lanes (Oetken et al., 2004). The use of TBT antifoulants on small boats was banned in 1982, and since 1987 similar legislation was adopted by other countries. The latest legislative act is of the International Marine Organisation, banning the use of antifouling paints containing TBT on any boat by 1 January 2003 and forbidding its presence on ship hulls after 2008 by the International Convention on the Control of Harmful Antifouling Systems on Ships (IMO, 2002). However, both IMO restrictions have not yet been ratified by the required number of member nations. Nevertheless, ambient TBT concentrations dropped in most of the previously highly contaminated areas so that snails were able to re-colonise sites at which extinction occurred earlier (Birchenough et al., 2002; Huet et al., 2004). In remote regions prosobranch populations without any imposex signs can be found again today (Følsvik et al., 1999; Strand & Asmund, 2003; Jorundsdottir et al., 2005). However, in some coastal waters no decline in ambient TBT concentrations and biological effects was found or even an increase in specific regions (Oehlmann, 2004). Michel and Averty (1999) compared monitoring results from the 1980s with results from 1997 and found a significant reduction of organotin contamination. However, port facilities continue to be a major source of contamination and affect natural and cultivated mollusc populations in the vicinity. Comparable observations were made also in the surroundings of Roscoff and part of these results are communicated here.

The netted whelk (*Nassarius reticulatus* L., 1758), a dioecious prosobranch (Fretter & Graham, 1985), can be found along most European rock or sand coasts. *N. reticulatus* inhabits the subtidal zone down to a depth of about 35 m. Unless scavenging for food or searching for mating partners the netted whelk stays dug into the sandy ground (Fretter & Graham, 1985). Due to pollution, the population of the netted whelk has declined. Along the German coasts of the North Sea, for example, only a small protected population remained between Germany and Denmark (Schulte-Oehlmann et al., 2001).

In this paper, imposex in *N. reticulatus*, collected at different distances from a pollution hot spot in the coastal area of Roscoff, is investigated. Furthermore, imposex intensities in the same species sampled at four stations around Roscoff over a period of 17 years are reported. The results indicate only a slight recovery from imposex since 1989.

Materials and methods

Sampling

In 2004-2006, *Nassarius reticulatus* was sampled from the sediment during low tide along a transect in the intertidal zone near Roscoff, France. Each year, the sampling was conducted in March. The transect starts at Roscoff Harbour (site 1) going west with 7 main sampling sites (Fig. 1). On sampling site 5 the investigation was extended with 4 additional sites (distance of at least 30 m in each direction) for the analysis of small local differences. Furthermore, two reference populations were sampled: one on the northern side of the island Île Verte near Roscoff and at a small bay east of Roscoff (Beg an Fry, nearest harbour at Locquirec,

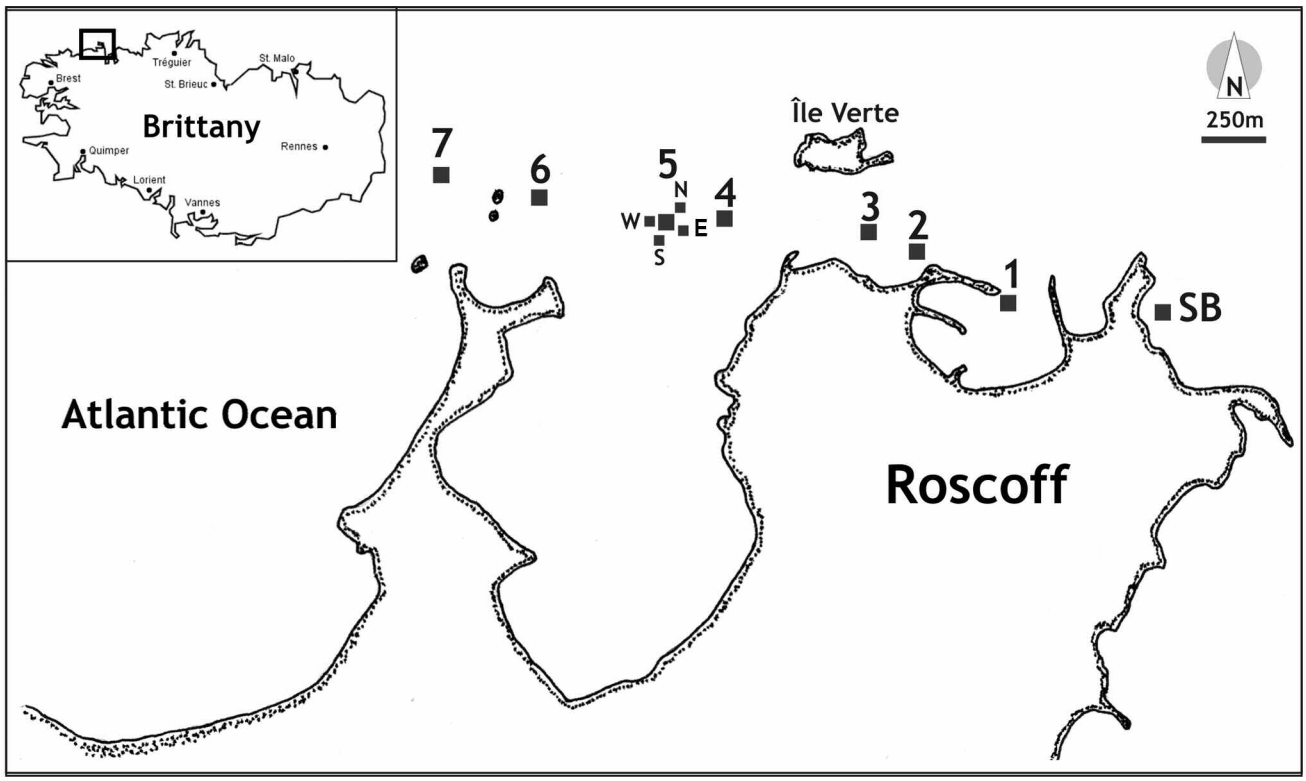

Figure 1. Map of Brittany (inset) and of the investigation area at Roscoff. The transect was drawn from site 1 (harbour) in western direction. At site 5 further sites were sampled (5W-west, 5N-north, 5E-east and 5S-south). SB = Sainte Barbe, additional sampling site in 2006.

Figure 1. Carte de la Bretagne (encart) et du secteur de recherche de Roscoff. La transversale a été dessinée du site 1 (port) vers l'ouest. Au site 5 d'autres sites ont été échantillonnés (5W-ouest, 5N-nord, 5E-est et 5S-sud). SB = Sainte Barbe, site d'échantillonnage additionnel en 2006.

Table 1. Location of the sampling sites (latitude and longitude), distance from harbour and sampling size of each gender for *Nassarius reticulatus* (2004-2006).

Tableau 1. Localisation des sites d'échantillonnage (latitude et longitude), distance du port et taille de l'échantillon de chaque genre de *Nassarius reticulatus* (2004-2006).

sampling site	latitude	longitude	distance from harbour [km]	males			females		
				2004	2005	2006	2004	2005	2006
1	48°43'33.2''	3°58'39.2''	0	12	14	11	20	18	21
2	48°43'39.9''	3°58'56.8''	0.42	15	19	18	15	13	14
3	48°43'41.8''	3°59'17.7''	0.82	18	15	16	14	17	16
4	48°43'46.2''	3°59'33.9''	1.20	16	12	13	14	20	19
5	48°43'43.8''	3°59'45.4''	1.41	13	15	30	17	13	22
5N	48°43'45.5''	3°59'45.1''	1.42	15	11	11	14	21	21
5E	48°43'43.9''	3°59'41.4''	1.33	13	13	8	17	19	24
5S	48°43'42.8''	3°59'46.8''	1.43	11	14	10	19	18	22
5W	48°43'44.5''	3°59'47.7''	1.46	11	5	26	18	27	15
6	48°43'47.5''	4°0'7.9''	1.89	14	15	14	15	17	18
7	48°43'51.0''	4°0'22.0''	2.20	15	12	8	15	20	24
Île Verte	48°43'51.3''	3°59'22''	1.05	17	13	16	15	19	16
Beg an Fry	48°42'4.2''	3°43'1.2''	-	10	8	6	22	24	26
Sainte Barbe	48°43'32.9''	3°58'8.6''	-	-	-	12	-	-	20

about 5 km linear distance further east). In 2006 the site Sainte Barbe (SB, Figure 1) was added to the investigation. Because of the clear age dependence of the morphology, snails with a shell height of approximately 2.5-3.0 cm were collected. It was tried to achieve a sample size of 32 snails at each collecting site, but that was not always possible (Table 1). The distance from the harbour was calculated by determining the exact location of the sampling sites by GPS (Geko™ 101, Garmin Ldt., Romsey, UK).

Imposex measurements

Netted whelks were narcotised in a solution of 7.5% $MgCl_2$ for two hours. Shell and aperture height were measured. With a vice the shell was broken and the soft body removed. The gender of each snail was determined based on the presence of the coiled seminal vesicle in males and an ingestion gland in females (Stroben et al., 1992a). The sexual maturity was defined by the presence of sperm in the seminal vesicle of males and of eggs in the gonadial and renal part of the oviduct of females. The size of the female pallial glands was measured to detect possible xenoestrogenic effects (Oehlmann et al., 2000). The imposex stage in females was determined according to Stroben (1994) and, if existent, the penis size measured. Furthermore, all whelks were investigated for parasites and excrescences of hyperplasic tissue on genital or other organs in the mantle cavity.

TBT measurements

TBT compounds were determined according to Stroben et al. (1992a). The complete tissues of 4-6 animals were homogenized in stoppered tubes and 10 ml concentrated HCl (Merck "suprapur") was added. After shaking for 30 min the homogenates were extracted with 10 ml hexane (pesticide grade) on an automatic shaker for 30 min and then centrifuged. TBT as Sn (TBT-Sn) was measured in the hexane extract using a Perkin-Elmer HGA-500 attached to a Perkin-Elmer 5000 AAS with background correction (wavelength 224.6 nm; slit 0.7 nm; injection volume 25 µl). TBT-Sn was determined in the hexane extract after shaking with 3 ml 1 N NaOH for 3 min. Internal standardization (standard addition with spiked samples) was employed. Recovery factors for TBT were 91.4 ± 8.4%. Additionally, certified reference material (CRM: PACS-1, National Research Council of Canada) for TBT was analysed. The analytical results were within the standard deviation of the certified values of the CRM. The detection limit (3σ) in a single sample was 8.8 ng TBT-Sn. All tissue concentrations are given on a dry weight basis as Sn. Unfiltered water samples of 0.5 or 1.0 L were taken in polycarbonate bottles at a depth of 0.5 m below water surface, acidified with 5 or 10 ml concentrated HCl (Merck "suprapur") and extracted with 5 or 10 ml hexane (pesticide grade) for 30 min. TBT-Sn was analysed as described for tissue analysis above. The detection limit (3σ) was 1.5 ng TBT-Sn.L^{-1}.

Statistical analyses

All statistical analyses were performed with the software program GraphPad Prism© Version 4.03 (GraphPad Software Inc., San Diego, CA, USA) for Windows XP and with STATeasy (Wissenschaftliche Auswertung, Hamburg, Germany). First, data were analysed with Kolmogorov-Smirnov normality test. The vas deferens sequence index (VDSI) was calculated as the mean of all imposex stages of a given sample (Stroben et al., 1992a). A VDSI of zero indicates a population with no imposex and the maximum value for a population - all females exhibiting the highest imposex stage of *N. reticulatus* - is 4.0. Differences between VDSI were calculated using ANOVA for classified values (STATeasy) with Tukey's post hoc test (differences between the years) and Student-Newman-Keuls post hoc test (differences between sampling sites). For males and females penis length differences between samples were analysed with Kruskal Wallis test followed by Dunn's post hoc test. A secondary imposex index, the relative penis length index (RPLI) was calculated by dividing the mean female penis length with the mean male penis length, multiplied with 100. However, this index is affected by seasonal variations of the male penis length which are related to the sexual cycle of the netted whelk so that the VDSI should be preferred (Stroben et al., 1996). The sex ratio was determined as proportion of females in the sample

Table 2. *Nassarius reticulatus*. Vas deferens sequence index (VDSI, with standard deviations in parentheses) over three years (2004-2006) at sampling sites near Roscoff.

Tableau 2. *Nassarius reticulatus*. Indice du Vas Deferens (VDS) (avec déviation standard) sur trois années (2004-2006) sur les sites d'échantillonnage près de Roscoff.

Sampling site (distance from harbour in km)	Vas deferens sequence index (VDSI)		
	2004	2005	2006
1 (-)	4.00 (0.00)	4.00 (0.00)	3.95 (0.22)
2 (0.42)	3.27 (0.44)	3.46 (0.50)	2.00 (1.41)
3 (0.82)	2.43 (0.90)	2.59 (0.91)	1.88 (1.05)
4 (1.20)	1.64 (1.29)	2.10 (1.14)	1.79 (1.15)
5 (1.41)	2.06 (1.00)	1.69 (1.14)	1.32 (1.33)
5N (1.42)	2.00 (1.32)	1.90 (1.11)	1.86 (1.12)
5E (1.33)	1.82 (1.34)	2.11 (1.12)	1.50 (1.22)
5S (1.43)	2.05 (1.23)	1.56 (1.21)	1.50 (1.31)
5W (1.46)	1.89 (1.05)	1.52 (1.37)	1.93 (1.18)
6 (1.89)	1.71 (1.10)	1.47 (1.24)	1.33 (1.11)
7 (2.20)	0.53 (0.72)	1.05 (0.97)	1.25 (0.97)
Île Verte	1.00 (0.97)	1.05 (1.10)	0.75 (0.43)
Beg an Fry	0.04 (0.20)	0.14 (0.34)	0.00 (0.00)
Sainte Barbe	-	-	1.90 (1.14)

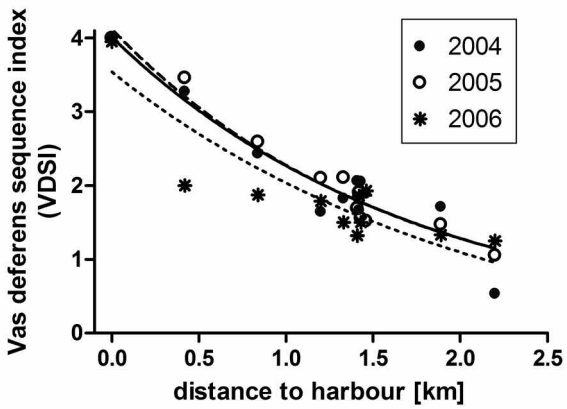

Figure 2. Vas deferens sequence index (VDSI) of *Nassarius reticulatus* collected in 2004 (closed circles), 2005 (open circles) and 2006 (black stars). Pearson correlation is significant with $p < 0.01$ ($r^2 = 0.901$, 2004; $r^2 = 0.945$, 2005 and $r^2 = 0.673$, 2006). Non-linear regression (2004: solid line, 2005: dashed line, 2006: dotted line) is performed with one phase exponential decay ($r^2 = 0.392$, 2004, $r^2 = 0.343$, 2005 and $r^2 = 0.239$, 2006). All sampling sites are significantly different from the harbour ($p < 0.05$).

Figure 2. Indice du Vas deferens (VDSI) de *Nassarius reticulatus* récoltés en 2004 (cercles fermés), en 2005 (cercles ouverts) et 2006 (étoiles noires). La corrélation de Peason est significative avec $p < 0{,}01$ ($r^2 = 0{,}901$, 2004; $r^2 = 0{,}945$, 2005 et $r^2 = 0{,}673$, 2006). La régression non linéaire (2004 : trait plein ; 2005 : tirets ; 2006 : pointillés) est réalisée avec une dégradation exponentielle de phase ($r^2 = 0{,}392$, 2004, $r^2 = 0{,}343$, 2005 and $r^2 = 0{,}239$, 2006). Tous les sites d'échantillonnage sont significativement différents du port ($p < 0{,}05$).

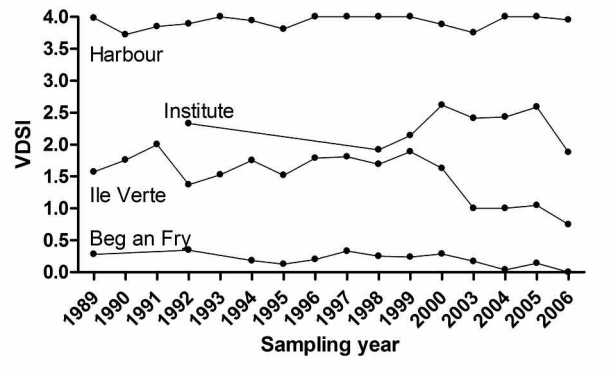

Figure 3: Vas deferens sequence index (VDSI) of *Nassarius reticulatus* collected at four sites in Brittany since 1989. Sampling was performed every year in March.

Figure 3. Indice du Vas deferens (VDSI) de *Nassarius reticulatus* récoltés sur quatre sites en Bretagne depuis 1989. Les échantillons ont été réalisés chaque année en mars.

and differences were tested with Fisher's exact test. Both RPLI and the sex ratio were recorded but will not be discussed in this work. The level of significance was defined with $p < 0.05$. Statistically significant differences are indicated in the tables and figures by asterisks with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Results

The distances between the sampling sites and the harbour are shown in Table 1 along with exact locations and numbers of males and females. The minimal range was 0.42 km (site 2) and the maximal distance was 2.2 km (site 7). At each sampling site *Nassarius reticulatus* was found with a minimum of 28 (site 5 in 2005) and a maximum of 52 animals (site 5 in 2006). The numbers of males and females were mostly evenly distributed, but at a few sites (5W in 2005; 5E, 7 and Beg an Fry in 2006) more females were sampled. The VDSI shows a decrease with distance from harbour (Fig. 2), the correlation being significant ($p < 0.001$ in 2004 and 2005 and $p < 0.01$ in 2006). This observation was confirmed for all sampling years. For all years, the VDSI at the sampling sites was significantly lower than at the harbour ($p < 0.05$). In 2004 and 2005, all females sampled at the harbour had the maximum imposex stage 4.0 (Table 2). In contrast to the general decline during the three sampling years, the VDSI at site 7 increased from 0.53 (2004) to 1.25 (2006). At site 5W values were slightly lower in 2005 than in 2004 but increased again in 2006, while at site 4 values increased from 2004 to 2005 and dropped again in 2006. The VDSI at the reference site Beg an Fry was 0.04 (2004) to 0.14 (2005) and reached a value of 0 in 2006. Furthermore, no significant differences between the values on site 5, 5N, 5E, 5S and 5W were detected for all years. The only significant difference ($p < 0.05$) of the VDSI between the years was found at site 2, the VDSI of 2006 being lower than in the previous years (Table 2). The VDSI of 1.9 at the site Sainte Barbe was at the same level as the VDSI at sites 2, 3, 5N and 5W in 2006.

In addition to the determination of the VDSI, the penis length of females and males was measured (Table 3). This parameter was not normally distributed for some groups and so the median was used for statistical analyses. The female penis length exhibited a relationship with distance from the harbour (Table 3). All sites, with the exception of site 2 in 2004 and 2005, were significantly different from the harbour. This fact was also demonstrated with a significant negative correlation between female penis length and distance from harbour ($p < 0.01$). When comparing the results for each sampling site over the period 2004 to 2006, no differences between the median female penis lengths were detected. Furthermore, no significant differences of the female penis length between sampling site 5 and the subsampling sites were detected.

Table 3: *Nassarius reticulatus*. Median penis length with 25% and 75% percentile of male and female snails sampled in 2004 until 2006 at sampling sites near Roscoff. Asterisks show significant differences from the harbour (Kruskal-Wallis and Dunn's Multiple Comparison Test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Tableau 3. *Nassarius reticulatus*. Médiane des longueurs de pénis avec 25% et 75% de mâles et femelles échantillonnés de 2004 à 2006 sur les sites d'échantillonnage près de Roscoff. Les astérisques montrent les différences significatives du port (Kruskal-Wallis et test de comparaison multiple de Dunn, * $p < 0{,}05$, ** $p < 0{,}01$, *** $p < 0{,}001$).

sampling site (distance from harbour in km)	female penis length [mm] (median with 25% and 75% percentile)			male penis length [mm] (median with 25% and 75% percentile)		
	2004	2005	2006	2004	2005	2006
1 (-)	9.10 (6.00/11.10)	9.60 (7.70/10.40)	6.80 (2.95/7.60)	13.30 (13.00/15.40)	15.90 (13.60/17.50)	16.00 (15.60/17.50)
2 (0.42)	1.00 (1.00/2.00)	1.80 (1.10/2.80)	0.50 (0.00/0.90) **	16.00 (15.00/17.00)	15.00 (14.30/16.00)	14.50 (13.00/15.50)
3 (0.82)	0.65 (0.00/0.90) **	0.60 (0.15/1.05) **	0.35 (0.00/0.50) ***	18.20 (17.10/19.50) ***	16.00 (13.10/17.50)	15.60 (14.50/16.50)
4 (1.20)	1.00 (0.00/1.00) **	0.10 (0.00/0.50) ***	0.50 (0.20/0.50) **	17.00 (15.80/17.00)	13.30 (12.60/14.30)	14.00 (13.50/15.00)
5 (1.41)	1.00 (0.00/1.00) **	0.00 (0.00/0.35) ***	0.00 (0.00/0.45) ***	18.00 (16.50/18.00) *	13.10 (0.80/14.80)	14.20 (13.10/15.10)
5N (1.42)	0.50 (0.00/0.50) ***	0.10 (0.00/0.30) ***	0.30 (0.00/0.75) ***	18.00 (17.00/19.50) **	15.00 (14.50/15.20)	14.00 (12.50/14.50)
5E (1.33)	0.50 (0.00/2.00) ***	0.30 (0.00/0.30) ***	0.00 (0.00/0.20) ***	18.00 (16.50/21.00) **	16.50 (15.40/17.10)	13.10 (11.50/13.20)**
5S (1.43)	0.50 (0.00/1.00) ***	0.10 (0.00/0.60) ***	0.00 (0.00/0.35) ***	17.00 (16.00/18.00)	13.80 (11.50/14.70)	14.20 (12.40/15.00)
5W (1.46)	0.50 (0.25/1.00) **	0.00 (0.00/0.20) ***	0.20 (0.00/0.80) ***	16.00 (16.00/18.00)	15.10 (13.40/16.20)	14.00 (13.00/15.01)
6 (1.89)	1.00 (0.00/1.00) *	0.00 (0.00/0.10) ***	0.00 (0.00/0.20) ***	19.00 (17.80/19.50) ***	14.50 (13.00/15.50)	17.00 (15.00/18.30)
7 (2.20)	0.00 (0.00/0.00) ***	0.10 (0.00/0.15) ***	0.00 (0.00/0.15) ***	18.00 (18.00/20.00) ***	14.00 (13.20/14.30)	14.90 (13.80/15.80)
Île Verte	0.00 (0.00/0.30)	0.00 (0.00/0.00)	0.00 (0.00/0.00)	14.90 (14.40/15.90)	16.40 (15.40/17.00)	16.00 (14.70/16.70)
Beg an Fry	0.00 (0.00/0.00)	0.00 (0.00/0.00)	0.00 (0.00/0.00)	16.80 (15.40/18.00)	16.70 (14.10/18.30)	15.80 (8.75/16.90)
Sainte Barbe	—	—	0.50 (0.0/0.80)	—	—	15.90 (14.80/16.70)

In 2004, the median size of the male penis length was significantly different from the harbour at site 3, 5, 5N, 5E, 6 and 7, the median penis length increasing with the distance. These results were not confirmed in 2005 and 2006. In 2005, no significant differences to the harbour were found, and in 2006 only site 5E was significantly different from the harbour (Table 3). At sites 4 ($p < 0.05$), 5 ($p < 0.01$), 6 ($p < 0.01$) and 7 ($p < 0.01$) significant differences in the male penis length were detected comparing the results of 2004 and 2005 (significance level not shown). Significant differences at sites 5 ($p < 0.01$), 5E ($p < 0.01$) and 5N ($p < 0.01$) were found comparing the years 2004 and 2006 (significance level not shown). In 2005, the median penis length of males was generally less than in 2004. The penis length at site 5 and the subsampling sites for all three years did not differ significantly from each other.

Figure 3 shows the development of the VDSI since the first sampling in 1989 for 4 selected sites. The results show a relatively constant high level at the harbour sampling site (3.92 ± 0.024) and a relatively constant low level at the first reference site Beg an Fry (0.20 ± 0.029). Until 2005, the sampling sites Île Verte (1.51 ± 0.092) and Beg an Fry showed a decrease of the VDSI since the late 1990s. In contrast, VDSI values increased at Roscoff Institute (site 3) from 2.29 ± 0.100 up to a VDSI of 2.59 in 2005, followed again by a decrease to 1.88 in 2006. In the same year VDSI values also decreased at all other sampling sites.

During the entire investigation period TBT concentrations in the water varied from 1.5 ng as $Sn.L^{-1}$ (Beg an Fry)

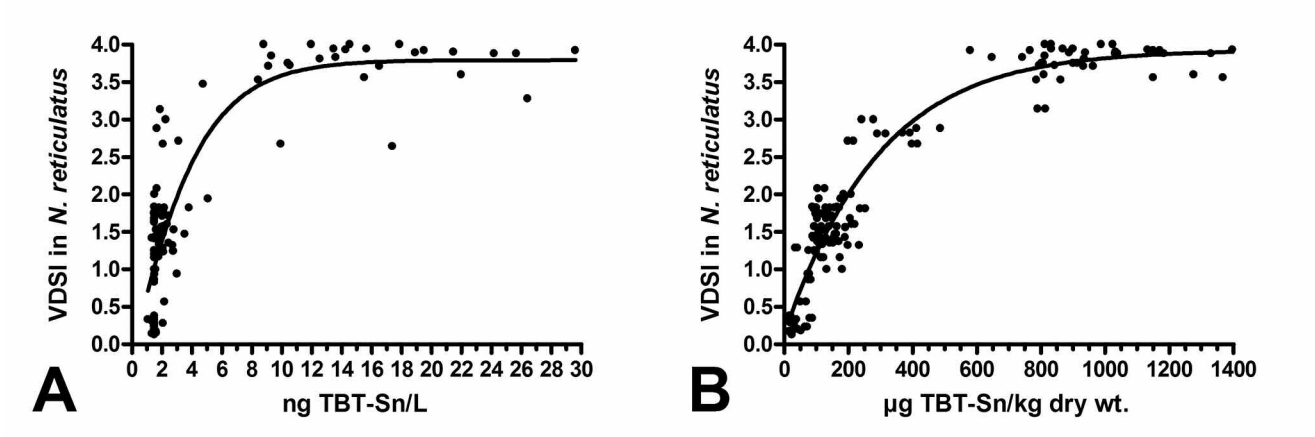

Figure 4. Vas deferens sequence index (VDSI) of *Nassarius reticulatus* correlated with TBT-Sn concentrations in water (A) and body tissue (B). Non-linear regression is performed (A: $r^2 = 0.758$, n = 107, $p < 0.0001$; B: $r^2 = 0.920$, n = 158, $p < 0.0001$).

Figure 4. Indice du Vas deferens (VDSI) de *Nassarius reticulatus* corrélé avec les concentrations de TBT-Sn dans l'eau (A) et dans les tissus (B). Une régression non linéaire est réalisée (A: $r^2 = 0{,}758$, n = 107, $p < 0{,}0001$; B: $r^2 = 0{,}920$, n = 158, $p < 0{,}0001$).

to 18.9 ng as $Sn.L^{-1}$ (harbour; data not shown). The body burden of female *N. reticulatus* ranged from 24.6 ng $Sn.g^{-1}$ dry wt. (Beg an Fry) to 1024 ng $Sn.g^{-1}$ dry wt. (harbour; data not shown). Comparing the pooled data of the VDSI in *N. reticulatus* with the amount of TBT-Sn in water and snail tissue results in a highly positive correlation ($r^2 = 0.758$ and 0.920, respectively; Fig. 4). Based on the significant concentration response relationship in figure 4A, the VDSI values at the four stations provided in figure 3 and assuming that no other androgenic compounds contribute to the observed imposex levels in *N. reticulatus* (cf. Discussion), even today aqueous TBT concentrations beyond 12 ng as $Sn.L^{-1}$ have to be expected for Roscoff Harbour. At Roscoff Institute, TBT concentrations dropped slightly from 2.5-5 ng as $Sn.L^{-1}$ in the early 1990s to 1.8-4 ng as $Sn.L^{-1}$ in 2006. In contrast, at the two other stations the improvement was more pronounced with a decline from 1.2-2.6 in the 1990s to 0.5-1.0 ng TBT-$Sn.L^{-1}$ today for the Île Verte site and from < 0.8 in the 1990s to < 0.1 ng TBT-$Sn.L^{-1}$ today for Beg an Fry.

Discussion

In the study area, sites 2-7 receive organotin contamination mainly with the water from the harbour when the tide goes out. Consequently, the transect was oriented westwards. The sampling site Île Verte was used as a local reference although it is still slightly affected by low tide current from the harbour. Beg an Fry served as a remote reference station, representing the background contamination and effect level for northern Brittany.

Nassarius reticulatus shows a maximum imposex development with a VDSI of 4.00 (Stroben et al., 1992a). Compared to other marine snails (*Hydrobia ulvae* (Pennant, 1777)*, Littorina littorea* (L., 1758)*, Nassarius incrassatus* (Ströhm 1768)) the netted whelk is more sensitive to TBT pollution (Mensink et al., 1996; Oehlmann et al., 1998; Barroso et al., 2000). *N. reticulatus* is less sensitive when compared with the dog whelk *Nucella lapillus* (L., 1758) and *Ocenebra erinacea* (L., 1758) at lower concentrations (Huet et al., 1995) and also less sensitive to *Ocinebrina aciculata* (Lamarck, 1822)*, Trivia arctica* (Pulteney, 1799) and *T. monacha* (da Costa, 1778) (Oehlmann et al., 1996). However, *N. reticulatus* showed the best discrimination of sites with different and higher TBT pollution (Huet et al., 1995; Barroso et al., 2000).

Not much is known about the habitat fidelity of adult *Nassarius reticulatus*, but according to Stroben (1994) their mobility as adults is rather limited. Coastal hydrography and structure of the area also limits the movement of the snails, especially at site 5. The distance between the sites of the transect should enable the appraisal of site-dependent answers to TBT contamination, although with some restrictions for site 5 and its sub-sites (5N, 5E, 5S, 5W). The narrow sampling scheme at site 5 with a distance of 30-125 m between the sub-sites was introduced in 2004 when first results indicated a VDSI increase for station 5 in the transect. The sub-sites were sampled to address the assumption of a possible sedimentation area for suspended particulate matter contaminated with organotin compounds around site 5. However, in 2005 and 2006 the observation of increased VDSI values at site 5 and its sub-sites when compared with site 4 was not approved.

Comparing all sites on imposex variation we found that the VDSI did not differ significantly between the years,

except for site 2. Depending on the site, the VDSI either increased (7) or decreased (1, 5 and 6). At sites 2-4 and at Île Verte, there is an increase from 2004 to 2005, but a decrease in 2006. The VDSI at the site Sainte Barbe is probably due to the fact that the ferry to England and Ireland passes north of this bay. An influence of the harbour is not very likely.

The significantly increasing median penis length of the males at sites 3, 5, 5N, 5E, 6 and 7 in 2004 can be regarded as sampling site dependant as no general correlation between the median penis length and the distance from harbour was detected and this finding was not confirmed in the following years.

The water concentrations monitored at Roscoff since 1989 (1.5-18.9 ng $Sn.L^{-1}$) are lower than those found by Barroso et al. (2000) along the Portuguese coast in 1998 (9-42 ng $Sn.L^{-1}$), but female TBT body burdens are in the same range as found by Barroso et al. (2002a), Sousa et al. (2005) and Barreiro et al. (2001). Sousa et al. (2005) and Barroso et al. (2002a) also described a highly significant relationship between female TBT residues and TBT concentrations in water and sediment, respectively. However, according to Stroben et al. (1992b), the food may contribute to more than half of the TBT body burden.

The half-life of TBT can vary from a few days to a few weeks in water (Jacobsen, 2000), but accumulated in sediment under anaerobic conditions the half-life may be even beyond decades (IMO, 2002). Harbours, ports and estuaries with heavily sedimented bottoms can be contaminated with TBT for several years (De Mora et al., 1995). Therefore, our observations probably illustrate still high TBT concentrations in the sediments and a remobilisation of TBT from sediments due to turbulences during tide turning. It may also be an indication that the restrictions on the use of antifouling paints containing TBT were not yet fully effective. However, a document of the International Maritime Organisation (2002) mentioned that since the regulation of organotin, TBT concentrations in the water on the Atlantic coast decreased, the sediment core suggested improvement and that organotin concentrations and shell malformations in Pacific oysters (*Crassostrea gigas*) decreased. Extending the restrictions to vessels larger than 25 m and shipyards (probably the main sources of organotin contamination today) according to the International Convention on the Control of Harmful Antifouling Systems on Ships (IMO, 2002) may contribute to a further reduction of TBT levels in the marine environment.

Although it is accepted that imposex is induced almost exclusively by TBT under field conditions (DeFur et al., 1999), the fungicide triphenyltin (TPT) has also proven to induce imposex in at least two species, the marine rock shell *Thais clavigera* (Küster, 1858) (Horiguchi et al., 1997) and *Marisa cornuarietis* (L., 1758) (Schulte-Oehlmann et al., 2000). The findings for *Marisa* were recently confirmed by Albanis et al. (2006): EC_{10} values for imposex induction and reduced egg production by TPT were 15.9 and 0.625 ng TPT-$Sn.L^{-1}$, respectively. While Schulte-Oehlmann et al. (2000) did not find any evidence for imposex induction in *N. lapillus* and *N. reticulatus* under TPT exposure, Barroso et al. (2002b) report an increase of imposex incidences in female *N. reticulatus* by 24-44% when exposed to nominal concentrations between 100 and 500 ng TPT-$Sn.L^{-1}$. More recently it was shown that a number of further androgenic compounds like methyltestosterone, letrozole® and fenarimol are able to induce imposex at $ng.L^{-1}$ concentrations (Albanis et al., 2006). The evidence for other man-made chemicals such as PCBs and PAHs to induce imposex is rather weak and largely based on correlations between virilisation levels observed in the field and residues in water and/or snails (e.g. Maran et al., 2006). However, the temporal concurrence of the introduction of TBT-based antifouling paints with the first occurrence of imposex, the good correlation between imposex levels and ambient TBT concentrations and especially the recovery from imposex after the (partial) ban of TBT-based paints strengthen the assumption that organotin compounds have been the dominating cause for observed imposex levels in the field during the last two decades.

Imposex is considered as an irreversible phenomenon in many prosobranch species and therefore a reliable tool for biomonitoring TBT pollution, although there would be only a slow decline of imposex in the population. Nowadays, there are hints that the females can recover from the TBT stress, at least when regarding the female penis length (Sousa et al., 2005). A study of Tester et al. (1996) also suggests a recovery from TBT pollution, recommending the female penis length, although less precise than the VDSI, as monitoring tool for the recovery because of its sensitivity to declining TBT levels.

Conclusion

Our study has shown that organotin pollution remains a cause for concern for coastal waters in Northern Brittany, especially at harbour locations. However, it seems as if the ban on TBT is showing its first results, as the VDSI at most sites decreased in 2006. Monitoring imposex in the *Nassarius reticulatus* population of the coastal area of Roscoff should be continued to see if the general ban on TBT has a positive effect on the recovery and development of non-target organisms.

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References

Albanis T., Allera A., Bachmann J., Barbaglio A., Berntsson P., Dittmann N., Carnevali D.C., Ciceri F., Dagnac T., Duft M., Falandysz J., Galassi S., Hala D., Janer G., Jeannot R., Jobling S., King I., Klingmüller D., Kloas W., Kusk K.O., Lavado R., Lo S., Lutz I., Oehlmann J., Oredsson S., Porte C., Rand-Weaver M., Sakkas V., Schmitt C., Schulte-Oehlmann U., Sugni M., Tyler X., van Aerle R., van Ballegoy C. & Wollenberger L. 2006. Executive summary on the project results of COMPRENDO – Comparative Research on Endocrine Disrupters (EU project no. EVK1-CT-2002-00129). Available online under http://www.comprendo-project.org

Barreiro R., González R., Quintela M. & Ruiz J.M. 2001. Imposex, organotin bioaccumulation and sterility of female *Nassarius reticulatus* in polluted areas of NW Spain. *Marine Ecology Progress Series*, **218**: 203-212.

Barroso C.M., Moreira M.H. & Gibbs P.E. 2000. Comparison of imposex and intersex development in four prosobranch species for TBT monitoring of a southern European estuarine system (Ria de Aveiro, NW Portugal). *Marine Ecology Progress Series*, **201**: 221-232.

Barroso C.M., Moreira M.H. & Bebianno M.J. 2002a. Imposex, female sterility and organotin contamination of the prosobranch *Nassarius reticulatus* from the Portuguese coast. *Marine Ecology Progress Series*, **230**: 127-135.

Barroso C.M., Reis-Henriques M.A., Ferreira M.S. & Moreira M.H. 2002b. The effectiveness of some compounds derived from antifouling paints in promoting imposex in *Nassarius reticulatus*. *Journal of the Marine Biological Association of the United Kingdom* **82**: 249-255.

Birchenough A.C., Evans S.M., Moss C. & Welch R. 2002. Re-colonisation and recovery of populations of dogwhelks *Nucella lapillus* (L.) on shores formerly subject to severe TBT contamination. *Marine Pollution Bulletin,* **44**: 652-659.

Blaber S.J.M. 1970. The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* (L.). *Proceedings of the Malacological Society of London,* **39**: 231-233.

DeFur P.L., Crane M., Ingersoll C. & Tattersfield L. (eds.) 1999. Endocrine Disruption in Invertebrates: Endocrinology, Testing, and Assessment. Proceedings of the Workshops on Endocrine Disruption in Invertebrates, 12-15 December 1998, Noordwijkerhout, The Netherlands. Pensacola, SETAC Press.

De Mora S.J., Stewart C. & Phillips D. 1995. Sources and rate of degradation of tri(n-butyl)tin in marine sediments near Auckland, New Zealand. *Marine Pollution Bulletin.,* **30**: 50-57.

Følsvik N., Berge J.A., Brevik E.M. & Walday M. 1999. Quantification of organotin compounds and determination of imposex in populations of dogwhelks (*Nucella lapillus*) from Norway. *Chemosphere,* **38**: 681-691.

Fretter V. & Graham A. 1985. The prosobranch molluscs of Britain and Denmark. Part 8 — Neogastropoda. *Journal of Molluscan Studies, Supplement,* **15**: 435-556.

Horiguchi T., Shiraishi H., Shimizu M. & Morita M. 1997. Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*. *Environmental Pollution,* **95**: 85-91.

Huet M., Fioroni P., Oehlmann J. & Stroben E. 1995. Comparison of imposex response in three prosobranch species. *Hydrobiologia,* **309**: 29-35.

Huet M., Paulet Y.M., Clavier J. 2004. Imposex in *Nucella lapillus*: A ten year survey in NW Brittany. *Marine Ecology Progress Series,* **270**: 153-161.

IMO (International Maritime Organisation) 2002. Anti-fouling systems. Focus on IMO, 31 pp, www.imo.org.

Jacobsen J.A. 2000. Organotin compounds in the Danish marine environment: Analysis and fate studies. *PhD thesis*, University of Roskilde, Denmark, 118 pp.

Jenner M.G. 1979. Pseudohermaphroditism in *Illyanassa obsoleta* (Mollusca: Neogastropoda). *Science*, **205**: 1407-1409.

Jorundsdottir K., Svavarsson K. & Leung K.M.Y. 2005. Imposex levels in the dogwhelk *Nucella lapillus* (L.) - continuing improvement at high latitudes. *Marine Pollution Bulletin,* **51**: 744-749.

Maran C., Centanni E., Pellizzato F. & Pavoni B. 2006. Organochlorine compounds (polychlorinated biphenyls and pesticides) and polycyclic aromatic hydrocarbons in populations of *Hexaplex trunculus* affected by imposex in the Lagoon of Venice, Italy. *Environmental Toxicology and Chemistry,* **25**: 486-495.

Mensink B.P., van Hattum B., Vethaak A.D. & Boon J.P. 1996. The development of imposex in relation to organotin contamination in the common whelk *Buccinum undatum*. *NIOZ. RAP,* **1996-3**: 38 pp.

Michel P. & Averty B. 1999. Contamination of French coastal waters by organotin compounds: 1997 update. *Marine Pollution Bulletin,* **38**: 268-275.

Oehlmann J. 2004. Biological effects of contaminants: Use of intersex in the periwinkle (*Littorina littorea*) as a biomarker of tributyltin pollution. *ICES Techniques in Marine Environmental Sciences*, **37**: 30 pp.

Oehlmann J., Stroben E., Schulte-Oehlmann U., Bauer B., Fioroni P. & Markert B. 1996. Tributyltin biomonitoring using prosobranchs as sentinel organisms. *Fresenius Journal of Analytical Chemistry*, **354**: 540-545

Oehlmann, J., Stroben E., Schulte-Oehlmann U. & Bauer B. 1998. Imposex development in response to TBT pollution in *Hinia incrassata* (Ströhm, 1768) (Prosobranchia, Stenoglossa). *Aquatic Toxicology*, **43**: 239-260

Oehlmann J., Schulte-Oehlmann U., Tillmann M. & Markert B. 2000. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol A and octylphenol as xeno-estrogens. *Ecotoxicology,* **9**: 383-397.

Oetken M., Bachmann J., Schulte-Oehlmann U. & Oehlmann J. 2004. Evidence for endocrine disruption in invertebrates. *International Review of Cytology,* **236**: 1-44.

Rexrode M. 1987. Ecotoxicity of tributyltin. *Proceedings Oceans*, **87**: 1443-1455.

Schulte-Oehlmann U., Tillmann M., Markert B., Oehlmann J., Watermann B. & Scherf S. 2000. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part II: Triphenyltin as a xeno-androgen. *Ecotoxicology,* **9**: 399-412.

Schulte-Oehlmann U., Duft M., Tillmann M., Markert B. &

Oehlmann J. 2001. *Biologisches Effektmonitoring an Sedimenten der Elbe mit* Potamopyrgus antipodarum *und* Hinia (Nassarius) reticulata *(Gastropoda: Prosobranchia).* Forschungsbericht für die Arbeitsgemeinschaft für die Reinhaltung der Elbe (ARGE), Hamburg, 46 pp.

Smith B.S. 1971. Sexuality in the American mudsnail, *Nassarius obsoletus* Say. *Proceedings of the Malacological Society of London,* **39**: 377-378.

Sousa A., Mendo S. & Barroso C. 2005. Imposex and organotin contamination in *Nassarius reticulatus* (L.) along the Portugese coast. *Applied Organometallic Chemistry*, **19**: 315-323.

Strand J. & Asmund G. 2003. Tributyltin accumulation and effects in marine molluscs from West Greenland. *Environmental Pollution,* **123**: 31-37.

Stroben E. 1994. *Imposex und weitere Effekte von chronischer TBT-Intoxikation bei einigen Mesogastropoden und Bucciniden (Gastropoda, Prosobranchia).* Cuvillier Verlag: Göttingen. 193 pp.

Stroben E., Oehlmann J. & Fioroni P. 1992a. The morphological expression of imposex in *Hinia reticulata* (Gastropoda: Buccinidae): A potential biological indicator of tributyltin pollution. *Marine Biology,* **113**: 625-636.

Stroben E., Oehlmann J. & Fioroni P. 1992b. *Hinia reticulata* and *Nucella lapillus*. Comparison of two gastropod tributyltin bioindicators. *Marine Biology,* **114**: 289-296.

Stroben E., Oehlmann J., Schulte-Oehlmann U. & Fioroni P. 1996. Seasonal variations in the genital ducts of normal and imposex-affected prosobranchs and its influence on biomonitoring indices. *Malacological Review,* **Supp. 6**: 173-184.

Tester M., Ellis D.V. & Thompson J.A. 1996. Neogastropod imposex for monitoring recovery from marine TBT contamination. *Environmental Toxicology and Chemistry*, **15**: 560-567.