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The rough tingle *Ocenebra erinacea* (Neogastropoda: Muricidae): an exhibitor of imposex in comparison to *Nucella lapillus*

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ABSTRACT: The muricid gastropod *Ocenebra erinacea* exhibits imposex (occurrence of male parts in addition to the female genital duct), a phenomenon which is caused by tributyltin (TBT)-compounds leached from ships' antifouling paints. Five stages of imposex development (1–5) with two different types at stage 1 can be distinguished and are documented with SEM-photographs for the first time. Four additional alterations of the genital tract are shown. Close to harbours and marinas, *O. erinacea* females exhibit malformations of the pallial oviduct, which seem to inhibit copulation and capsule formation resulting in sterilization. The TBT accumulation in the whole body and the accumulation pattern in single tissues are described; contrary to other prosobranchs no sex-related differences were found. The VDS, uncubed RPS and average female penis length of a population were analysed regarding their quality as parameters for TBT biomonitoring. On the background of the ecology of this species the VDS is chosen as the best index. Only in highly polluted areas should the uncubed RPS be used as a secondary parameter. A statistical study, based on the analysis of natural populations of *Nucella lapillus* and *O. erinacea*, allows a comparison of the specific TBT sensitivity of the two bioindicators. Dogwhelks exhibit a greater TBT sensitivity, namely at slightly polluted sites, nevertheless even here rough tingles develop obvious imposex characteristics.

INTRODUCTION

The European rough tingle *Ocenebra erinacea* is a gonochoristic species, but as in many other prosobranch gastropods during the past two decades females have been found to exhibit male sex characters, generally a penis and/or a vas deferens (for review Fioroni et al., 1991). This phenomenon was termed imposex (Smith, 1971) or pseudohermaphroditism (Jenner, 1979), and is induced under the influence of TBT (tributyltin) compounds used as biocides in antifouling paints at the ppt (parts per trillion: ng/l)-level.

Since 1970, *Nucella lapillus* (Blaber, 1970; Gibbs et al., 1987; Oehlmann et al., 1991), *Ilyanassa obsoleta* (Smith, 1971, 1980, 1981a, b, c, d; Bryan et al., 1989a), *Thais emarginata* (Houston, 1971), *Hinia (Nassarius) reticulata* (Féral, 1974a) and *O. erinacea* (Féral, 1974b, 1976a, b, c, 1980; Gibbs et al., 1990) were the first discovered penis-bearing neogastropods. The information about imposex development and expression in the rough tingle is rather incomplete. The studies of Féral (1974b, 1976a, b, c, 1980) give only data about the portion of penis-bearing females in the populations. Gibbs et al. (1990) reports on TBT-induced sterilization in *O. erinacea*, but unfortunately does not give an exact staging of the imposex expression.

The distribution of *O. erinacea* extends northwards from the Mediterranean, Madeira and the Azores to the south and west coasts of Britain and Ireland. The rough tingle can also be found in the southern but not in the northern part of the North Sea. The species is predominantly sublittoral with a breeding immigration to the intertidal zone during spring (Fretter & Graham, 1985), but in France part of the populations remains intertidal throughout the year. *O. erinacea* is a predator with a wide range of prey species such as *Cardium, Venus,* barnacles, *Crepidula* and tubicolous worms. Oysters and mussels (*Mytilus edulis*) are also taken but do not appear to be preferred even when common in a habitat. As only incomplete information about imposex development in *O. erinacea* is available (Féral, 1974b ff.; Fioroni et al., 1990f; Gibbs et al., 1990), the objectives of this study are to set up an imposex classification system based on this species, to introduce the rough tingle as an indicator of TBT pollution and to find a suitable index for TBT biomonitoring. Additionally, a comparison of the TBT sensitivity of *O. erinacea* and *N. lapillus*, based on field analyses, is made.

MATERIAL AND METHODS

The external topography of 1657 specimens of *Ocenebra erinacea*, collected at 19 stations on the French coast between the Île d'Yeu and Coutainville up to November 1991, was examined. Complete series of histological sections exist for 25 specimens. For each sample on a site, 30 (if possible) or more adult rough tingles were collected and narcotized using 7 % $MgCl_2$ in distilled water. The shell and aperture height and, after cracking the shell with a vice, the external properties of the genital tract including vas deferens extension and penis length were measured to the nearest 0.1 mm. For serial sections and for scanning electron microscopy, the specimens were fixed in Bouin's fluid and then preserved in 70 % ethanol. After embedding in paraplast, serial sections (7–10 µm) were made and stained with azan after Heidenhain, haemalun-chromotrope, trichrome after Goldner, alcian blue and the PAS-reaction. Specimens for SEM were dehydrated via graded ethanol series, critical point dried, coated with gold and examined with a Hitachi scanning electron microscope S-530.

For all populations, the following indices for TBT biomonitoring were calculated:

(1) VDS (vas deferens sequence) index is calculated as the average imposex stage (according to our general scheme of imposex development in prosobranchs: Fig. 1) of a population;

(2) uncubed RPS (relative penis size) index is defined as (median length of female penis/median length of male penis) $\times 100$ (Stroben et al., 1992a)

(3) average female penis length of a population.

Our general system of imposex classification (stage 1–6) (Fig. 1; Fioroni et al., 1991; Oehlmann et al., 1991; Stroben et al., 1992a) is valid for the description of imposex in all known imposex-affected prosobranch species, including *O. erinacea*. The cubed RPS (Gibbs et al., 1987) is rejected, because this index proved to be unreliable (Oehlmann et al., 1991; Stroben et al., 1992a; see below).

For organotin analyses the whole bodies of 2–5 animals or single tissues of up to 20 animals were pooled and analysed according to Stroben et al. (1992b). Recovery factors

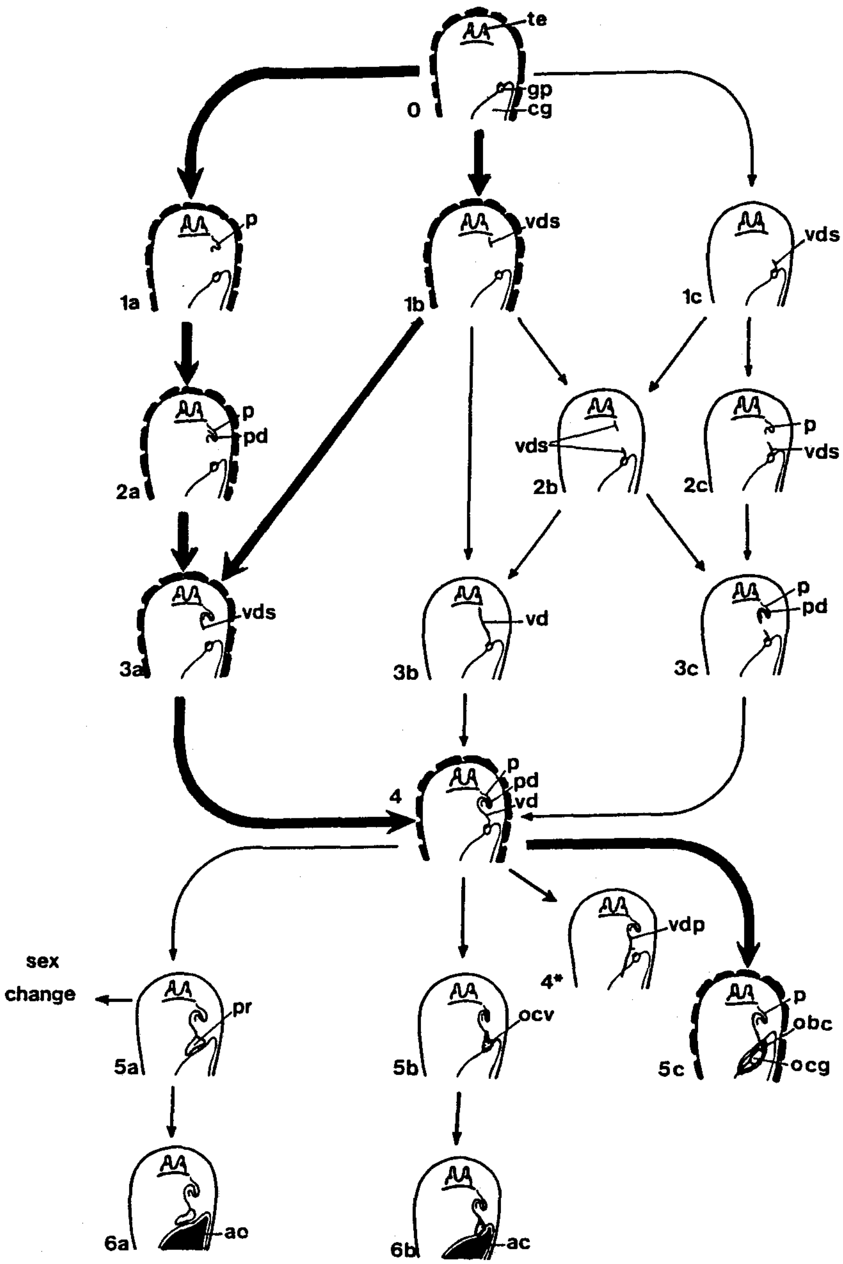

Fig. 1. General scheme of imposex evolution in prosobranchs. Imposex stages of *Ocenebra erinacea* in bold. Abbreviations: ac, aborted capsules; cg, capsule gland; gp, genital papilla; obc, open bursa copulatrix; ocg, open capsule gland; ocv, occlusion of the vulva; p, penis; pd, penis duct; pr, prostate; te, tentacle; vd, vas deferens; vdp, vas deferens passage into capsule gland; vds, vas deferens section

for DBT were 92.3 ± 9.4 % and 91.4 ± 8.4 % for TBT. The detection limit (3σ) in a single sample was 7.4 and 8.8 ng DBT-Sn and TBT-Sn respectively. All DBT and TBT tissue concentrations are given on a dry weight basis.

RESULTS

Normal genital system and imposex classification

The anatomy and histology of the normal female (= stage 0; ♀ without any male characteristics; Figs 2c, d) and of the male genital tract (Figs 2a, b) are described by Fretter (1941) and Fretter & Graham (1962). Some information is also given by Fioroni et al. (1990) and Gibbs et al. (1990). The various frequencies of males, pure females and of the imposex stages and types are represented in Table 1.

The imposex phenomenon affects the ⚥ (= pseudandric ♀; Jenner, 1979) with additional male parts. *Ocenebra erinacea* demonstrates two different types (a, b) of imposex expression in the stage 1, and only one type in the stages 2–5 (Fig. 1).

Stage 1: Type a: Tiny penis without a penis duct, behind the right ocular tentacle (Figs 2e, f). Type b: No penis but a short, distal vas deferens tract behind the right ocular tentacle (Fig. 3a).

Stage 2 (only type a): Penis with a closing or closed penis duct behind the right ocular tentacle (Fig. 3b).

Stage 3 (only type a): Penis with penis duct continuing in an incomplete distal tract of the vas deferens that grows out successively against the vaginal opening (Figs 3c, d).

Stage 4: Penis with a penis duct and a continuous vas deferens from the penis up to the vulva (Figs 3e, f). Within this stage, the least-affected females have a vaginal opening (vulva) sited terminally on a prominent genital papilla (Fig. 3e) and a large bursa copulatrix which extends for up to one-third of the length of the capsule gland. In more affected individuals, the bursa is reduced in size, also the genital papilla appears smaller than normal and is finally lacking completely (Fig. 3f). This represents the last fertile stage of imposex. Fertility is evident from a functional ovary and the existence of all functioning glands in the pallial oviduct assisted by a normal ventral pedal gland. Even if the genital papilla is modified, the copulation capability is conserved. This is demonstrated by sperm filled bursae copulatrices before or during the spawning season. The penis length is extremely variable at this stage (Table 1).

Stage 5 (only type c): As in stage 4, a penis (with a duct) and a continuous vas deferens can be found, but the ontogenetic closure of the pallial oviduct is incomplete. Either the size reduced bursa copulatrix alone (stage 5c [B]; Figs 4a, b) or additionally the capsule gland (stage 5c [B/C]; Figs 4c, d) are split ventrally exposing the internal lobes of the capsule gland for up to two-thirds of its length. These malformations of the pallial oviduct affect its function. The protrusion of the bursa copulatrix inhibits copulation because sperms might be spilled into the mantle cavity and the ventrally open capsule gland prevents the formation of intact capsules. This is evident from the occurrence of irregularly-shaped horny capsular material that can be found during and after the spawning season within the capsule gland.

Table 1. *Ocenebra erinacea*. Biometrical data of males, females and the different imposex stages. *All* data are based on narcotised animals. For definition of imposex stages, compare text. s.d.: standard deviation; vd: vas deferens

Sex or imposex stage	Number of specimens	% of total	% of females	Shell *height* (mm ± s.d.)	Aperture *height* (mm ± s.d.)	Penis length (mm ± s.d.)	Albumen gland (mm ± s.d.)	Ingestion gland (mm ± s.d.)	Capsule gland (mm ± s.d.)	% with ex-crescenses on penis and/or vd	% para-sited	% sexually mature
♂	744	44.9		26.18 3.20	16.51 2.36	5.44 1.55	– –	– –	– –	0.54	1.21	88.44
0	32		3.50	28.92 3.13	17.07 3.47	0 0	3.43 1.03	3.30 1.14	11.43 2.92	0	0	84.38
1a	42		4.60	29.49 4.06	18.32 3.52	0.24 0.09	3.31 0.69	2.89 0.84	10.38 1.82	0	0	78.75
1b	10		1.10	29.70 3.18	18.48 2.08	0 0	3.32 1.01	3.00 1.23	10.93 2.47	0	10.0	90.00
2a	17		1.86	28.23 4.35	17.48 4.04	0.63 0.25	2.81 0.87	2.58 0.97	8.92 2.05	0	0	64.71
3a	336		36.80	28.10 3.44	17.58 2.89	0.94 0.29	3.01 0.93	2.83 1.09	9.32 2.66	0.30	0	89.88
4	458		50.16	28.34 3.34	17.75 2.59	2.10 0.81	3.13 0.84	2.96 0.98	9.49 2.31	0.44	1.75	92.79
5c (B)	12		1.32	28.30 2.80	17.56 1.75	2.92 0.74	2.84 1.04	2.80 1.30	8.30 2.79	0	8.33	83.33
5c (B/C)	6		0.66	31.70 3.45	19.13 2.13	4.35 1.19	2.73 0.73	2.25 1.10	7.70 1.65	16.67	0	83.33
♀	913	55.1	100.0	28.36 3.44	17.70 2.81	1.49 0.97	3.09 0.89	2.91 1.03	9.50 2.49	0.44	1.20	90.03

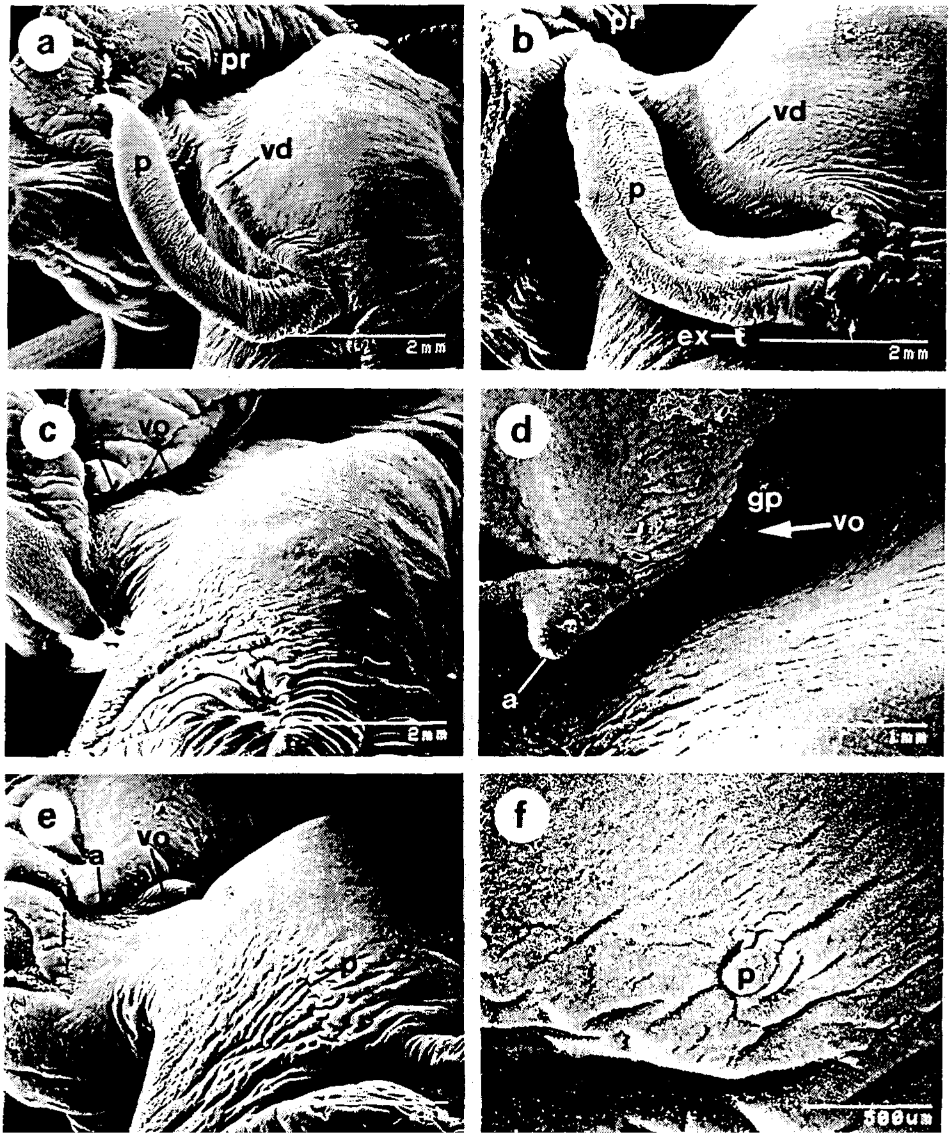

Fig. 2. *Ocenebra erinacea*. SEM photographs of male, normal female and imposex stage 1a. a: Male. b: Male with excrescences on the penis. c: Stage 0 (normal female). d: Detail of c (stage 0): genital papilla without adjacent male parts. e: Stage 1a. f: Detail of e (stage 1a): penis without penis duct. Abbreviations (see Fig. 1): a, anus; ex, excrescences; vo, vaginal opening (vulva)

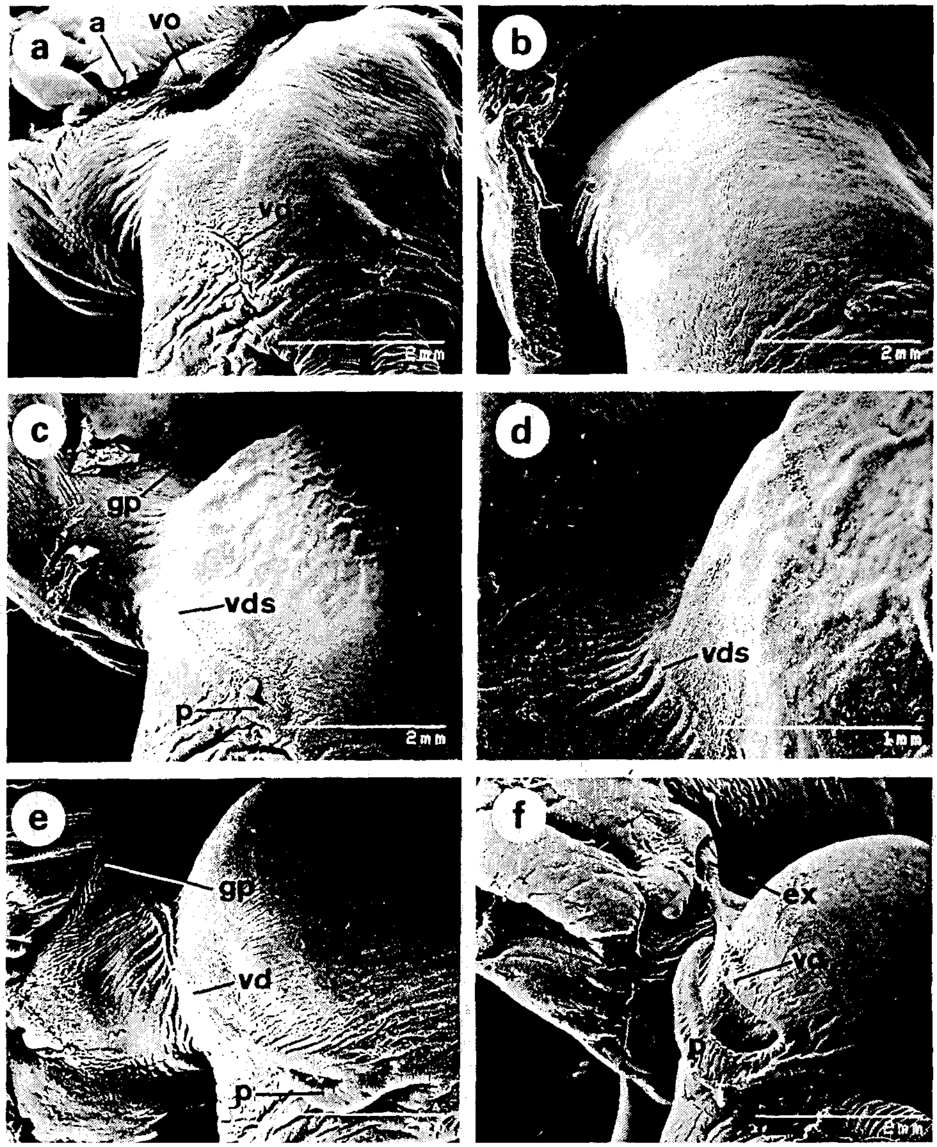

Fig. 3. *Ocenebra erinacea.* SEM-photographs of stages 1b–4. a: Stage 1b. b: Stage 2a. c: Stage 3a. d: Detail of c (stage 3a): vas deferens does not reach the vaginal opening. e: Stage 4 with well developed genital papilla. f: Stage 4 with reduced genital papilla and excrescences on the vas deferens in front of the vulva. Abbreviations (see Figs 1, 2)

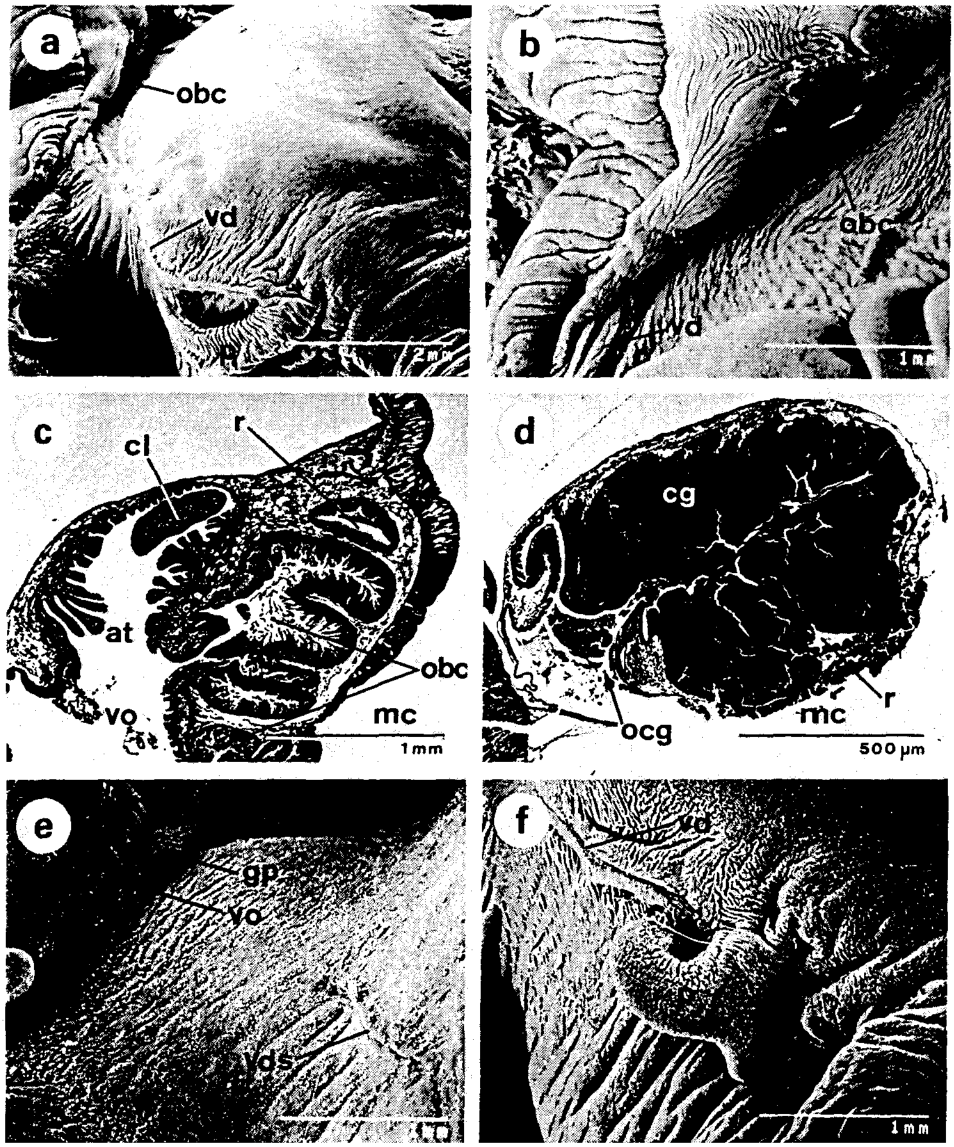

Fig. 4. *Ocenebra erinacea.* SEM photographs of stage 5c and of additional alterations: a: Stage 5c (B) with an open bursa copulatrix. b: Detail of a (stage 5c): vas deferens and internal folds of the open bursa copulatrix. c: Stage 5c (B/C): histological transverse section through the distal pallial oviduct with open atrium and bursa copulatrix. d: Stage 5c (B/C): histological transverse section through the open capsule gland. e: Stage 1a with an isolated vas deferens section between penis and vaginal opening. f: Stage 4 with a bifurcate penis. Abbreviations (see Figs 1–3): at, atrium, muscular vestibulum; cl, cephalic lobe of capsule gland; mc, mantle cavity; r, rectum

Some further morphological alterations are possible:

(1) In earlier stages (1a, 1b, 2a, 3a), the appearance of short vas deferens sections between the penis and the vagina (52 specimens, Fig. 4e).

(2) The formation of a coiled oviduct (1 individual), interpreted by Smith (1971, 1980, 1981a, b, c, d) in *Ilyanassa obsoleta* as a mimic seminal vesicle.

(3) The formation of a bifurcate penis (1 animal, Fig. 4f).

(4) Excrescences of hyperplasic tissue on the penis and/or the vas deferens (4 specimens). In one animal (Fig. 3f) this occurred on the vas deferens in front of the vulva, but the vaginal opening was still intact.

The masculinization effect of TBT on the female genital tract in *O. erinacea* is not only an enlargement of the female penis, but also a reduction of the extent of albumen, ingestion and capsule gland (Table 1, Fig. 6d). This reduction is significant on the $p < 0.05$ level for the capsule gland (length of capsule gland in mm = 10.63–0.34 × [imposex stage]; n = 854), but not for the albumen and ingestion gland (height of albumen gland in mm = 3.26–0.043 × [imposex stage]; n = 854; $p < 0.1$; height of ingestion gland in mm = 2.96–0.017 × [imposex stage]; n = 854; $p > 0.1$).

TBT accumulation

Tributyltin compounds can be found to a different extent in the tissues of *Ocenebra erinacea* (Table 2). The highest amount of TBT is accumulated by the kidney, followed by the digestive gland/gonad complex, the foot and the remaining organs and tissues. In areas likely to be contaminated by TBT (e.g. Roscoff harbor) due to vessel activity, the body burden in *O. erinacea* is 600–1100 ppb TBT-Sn and 200–1100 ppb DBT-Sn. Even at a number of sites away from sources of TBT contamination (e.g. Méan Mélen, Cap Fréhel, Beg an Fry) the organotin level in the whole body reaches 30–150 ppb TBT-Sn and 40–120 ppb DBT-Sn. In contrast to *Nucella lapillus* (unpublished data) and *Hinia (Nassarius) reticulata* (Stroben et al., 1992a), where females accumulated more TBT and DBT compared to males, the TBT and DBT body burden in *O. erinacea* shows no sex related differences (Figs 5a, b).

Table 2. *Ocenebra erinacea*. TBT concentration in pooled tissues of 15 males and 16 females from Roscoff harbour (France) (sample date: September 1990)

	Females			Males		
	Tissue bulk (% total wet wt)	TBT-Sn (ppb dry wt)	Body burden in each tissue (%)	Tissue bulk (% total wet wt)	TBT-Sn (ppb dry wt)	Body burden in each tissue (%)
Digestive gland/gonad	17.69	1566.8	57.08	21.30	1015.6	39.46
Foot	25.78	432.7	11.50	26.19	1241.7	29.82
Kidney	5.14	5364.9	18.51	4.41	4659.9	11.83
Remains	51.39	206.9	12.91	48.10	404.9	18.89
Whole animal	100.0	776.1	–	100.0	892.3	–

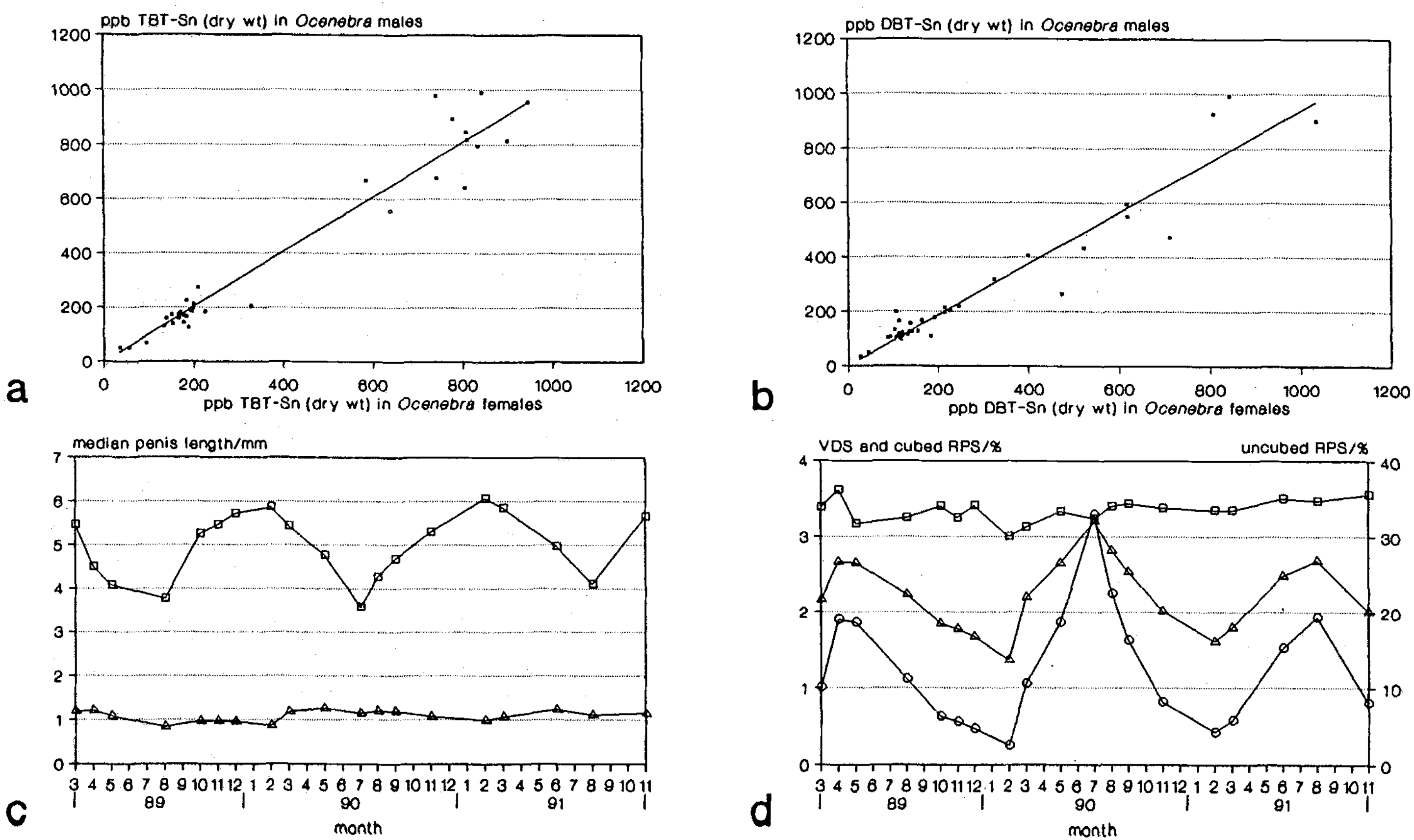

Fig. 5. *Ocenebra erinacea*. a: TBT fraction in males vs TBT fraction in females with linear regression ($y = 1.011\,x$; $n = 35$; $r = 0.975$; $p < 0.01$). b: DBT fraction in males vs DBT fraction in females with linear regression ($y = 0.989\,x$; $n = 35$; $r = 0.933$; $p < 0.01$). c: Variation of average male (□) and female (△) penis length at Île Verte (Roscoff) during 1989–1991. d: Variation of VDS (□), uncubed RPS (△) and cubed RPS (○) at Île Verte (Roscoff) during 1989–1991

Indices for TBT biomonitoring

During the 1970s it was sufficient to determine the portion of penis-bearing females in *Ocenebra erinacea* populations as a suitable index for TBT biomonitoring (Féral, 1974b, 1976a, b, c, 1980). But today at most sites, 100 % of the females are penis-bearing and none of the analysed populations was totally unaffected by imposex. Meanwhile, other indices for TBT biomonitoring have been developed (e.g. Gibbs et al., 1987; Oehlmann et al., 1991; Stroben et al., 1992a). The VDS (Fig. 6a), uncubed RPS (Fig. 6b) and the median female penis length (Fig. 6c) of natural populations were determined and tested for their quality and validity. The cubed RPS (Gibbs et al., 1987) was rejected, because it exhibited seasonal variations within one order of magnitude at most stations. These variations are a consequence of seasonal changes in the median male penis size. The maximum penis length is attained between November and March, the minimum penis length in late summer (July, August) during sexual repose of males. In contrast to the male, the median female penis size at a given station is more or less constant throughout the year (Fig. 5c). Due to the fact that the RPS relates the average female penis length with the inconstant median male penis size, the RPS exhibits seasonal changes (Fig. 5d). On the other hand, the VDS index remains relatively constant, without seasonal effects, and is clearly the better parameter. The correlations between TBT body burden in *O. erinacea* females and the VDS, uncubed RPS and average female penis length (Figs 6a–c) were highly significant ($p < 0.01$). All three parameters are suitable indices for TBT biomonitoring, but the VDS demonstrates the greatest ecological evidence (see "Discussion"). Another reason for the preference of the VDS to the uncubed RPS and average female penis length is the occurrence of relative high portions (40–60 %) of the penisless imposex stage 1b at slightly polluted sites (e.g. Méan Mélen). A calculation of the uncubed RPS and the average female penis length alone may lead to an underestimation of the TBT pollution at such a site.

Comparison of the TBT sensitivity of *Ocenebra erinacea* and *Nucella lapillus*

The fact that *Ocenebra erinacea* often cohabits the same sites as *Nucella lapillus* allows a direct comparison of the TBT sensitivity of both species on the basis of imposex development and accumulation of organotin compounds. The population data (VDS, uncubed RPS, average female penis length, TBT-Sn body burden) of *O. erinacea* were calculated and plotted against the corresponding values of *N. lapillus*. Regressions were calculated (Figs 7a–d) and found to be highly significant ($p < 0.01$). The linear regression for DBT-Sn body burden was also calculated (ppb DBT-Sn in *O. erinacea* = 131.55 + 0.18 × [ppb DBT-Sn in *N. lapillus*]; $n = 71$; $r = 0.709$; $p < 0.01$).

Lower TBT exposure results in a higher increase of all TBT biomonitoring parameters in *N. lapillus* compared to *O. erinacea*. However, even in areas only slightly exposed to TBT, female rough tingles develop obvious imposex characteristics. In *O. erinacea* the threshold for the induction of imposex is slightly higher than that in *N. lapillus*. VDS development in *O. erinacea* is initiated with a short delay, but is parallel to the increase in dogwhelks (Fig. 7a). VDS values in the rough tingle are generally 0.5–0.7 lower than in *N. lapillus*. A VDS of 5.0 is the maximum value attainable in *O. erinacea*, because stage 5c (B/C) represents the recent final point of imposex development in this species. As

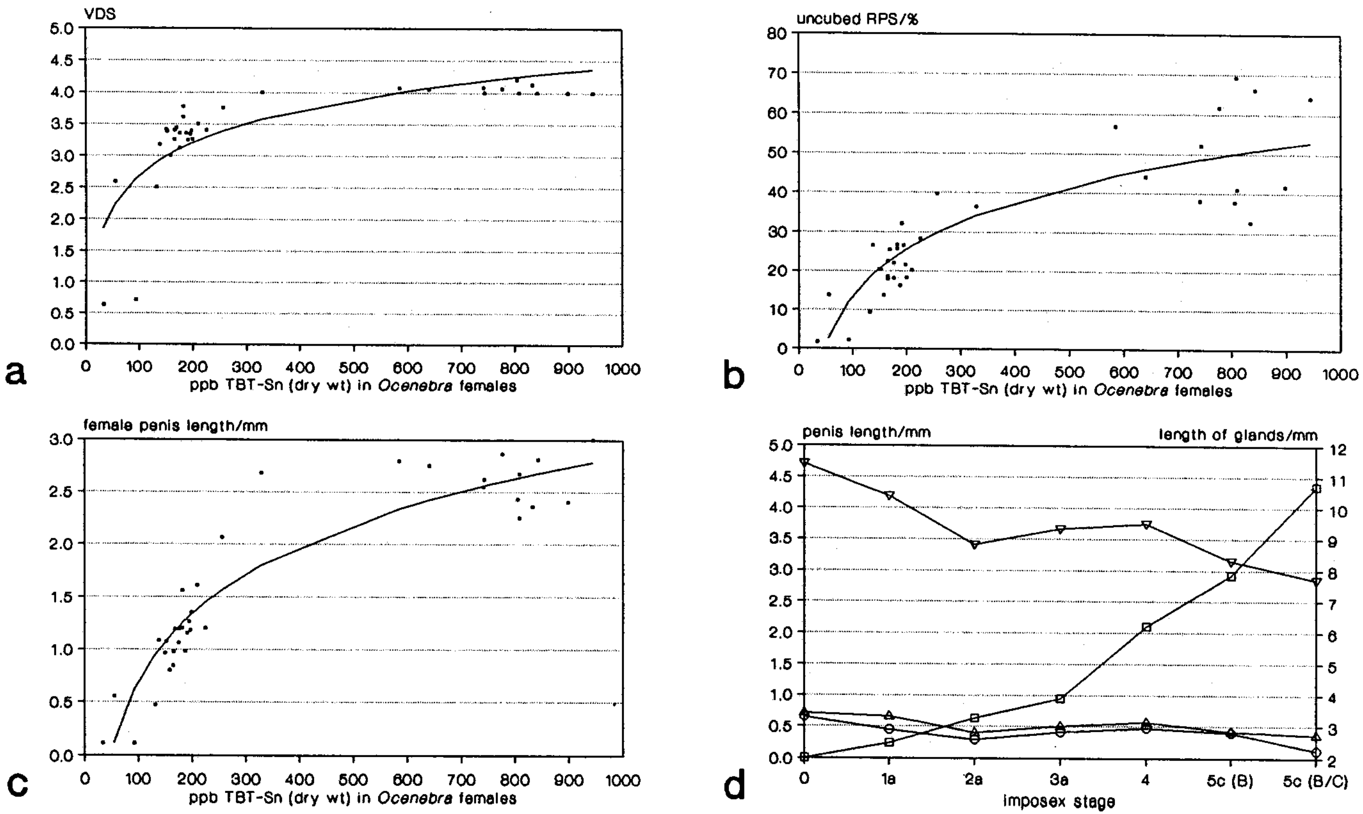

Fig. 6. *Ocenebra erinacea*. Relationship between TBT body burden of females and imposex indices. a: VDS index with logarithmic regression ($y = -0.761 + 0.749$ [ln(x)]; $n = 37$; $r = 0.807$; $p < 0.01$). b: Uncubed RPS index with logarithmic regression ($y = -67.55 + 17.58$ [ln(x)]; $n = 37$; $r = 0.865$; $p < 0.01$). c: Average female penis length with logarithmic regression ($y = -3.613 - 10.51$ [ln(x)]; $n = 37$; $r = 0.891$; $p < 0.01$). d: Relationship of imposex stage and average length of female penis (□), capsule gland (▽), and height of albumen (△) and ingestion gland (○)

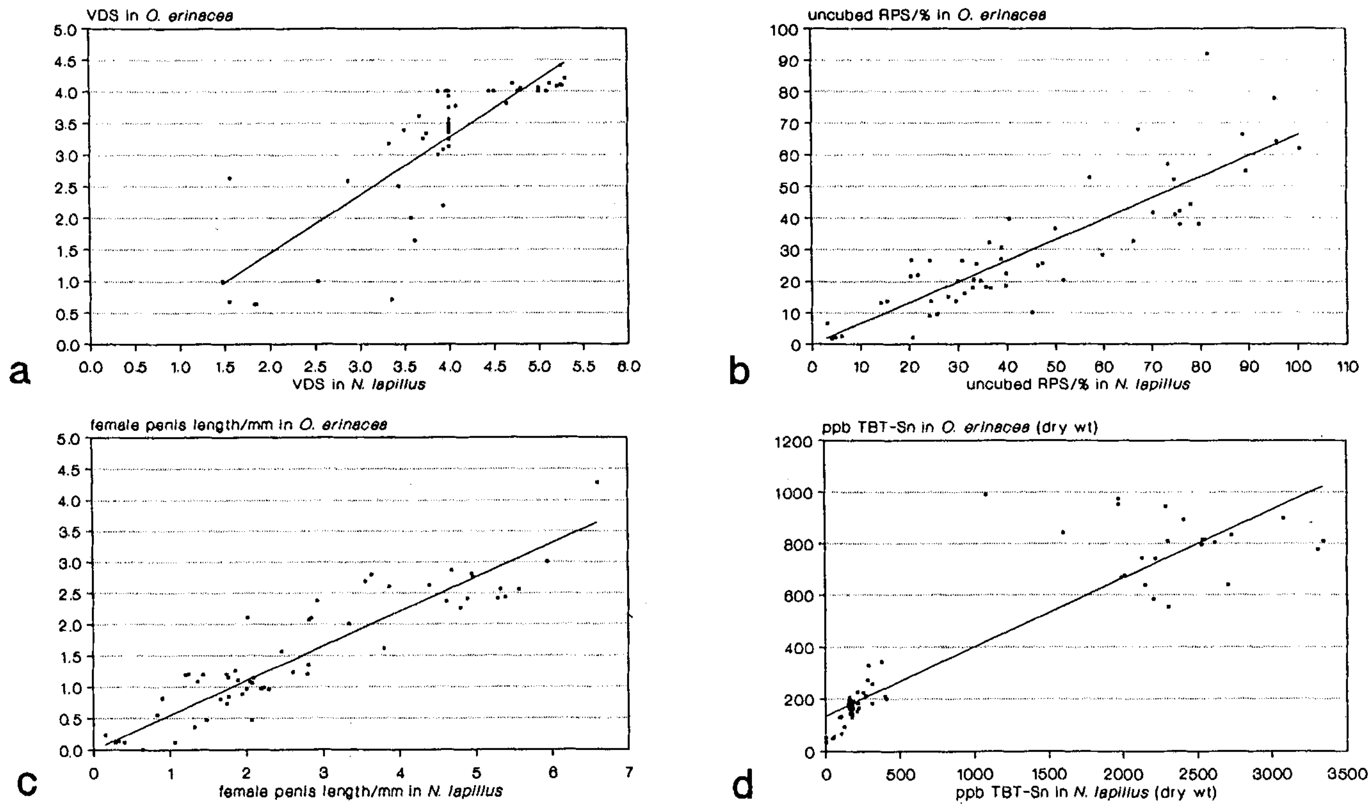

Fig. 7. *Ocenebra erinacea* and *Nucella lapillus*. Relationship between species-related imposex parameters and TBT body burden. a: VDS with linear regression ($y = -0.382 + 0.913\ x$; $n = 56$; $r = 0.841$; $p<0.01$). b: Uncubed RPS with linear regression ($y = 0.661\ x$; $n = 56$; $r = 0.870$; $p<0.01$). c: Average female penis length with linear regression ($y = 0.551\ x$; $n = 56$; $r = 0.913$; $p<0.01$). d: TBT-Sn body burden with linear regression ($y = 135.99 + 0.265\ x$; $n = 71$; $r = 0.923$; $p<0.01$)

shown in Figure 8, the VDS development of *O. erinacea* is linear at less and moderately polluted sites, while in *N. lapillus* a plateau is reached when the VDS attains values around 4.0. On severely polluted coasts, the VDS development of the dogwhelk increases again, but reaches a stage of equilibrium in the rough tingle at values above 4.0. The same relation as for the VDS increase in both species can be described for the two other indices. Generally, *O. erinacea* attains only 66.1 % and 55.1 % of the *N. lapillus* values for the uncubed RPS and the average female penis length, respectively (Figs 7b, c).

The statistical analysis of organotin uptake shows that, in comparison to dogwhelks, the rough tingle accumulates DBT and TBT to a lesser extent (Fig. 7d). This effect is rather obvious at highly polluted sites (e.g. Roscoff harbor), where values of TBT body burden in *O. erinacea* attain only one-third of the values measured in the tissues of *N. lapillus*. At slightly (e.g. Méan Mélen, Cap Fréhel) and moderately contaminated sites (e.g. Île Verte), rough tingles contain only about 90 % of the TBT body burden of dogwhelks.

DISCUSSION AND CONCLUSION

A first detailed report on TBT-induced reproductive abnormalities in female *Ocenebra erinacea* was presented by Gibbs et al. (1990), but unfortunately without an exact staging of imposex expression. Our study gives a description with all stages of imposex development in the rough tingle documented with SEM and histological photographs.

The TBT sensitivity of this species is slightly lower than that of the dogwhelk *Nucella lapillus*, but higher compared to *Hinia reticulata* (Stroben et al., 1992b). The number of imposex stages and types realized in *O. erinacea* is more restricted than in *N. lapillus*. The final point of imposex development is stage 5c when a ventrally split bursa copulatrix and capsule gland are attained. It seems highly improbable that the reproductive

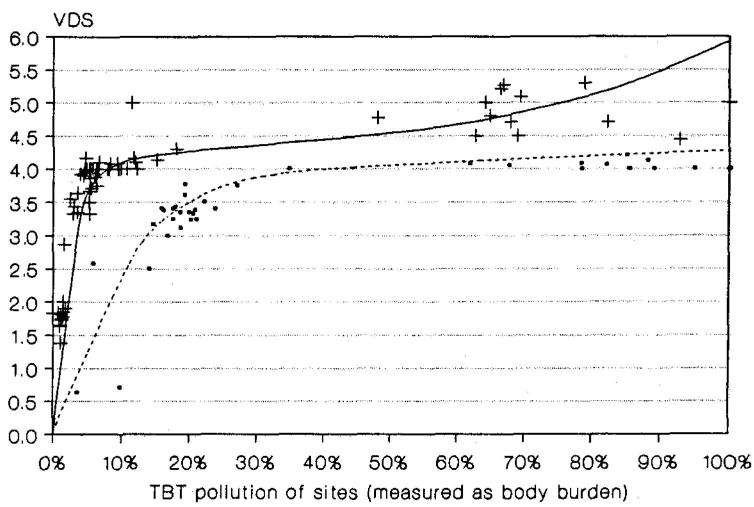

Fig. 8. Relationship between VDS and relative TBT pollution of sites in *Ocenebra erinacea* (• and dashed line) and *Nucella lapillus* (+ and solid line). Lines are eye-fitted

performance of rough tingle females is unaffected by these malformations of the pallial oviduct, as already supposed by Gibbs et al. (1990). In contrast to these authors, who found up to 100 % of females with a split oviduct, the portion of stage 5c females attains a maximum value of 40 % in our analysed populations in direct proximity to marinas. Because of this situation, the question as to whether or not reproductive capability at the most affected sites is restricted has yet to be answered. The portion of juvenile and subadult rough tingles close to harbors is less compared to populations far away from TBT sources. The reproductive success in the proximity to harbors is probably due to the relatively high numbers of still fertile (i.e. imposex stage 4) females.

TBT-induced sterilization is not only reported for *O. erinacea* (Gibbs et al., 1990; this study), but occurs also in *N. lapillus* (Gibbs et al., 1987; Oehlmann et al., 1991), *Nucella lima* (Short et al., 1989), *Nucella lamellosa* (Bright & Ellis, 1990), *Thais haemastoma* (Spence et al., 1990), *Urosalpinx cinerea* (Gibbs et al., 1991) and *Ocinebrina aciculata* (in prep.). The sterilization effect of advanced imposex is different in the muricids. In *N. lapillus, N. lima, N. lamellosa* and *T. haemastoma* the oviduct is blocked, but in *O. erinacea* and *U. cinerea* the oviduct is more or less completely split, instead of the normal closed tube. *O. aciculata* links both possibilities of sterilization, because not only a blocking or loss of the vulva, but also a splitting of the pallial oviduct occurs. Oviduct blockage of the *N. lapillus* type causes high female mortalitiy (Oehlmann et al., 1991) and consequently male-biased sex ratios in the populations. Sterilization due to the open structure of the pallial oviduct provokes poor recruitment of juveniles, but not high female mortality. Thus, the sex ratio in imposex-affected *O. erinacea* populations does not change towards male dominance. Contrary to Gibbs et al. (1990) we did not observe any indications for a sex change like those observed in *N. lapillus* (Gibbs et al., 1988; Oehlmann et al., 1991) and *O. aciculata* (Fioroni et al., 1991). In contrast to these muricids, imposex-affected mesogastropods (Fioroni et al., 1991) and buccinids (Stroben et al., 1992a) show no restrictions in fertility and no TBT-induced sterilization. The tendency towards a diminution of the capsule and, with restrictions, the albumen gland in advanced imposex stages may cause a reduced reproductive success. Further study on this subject has to be carried out.

For *O. erinacea*, as for other imposex-affected prosobranch species (Fioroni et al., 1991), the spatial distribution of imposex in relation to boating activity suggests that the rough tingle has potentials as bioindicator of TBT contamination. We found a significant correlation ($p < 0.01$) between TBT body burden and the three described imposex indices (VDS, uncubed RPS, female penis length) (Figs 6a–c).

The VDS is the most informative parameter for TBT biomonitoring and exhibits a greater reliability than especially the cubed RPS after Gibbs et al. (1987):

(1) The VDS shows the greatest ecological evidence, because the portion of sterilized females in a population can easily be recognized when values above 4.0 are attained.

(2) In contrast to the median male penis length and consequently also to the uncubed and especially cubed RPS, the VDS and the average female penis length exhibit no seasonal changes but remain more or less constant throughout the year.

(3) The occurrence of an imposex type lacking a penis (stage 1b) supports our preference for the VDS because the RPS calculation in populations with a high amount of stage 1b could lead to an underestimation of TBT exposure (see results).

Therefore we suggest the VDS as the best index for TBT biomonitoring. Only in highly polluted areas can the uncubed RPS be used as a secondary parameter because here VDS development comes into an equilibration above a value of 4.0. In this case, the uncubed RPS gives a good differentiation between the TBT exposure of such highly contaminated populations. Our general scheme of imposex evolution in prosobranchs (Fioroni et al., 1991; Oehlmann et al., 1991; this paper) proved its validity for describing imposex in *O. erinacea*. Even imposex stages clearly described in the literature for other species, e.g. *Searlesia dira, Ocenebra lurida, Colus halli* (Bright & Ellis, 1990) and *N. lima* (Short et al., 1989) can also be classified using this scheme. This leads to the possibility of comparing the TBT sensitivity of different prosobranch species on the VDS basis (e.g. Stroben et al., 1992b).

Gibbs et al. (1990) (basing on 22 analyses) report on sex related differences of TBT accumulation in *O. erinacea*. TBT concentrations in females were about 12 % higher than those in males. Similar differences occur in dogwhelks (own unpublished observations), *H. reticulata* (Stroben et al, 1992a) and *Trivia artica* (own unpublished observations). In this study, considering 35 samples, the TBT and DBT accumulation in male and female *O. erinacea* were comparable (Figs 5a, b). We also determined in 40 samplings at 5 sites differences in the TBT accumulation pattern of single tissues and organs (Table 2) compared to Gibbs et al. (1990) (1 analysed population), who found the highest amounts in the pallial oviduct of females and the digestive gland/gonad complex of males. We are not able to explain this discrepancy at the moment, but it has to be pointed out that the same pattern of TBT accumulation by the tissues described here was also found in *H. reticulata* (Stroben et al., 1992a), *N. lapillus, Murex brandaris* and *Murex trunculus* (own unpublished data).

Like *N. lapillus*, the rough tingle is also a suitable TBT bioindicator (Gibbs et al., 1987; Oehlmann et al., 1991; this study), but dogwhelks exhibit a greater TBT sensitivity, namely in slightly polluted areas; nevertheless, even here *O. erinacea* develops obvious imposex characteristics. The effect of TBT on imposex development, measured as an increase of the VDS, uncubed RPS and the average female penis length, was generally higher in *N. lapillus*. This may be due to the more subtidal distribution of rough tingles in Roscoff harbor, the most polluted site in our study area, and a consequently higher TBT exposure of the more intertidal living dogwhelks. Another explanation for these differences may lie in the levels of accumulated TBT by the specific prey of dogwhelks and rough tingles. *N. lapillus* feeds mainly on balanids and mussles, which proved to be an important source of tissue TBT (Bryan et al., 1989b). The question as to whether or not the diet of *Ocenebra erinacea*, e.g. *Cardium, Venus, Crepidula* and tubicolous worms, have lesser TBT body burdens compared to *N. lapillus*' prey has yet to be answered. Generally, the differences in development of the various imposex parameters between *O. erinacea* and *N. lapillus* can be described by using the mathematical equations given in Figure 7. Thus, there exists a fundamental comparability between both TBT bioindicators, and even if one of them is absent at a given site, the biological effects on this species are predictable basing on the results of the second species (Fig. 8).

The main advantages of dogwhelks as bioindicators are the higher TBT sensitivity and the broader already existing data base regarding imposex development and TBT ecotoxicology. But *O. erinacea* with its more southern distribution allows one to obtain information on TBT pollution in regions where dogwhelks are lacking (e.g. coast of Portugal and the Mediterranean). If dogwhelks and rough tingles are analysed as indicator species wherever both cohabit the same sites, this would result in a broader data base on TBT effects and TBT exposure of the analysed region.

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