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**The genital systems of *Trivia arctica* and  
*Trivia monacha* (Prosobranchia, Mesogastropoda)  
and tributyltin induced imposex<sup>1</sup>**

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**Summary**

The mesogastropod prosobranchs *Trivia arctica* and *T. monacha* collected along the coast of Brittany and Normandy between 1988 and 1992 exhibit imposex or pseudohermaphroditism (occurrence of male parts in addition to the female genital system) in response to tributyltin (TBT) pollution. Beside some normal females (stage 0) different imposex stages according to the classification of FIORONI et al. (1991) (3a, 3b and 4 for *T. arctica* and 3b and 4 for *T. monacha*) (Fig. 1) can be distinguished and are documented with scanning electron micrographs for the first time. Additional alterations of the genital tract e. g. excrescences on the vas deferens, a coiled oviduct, a bi- or trifurcated penis or several penes (2 - 5) are described. Neither TBT-induced sterilization nor sex change occur. TBT accumulation in soft parts shows sex-related differences. The VDS (vas deferens sequence) index, uncubed RPS (relative penis size) index and average female penis length of a population are dependent from TBT concentrations in ambient sea water and TBT body burden. A statistical study, based on an analysis of natural populations of *T. arctica*, *T. monacha* and *Nucella lapillus* allows a comparison of the specific TBT sensitivity of the three TBT bioindicators. *N. lapillus* exhibits a lower threshold for imposex development and a higher TBT sensitivity, but both *Trivia* species proved to be suitable TBT bioindicators.

**1. Introduction**

The phenomenon of imposex (SMITH, 1971) or pseudohermaphroditism (JENNER, 1979), that is the occurrence of additional male parts in the female genital system, is analysed since many years. Imposex is induced by TBT (tributyltin) compounds, which are used as biocides in various formulations especially in marine antifouling paints. To date, carnivorous neogastropods (= Stenoglossa) like e. g. *Nucella lapillus* (GIBBS et al., 1987; OEHLMANN et al., 1991), *Ocenebra erinacea* (GIBBS et al., 1990; OEHLMANN et al., 1992a), *Ocenebrina aciculata* (OEHLMANN et al., 1992b), *Hinia (Nassarius) reticulata*

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<sup>1</sup> The results of this paper are part of E. STROBEN's dissertation at the Westfälische Wilhelms-University, Münster.

(STROBEN et al., 1992 a, b) and *H. incrassata* (FIORONI et al., 1990, 1991) are in the centre of interest.

Within the Mesogastropoda, the genus *Trivia* – in contrast to the species of *Littorina* (see "Discussion") – exhibits imposex and a rather high TBT sensitivity, comparable to neogastropods. It is therefore of some importance for TBT biomonitoring and necessitates a corresponding description, which is documented for the first time with histological photographs and scanning electron micrographs.

Only little attention was paid to the genital system of *Trivia arctica* and *T. monacha*. All subsequent statements on the normal genital systems of *T. arctica* and *T. monacha* (e.g. FRETTER, 1951; MORTON, 1960; FRETTER/GRAHAM, 1962; HYMAN, 1967; FRANC, 1968; PURCHON, 1968; GÖTTING, 1974; WEBBER, 1977) base on the classical study of FRETTER (1946) which gives neither histological figures nor a photographical documentation. Some additional information on South African species are outlined by GOSLINER/LILTVED (1982). Imposex expression and development was not described in detail before (FIORONI et al., 1991).

Both species can be found between tide marks of rocky shores, under stones and on ascidian colonies of *Diplosoma*, *Botryllus*, *Botrylloides* and *Polyclinum*. *Trivia arctica* is distributed throughout the Mediterranean, and in European Atlantic waters up to Norway in the north. Though the species can be found between tide marks, the bulk of the populations lives subtidally. *T. monacha* exhibits a more southern distribution also from the Mediterranean up to the British Isles in the north (LEBOUR, 1931, 1933). Both species are carnivorous and feed on ascidians in their habitat. The capacity of *T. arctica* and *T. monacha* for dispersion is high because both species hatch as a free swimming planktonic "long distance" veliger (Echinospira).

The objectives of our work are to give a photographically based documentation of the genital system including imposex; topographical aspects are stressed, while the histochemistry of the different glandular cell types of the genital ducts are not worked out. We give furthermore the imposex classification (Fig. 1) and add informations concerning a suitable index for TBT biomonitoring.

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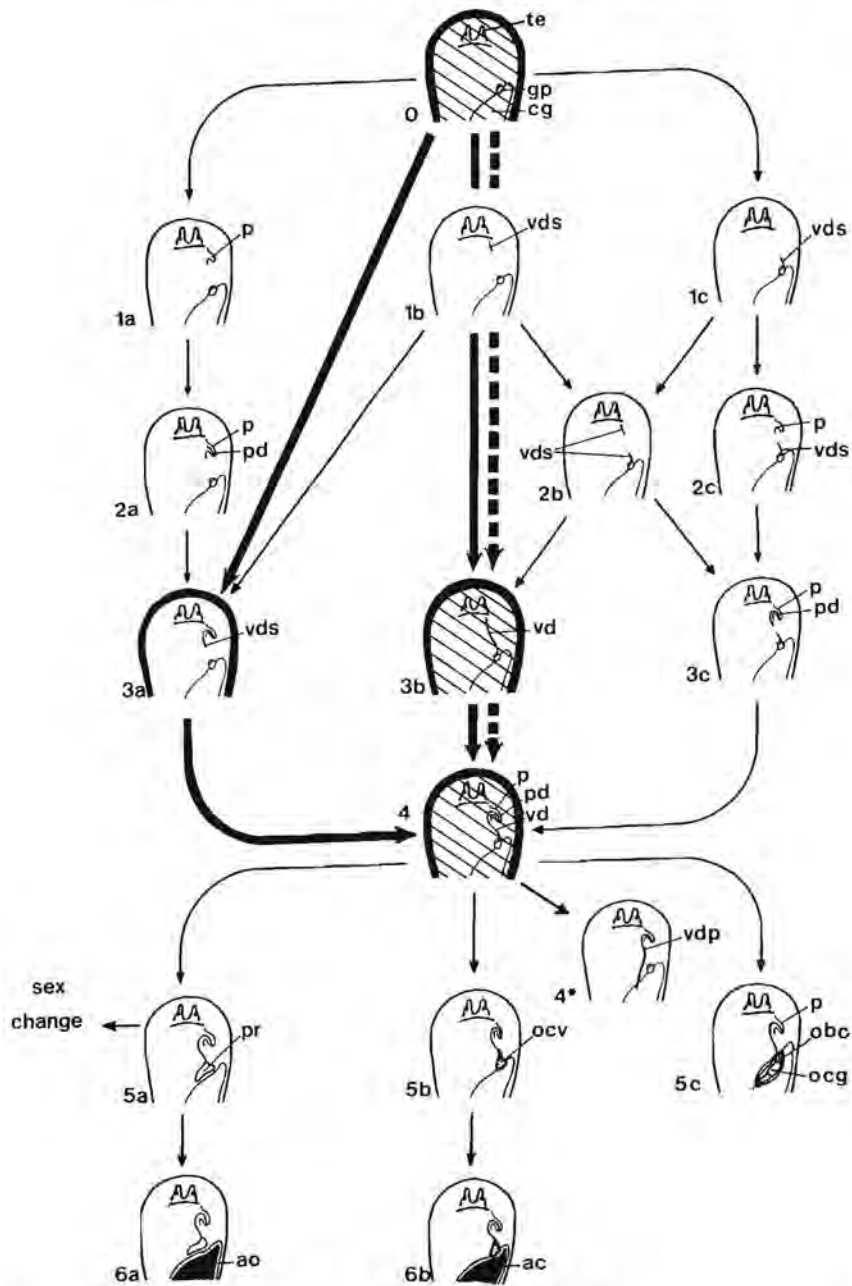


Fig. 1. General scheme of imposex evolution in prosobranchs. Imposex stages of *Trivia arctica* (in bold) and *T. monacha* (in hatching and dashed lines). Abbreviations: ac: aborted capsules; cg: capsule gland; gp: genital papilla; obc: open bursa copulatrix; ocg: open capsule gland; ocv: occlusion of 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.

## 2. Material and Methods

The external topography of 1199 specimens of *Trivia arctica* and 127 of *T. monacha*, collected at 11 stations along the coast of Brittany and Normandy between March 1988 and March 1992 was analysed. A map indicating these stations and the calculated VDS values of the populations are given in OEHLMANN et al. (1992c). Complete series of histological sections exist for 15 respectively 23 specimens of these species.

The animals were narcotized using 7% MgCl<sub>2</sub> in distilled water. The height of the shell and its aperture were measured to the nearest 0.1 mm using a vernier calliper. The shell was cracked with a vice, and then the animals were analysed with a stereo microscope. The external dimensions of the genital tract including vas deferens extension and penis length were measured with an exactness of 0.1 mm. All individuals with an uncertain anatomy were fixed with Bouin's fluid and preserved in 70% alcohol. Owing to shrinkage, many details, especially the vas deferens, are then much more visible.

After embedding in paraplast, serial sections (7–10 µm) were made and stained with azan according to Heidenhain, haemalun-chromotrop, trichrome according to Goldner, alcian blue and the PAS-reaction (ROMEIS, 1968).

Animals for semithin sections (1 µm; LKB 2008 ultratome) were fixed in 2% OsO<sub>4</sub> dissolved in 0.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 70% sea water (pH 7.2–7.4) for 90 minutes at room temperature or overnight at 4°C and embedded in Spurr (SPURR, 1969) or styrol-methacrylate (KUSHIDA, 1969).

Specimens for SEM were fixed in Bouin's fluid, dehydrated via graded ethanol series, critical point dried, coated with gold and examined with a Hitachi scanning electron microscope S-530. The histological photographs were made with a Zeiss photomicroscope II.

When possible, 30 or more adult *Trivia arctica* and *T. monacha* were collected intertidally. The following indices for TBT biomonitoring were used: (1) The VDS (vas deferens sequence) index is calculated as the average imposex stage (according to Fig. 1) of a population. (2) The uncubed RPS index is defined as [(mean length of female penis)/(mean length of male penis) × 100] (STROBEN et al., 1992a). This index is called "uncubed" to differentiate it from the unsuited RPS (p. [12]) introduced by GIBBS et al. (1987) [(mean length of female penis<sup>3</sup>)/(mean length of male penis<sup>3</sup>) × 100]. (3) Average female penis length of a population.

Our general system of imposex classification (stage 1–6) (FIORONI et al., 1991; OEHLMANN et al., 1991, 1992a, b; STROBEN et al., 1992a, b) (Fig. 1) proved valid in describing imposex in all known prosobranch species with this phenomenon including *Trivia arctica* and *T. monacha*.

The determination of TBT and DBT (dibutyltin) compounds was based on STROBEN et al. (1992a). The analysis includes an extraction of TBT and DBT compounds with hexane, the elimination of DBT by washing the hexane extract with NaOH and quantification using atomic absorption spectroscopy (Perkin-Elmer HGA-500 attached to a Perkin-Elmer 5000 AAS with background correction; wave length 224.6 nm; slit 0.7 nm; injection volume 25 µl). Internal standardization (standard addition with spiked samples) was employed. Certified reference material (CRM: PACS-1, delivered by the National Research Council of Canada) was analysed additionally. Own results were within the standard deviation of the certified values for the CRM.

### 3. Results

The male and female genital systems were investigated in both species of *Trivia* with histological slide series, semithin sections and with help of SEM-techniques. Additionally, the male tract of imposex affected females was examined using the same techniques. Generally the previous results of FRETTER (1946) for the normal male and female genital systems can be confirmed. Therefore, in the following no complete description of the male and female tract is given, but only differences to the well-known results of FRETTER (1946) are communicated. Some further important facts are stressed.

#### 3.1 Male genital system

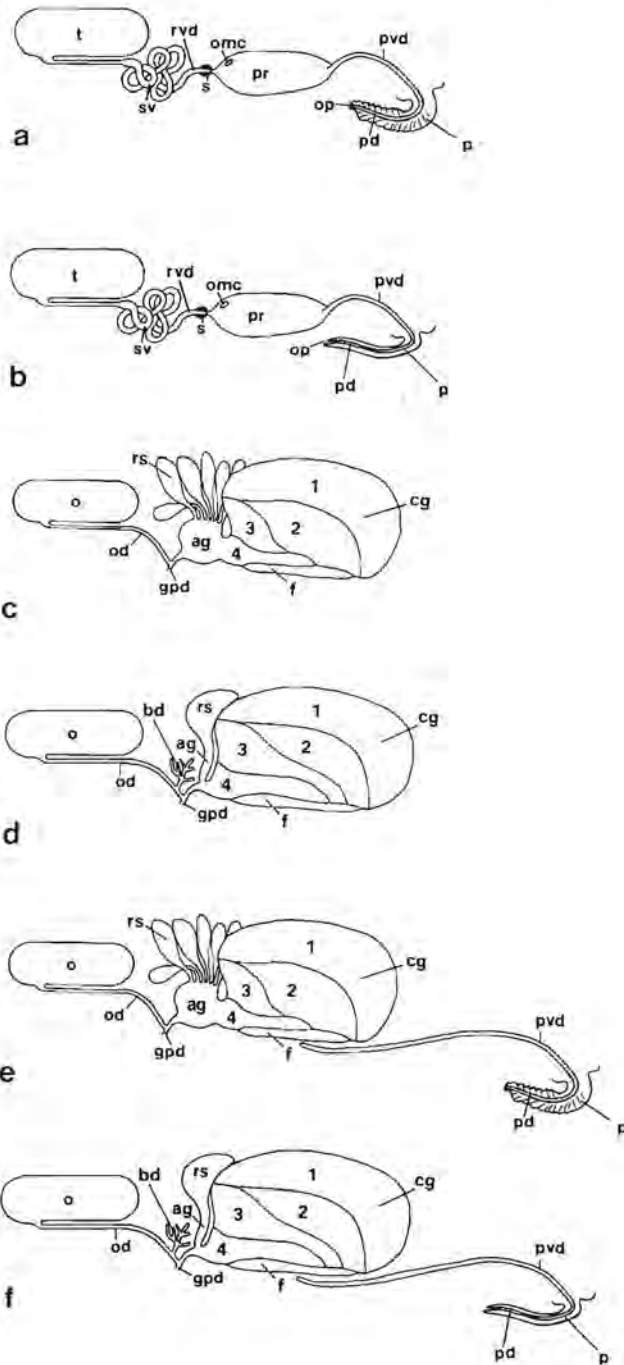
It consists (from proximal to distal) of the testicle (testis), the testicle duct (ductus testis), the seminal vesicle (vesicula seminalis), the renal portion of the vas deferens (with a sphincter), the prostate gland (prostata), the pallial portion of the vas deferens and the penis with its duct (Fig. 2 a, b).

Most of the results of FRETTER (1946) on British specimens could be confirmed, but cilia in the epithelial cells of the seminal vesicle could not be found (Fig. 3 a). Also the amoebocytes described by FRETTER (1946) between the central laying spermatozoa and in the coat of the vesicle were absent in French specimens.

Furthermore, the cellular inventory of the prostate gland differs between the two species: *Trivia arctica* has beside the ciliated supporting cells only one type, *T. monacha* two types of glandular cells, one with neutral mucosubstances, which lacks in *T. arctica*, the second with a mucous secretion. In contrast to FRETTER's (1941) description also the penial duct exhibits, like the pallial vas deferens, some mucous glandular cells.

Contrary to *Nucella lapillus* for instance, the sphincter of the vas deferens versus the prostate gland is poorly developed. *Trivia* has like *N. lapillus* (OEHLMANN et al., 1988) and other *Stenoglossa* a slit-like aperture of the prostate into the mantle cavity (Fig. 3 b). As in *Hinia reticulata*, the pallial part of the vas deferens is completely closed (Fig. 3 c) (STROBEN et al., 1992 a). In muricid gastropods (e.g. *N. lapillus*, *Ocenebra erinacea*, *Ocenebrina aciculata*) the pallial tract is also a closed tube, but it has fused epithelia connecting the lumina of the tract sections and the inner mantle epithelium as a remnant of the ontogenetic infolding of the pallial epithelium (OEHLMANN et al., 1991, 1992 a, b).

The penis is well developed with species-specific differences: it is cylindrical, smooth, long and pointed in *T. monacha* (Fig. 5 a), but leaf-like, broad, flattened and with a wavy surface in *T. arctica* (Fig. 4 a, b).



## 3.2 Female genital system

It consists (again from proximal to distal) of the ovary, the ovarian and renal portion of the oviduct (separated by the aperture into the gonopericardial duct), the albumen gland with receptaculum seminis, the capsule or nidamental gland (with 4 lobes), ventral channel and a ventral slit-like vaginal opening against the mantle cavity (Fig. 2c, d). In comparison with the description of FRETTER (1946), some differences as a gonopericardial duct in both species and only four lobes instead of five in the capsule gland were found. The cells of the ventral channel have an extensive border of microvilli neglected by FRETTER (1946).

At the junction of the ovarian and renal part of the oviduct arises the gonopericardial duct which opens by an inconspicuous ciliated funnel into the pericardium. This duct exhibits a ciliated epithelium with flattened or cubical cells and is folded like the renal portion of the oviduct (Fig. 3e, f).

The differences between the two species are considerable (Fig. 2c, d and 3d). The renal oviduct of *Trivia monacha* has additional blind ending diverticula with a flat or cubical epithelium and with cilia only in the distal part. These diverticula lack in *T. arctica*. In *T. monacha*, they are sometimes voluminous and contain then spermatozoa and vitelline material; the disintegration of superfluous spermatozoa respectively yolk substances is probable (Fig. 3d). Amoebocytes in the interior of the organ, mentioned by FRETTER (1946), were not present.

Dorsally, the spherical albumen gland of *Trivia arctica* runs into six blind ending and unfolded tubes, which represent the receptaculum seminis. Contrary to *T. arctica*, the slender but intensively folded albumen gland of *T. monacha* passes directly in the sac-like receptaculum seminis. This is surrounded by haemocoelic spaces, which are filled with amoebocytes.

None of both *Trivia* species exhibits a terminal vagina with a vaginal opening situated on a prominent genital papilla as it is realized in neogastropods as *Nucella lapillus* (OEHLMANN et al., 1991) and *Hinia reticulata* (STROBEN et al., 1992a). The female genital opening is a slit at the ventral side of the capsule gland; the ventral channel continues ventrally up to the distal end of the capsule gland.

Fig. 2. *Trivia arctica* (a, c, e) and *T. monacha* (b, d, f). Schematic representation of lateral views of genital tracts: male (a, b), female (c, d), and imposex stage 4 (e, f). Abbreviations: ag: albumen gland; bd: branching diverticula of oviduct; cg: capsule gland; f: female opening; gpd: gonopericardial duct; o: ovary; od: oviduct; omc: opening into the mantle cavity; op: opening of the penis; p: penis; pd: penis duct; pr: prostate; pvd: pallial vas deferens; rs: receptaculum seminis; rvd: renal vas deferens; s: sphincter; sv: seminal vesicle; t: testis; 1: dorsal lobe; 2: medioventral lobe; 3: posteriorioventral lobe; 4: caudal lobe.

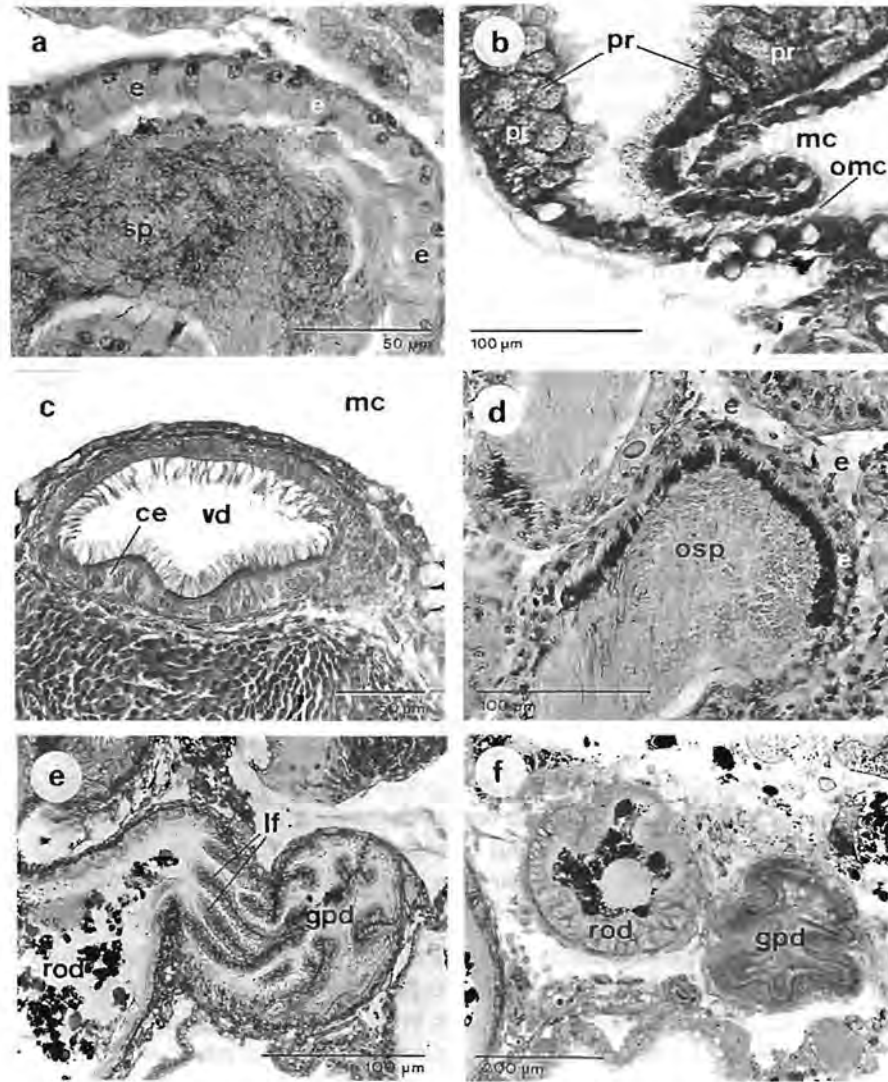


Fig. 3. *Trivia arctica* and *T. monacha*. Histological sections of male (a - c) and female (d - f) genital tract. (a) Transverse section of vesicula seminalis of *T. monacha*. (b) Transverse section of proximal part of the prostate of *T. arctica*. (c) Transverse section of pallial vas deferens of *T. monacha*. (d) Section through oviduct diverticula of *T. monacha*. (e) Section through gonopericardial duct running in renal oviduct of *T. monacha*. (f) Section through gonopericardial duct running parallel to renal oviduct of *T. monacha*. Abbreviations: ce: ciliated epithelium; e: unciliated epithelium; gpd: gonopericardial duct; lf: longitudinal folds; mc: mantle cavity; omc: opening into the mantle cavity; osp: orientated spermatozoa; pr: prostate; rod: renal oviduct; sp: spermatozoa; vd: vas deferens.



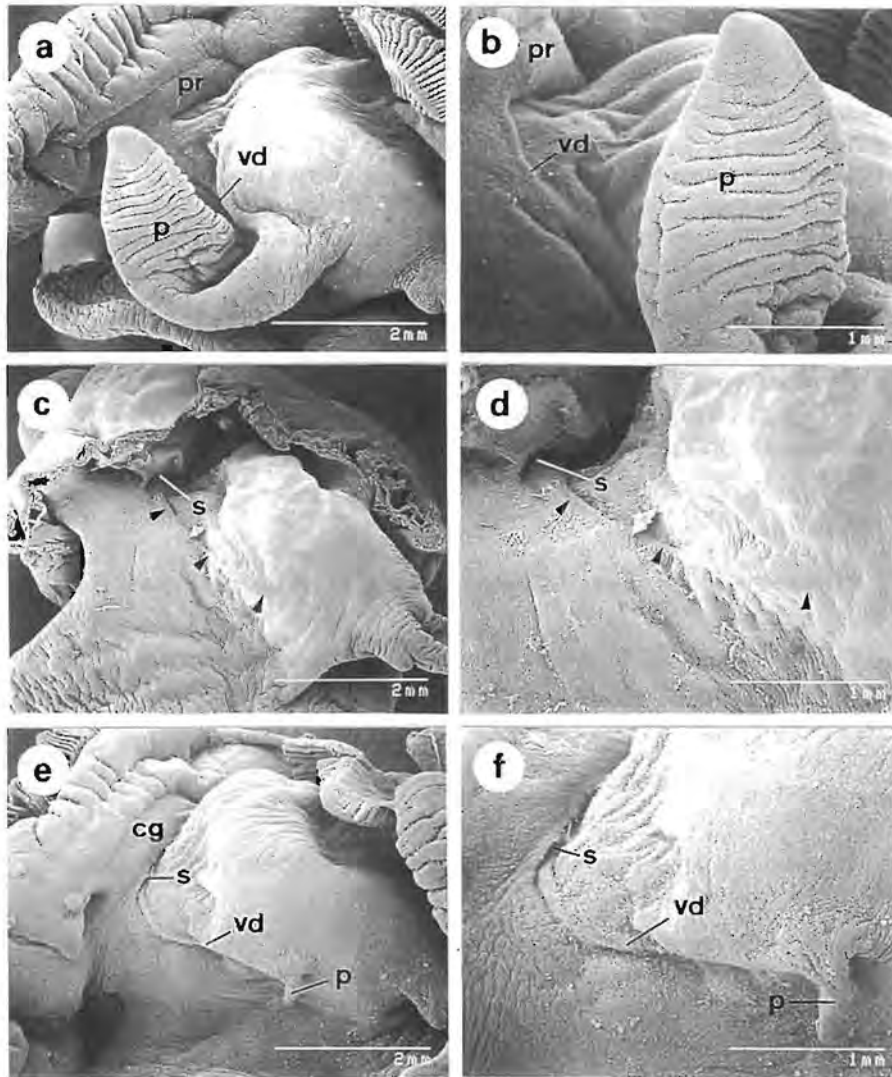


Fig. 4. *Trivia arctica*. Scanning electron micrographs of male and imposex stage 3a and 4. (a) Male during period of reproduction (March). (b) Detail of (a). (c) Stage 3a. (d) Detail of (c). (e) Stage 4. (f) Detail of (e). Abbreviations: cg: capsule gland; p: penis; pr: prostate; s: slit-like female opening; vd: vas deferens. Arrows: vas deferens.

### 3.3 Imposex classification and expression

The imposex phenomenon is characterized by a superimposition of additional male parts, i.e. a penis and/or vas deferens on females. Imposex development in prosobranchs can be generally described by an evolutive line with six stages (1 - 6) mostly with additional types (a - c; Fig. 1) (FIORONI et al., 1991; OEHLMANN et al., 1991, 1992a, b; STROBEN et al., 1992a, b). Stage 0 is a female unaffected by imposex and thus without any male characteristics (Fig. 5b); the stages 5 and 6 are characterized by sterilization due to malformations of the pallial oviduct.

We found in *Trivia arctica* the stages/types 0, 3a, 3b and 4, in *T. monacha* the stages/types 0, 3b and 4. The frequencies of the imposex stages and types are given in Tab. 1 for *T. arctica* and Tab. 2 for *T. monacha*. Imposex expression in this genus can be described as follows:

#### Stage 3

Type a: Penis with penis duct continuing in an incomplete distal tract of the vas deferens (Fig. 1).

Type b: Without penis. A vas deferens extends from the right ocular tentacle over the bottom of the mantle cavity to the slit-like female opening (Fig. 1; 4c, d; 5c).

#### Stage 4

Penis with a penis duct and a continuous vas deferens from the penis up to the vaginal slit (Fig. 1; 2e, f; 4e, f; 5d - f).

Stage 4 is the end of imposex development in *Trivia arctica* and *T. monacha*. In contrast to muricid gastropods there are no restrictions of fertility, and the histological structure of the ovary is completely normal. Furthermore, the existence of all functioning glands in the pallial oviduct section, assisted by a normal ventral pedal gland, allows the deposition of egg capsules. The female opening is unmodified and the capability of copulation is conserved.

The female penis length increases in all analysed prosobranch species generally from stage 0 to 6. The same has to be expected for both *Trivia* species. Differences of penis length between the penisless stage 3b (*Trivia arctica* and *T. monacha*) and the stages 3a (*T. arctica*) and 4 (*T. arctica* and *T. monacha*) are obvious. A Student's *t*-test analysis between stages 3a and 4 in *T. arctica* showed that penis length differences in both stages are not significant (492 degrees of freedom;  $t = 0.03$ ;  $p > 0.05$ ). The masculinization

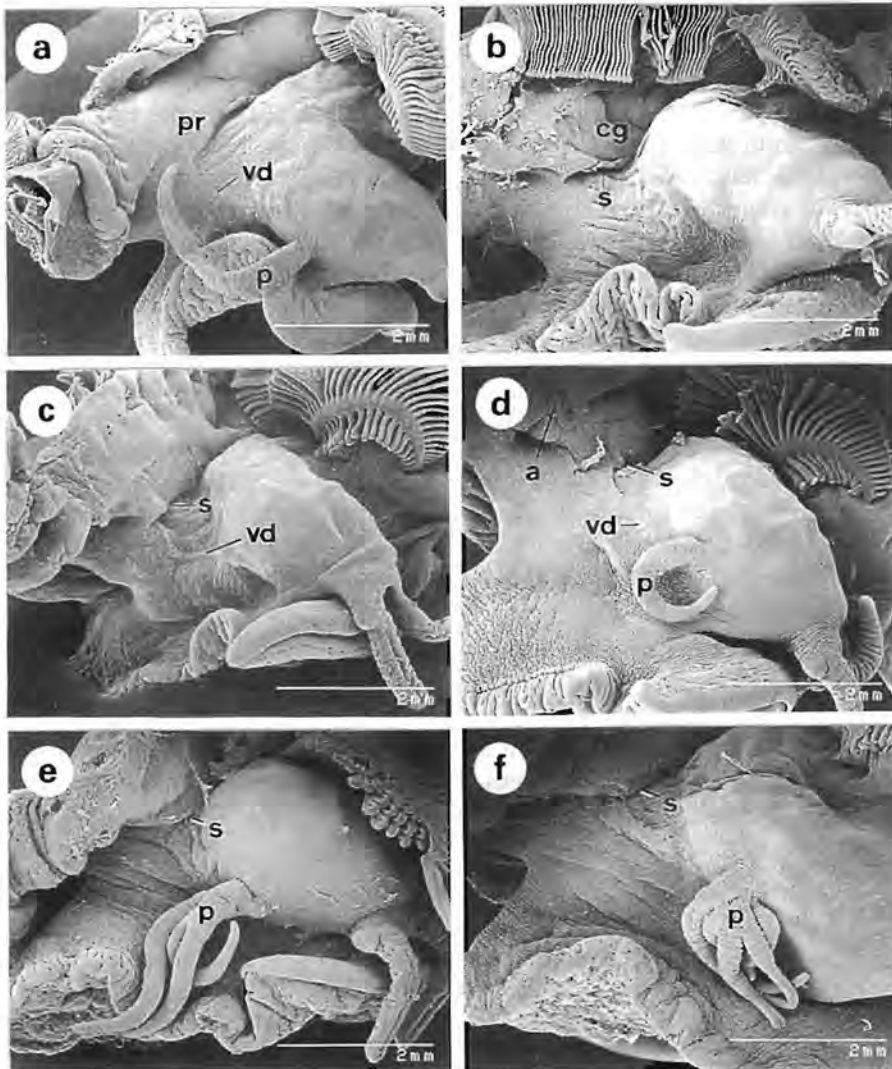


Fig. 5. *Trivia monacha*. Scanning electron micrographs of male, normal female (stage 0) and imposex stages 3 b, 4 and 4 with additional alterations. (a) Male. (b) Normal female (stage 0). (c) Stage 3 b. (d) Stage 4. (e) Stage 4 with a trifurcate penis. (f) Stage 4 with five penes. Abbreviations: a: anus; cg: capsule glands; p: penis; pr: prostate; s: slit-like female opening; vd: vas deferens.

effect of TBT on the female genital system of prosobranchs is not only an enlargement of the female penis length, but also a reduction of the extent of the female pallial glands (Tab. 1). A Student's *t*-test analysis proved that the

length of the capsule gland is smaller in higher imposex stages (stage 4 < 3b = 3a < 0;  $p < 0.05$  between stage 0 and 3a/3b;  $p < 0.005$  between stage 0 and 4;  $p < 0.0005$  between stage 3b and 4). The extension of the albumen gland and of the receptaculum seminis is also smaller in stage 4 compared to stage 3b ( $p < 0.001$  for the albumen gland and  $p < 0.05$  for the receptaculum). A statistical proof for the other imposex stages of *T. arctica* and all stages of *T. monacha* can not be given, because the number of analysed specimens is too small. There is no obvious dependence of the established imposex stage and the measured shell and aperture height, or to parasitisation or sexual maturity (Tab. 1, 2).

Some further morphological alterations are possible: for *Trivia arctica* (1) the formation of a coiled oviduct (3 specimens of stage 4); (2) excrescences of hyperplastic tissue on the vas deferens (1 individual of stage 4). For *T. monacha* the observed alterations were more frequent: (1) a coiled oviduct (1 specimen of stage 3b and 19 specimens of stage 4); (2) the formation of 2 to 5 penes (17 individuals of stage 4, Fig. 5f); (3) the formation of a bi- or trifurcate penis (1 female of stage 4, respectively, Fig. 5e). – A coiled oviduct was interpreted by SMITH (1971) in *Ilyanassa obsoleta* as mimic seminal vesicle.

Imposex affected females of the two species develop the species-specific penis forms (Fig. 2e, f; 5d–f). Also the different characteristics of the female genital tracts of the two species like the structure of the receptaculum seminis or the additional diverticula of the oviduct are conserved. The histological structure of the vas deferens and the penis resembles that of the corresponding formations in males.

### 3.4 TBT accumulation and imposex development

*Trivia arctica* and *T. monacha* accumulate TBT and DBT compounds in their natural environment. A correlation analysis (Fig. 6c) proved that the TBT body burden in *T. arctica* increases with the TBT content of the sea water. In areas likely to be contaminated by TBT (e.g. Roscoff harbor with 8.45 to 29.6 ng TBT-Sn/l) due to vessel activity, the body burden in *T. arctica* is 800 to 1800 µg TBT-Sn/kg and 400 to 1300 µg DBT-Sn/kg. Even at a number of sites away from sources of TBT contamination (e.g. Méan Mélen with < 1.50 to 1.65 ng TBT-Sn/l) the organotin level in the whole body reaches 30 to 40 µg TBT-Sn/kg and 20 to 65 µg DBT-Sn/kg. These values can be confirmed for *T. monacha*, which accumulated at Roscoff harbor 900 to 1300 µg TBT-Sn/kg and 800 to 900 µg DBT-Sn/kg, at Méan Mélen 30 to 40 µg TBT-Sn/kg and 20 to 65 µg DBT-Sn/kg. Because *T. monacha* was found in small numbers, only few organotin analyses and thus no correlation check were possible.

Table 1: *Trivia arctica*. Biometrical data of males, females and the different imposex stages. All data are basing 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		aperture height		penis length		albumen gland		receptaculum seminis		capsule gland		% with excrescences on penis and/or vd	% parasited	% sexual mature
				/mm	±s.d.	/mm	±s.d.	/mm	±s.d.	/mm	±s.d.	/mm	±s.d.	/mm	±s.d.			
♂	649	54.1		6.02	0.57	9.50	0.85	4.05	1.02	-	-	-	-	-	-	0.31	0	97.5
0	4		0.7	7.03	0.57	10.65	0.65	0	0	1.13	0.38	0.88	0.22	2.95	0.60	0	0	100.0
3a	5		0.9	6.26	0.44	9.98	0.74	1.76	0.73	0.80	0.12	1.00	0.09	2.50	0.38	0	0	100.0
3b	52		9.5	6.28	0.63	9.83	1.02	0	0	0.96	0.32	0.92	0.26	2.27	0.46	0	0	100.0
4	489		88.9	6.00	0.53	9.44	0.84	1.77	0.78	0.86	0.23	0.90	0.25	2.18	0.50	0.20	0	95.9
♀	550	45.9	100.0	6.04	0.55	9.49	0.88	1.59	0.98	0.87	0.23	0.90	0.25	2.19	0.50	0.18	0	96.4

Table 2: *Trivia monacha*. Biometrical data of males, females and the different imposex stages. All data are basing 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		aperture height		penis length		albumen gland		receptaculum seminis		capsule gland		% with excrescences on penis and/or vd	% parasited	% sexual mature
				/mm	±s.d.	/mm	±s.d.	/mm	±s.d.	/mm	±s.d.	/mm	±s.d.	/mm	±s.d.			
♂	54	42.5		5.68	0.67	9.42	1.06	3.72	1.09	-	-	-	-	-	-	0	0	94.4
0	4		5.5	6.25	0.50	10.40	0.74	0	0	1.10	0.07	0.83	0.24	2.68	0.79	0	0	100.0
3b	11		15.1	6.03	0.68	9.85	0.94	0	0	1.00	0.17	0.84	0.27	2.75	0.74	0	0	100.0
4	58		79.4	6.20	0.71	10.13	1.00	2.19	0.99	0.99	0.25	0.93	0.17	2.62	0.58	0	1.72	100.0
♀	73	57.5	100.0	6.18	0.70	10.10	0.98	1.74	1.25	1.00	0.23	0.90	0.21	2.64	0.62	0	1.37	100.0

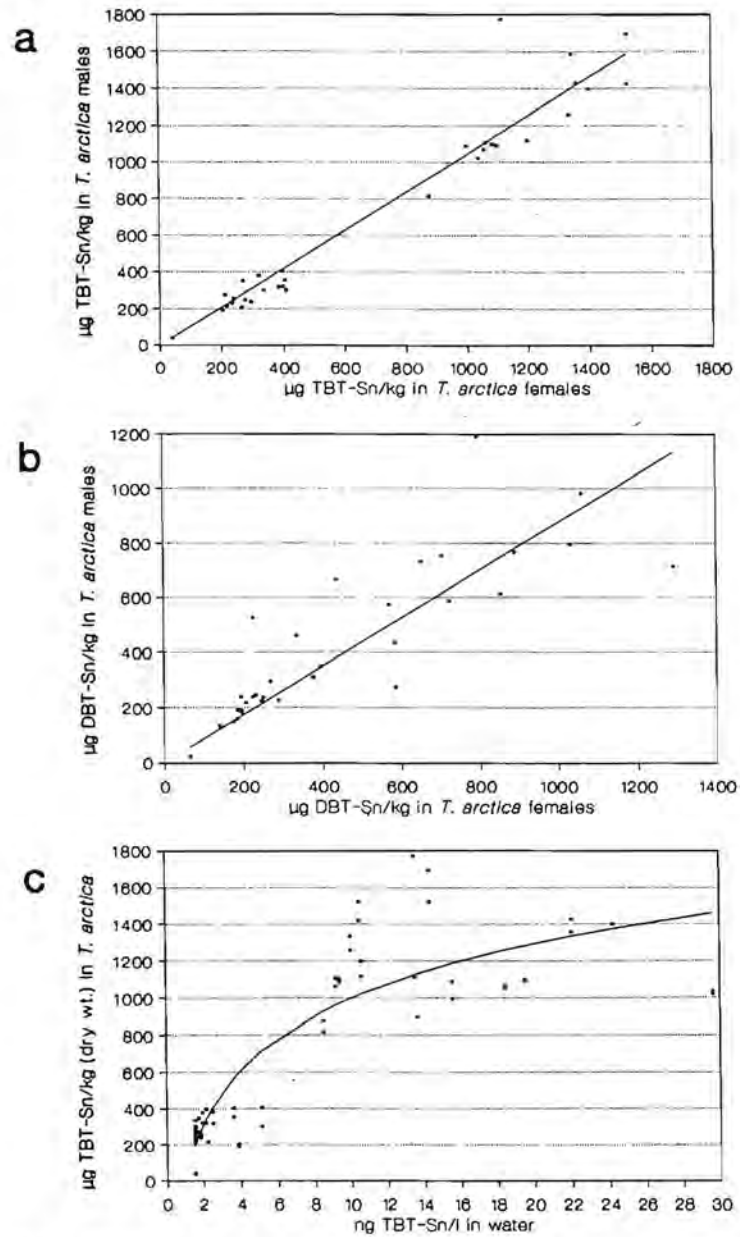


Fig. 6. *Trivia arctica*. Relationship between butyltin concentrations in males and females (a, b) and between TBT concentration in ambient sea water and TBT body burden. (a) TBT fraction in males vs TBT fraction in females (·) and calculated linear correlation (continuous line):  $\mu\text{g TBT-Sn/kg in males} = 1.004 \cdot \mu\text{g TBT-Sn/kg in females}$  ( $n = 32$ ;

Calculated biological concentrations factors (BCF; dry tissue/water) for TBT were also comparable: at Méan Mélen the BCF-values were  $2.0 \cdot 10^4$  to  $2.4 \cdot 10^4$  in *Trivia arctica* and *T. monacha*, at Roscoff harbor  $6.0 \cdot 10^4$  to  $9.5 \cdot 10^4$  for *T. arctica* and  $4.4 \cdot 10^4$  to  $1.1 \cdot 10^5$  for *T. monacha*. Moderately TBT polluted sites, e.g. Ile Verte at Roscoff, take an intermediate position concerning TBT body burden and BCFs.

A correlation analysis proved sex-related differences for the accumulation of butyltin compounds in *Trivia arctica* (Fig. 6 a, b). In the field, males contained 104% of the female TBT-Sn and 88% of the female DBT-Sn body burden, respectively. The differences in TBT accumulation are not very pronounced, but for most imposex affected prosobranch species sex-related variations are known. Mostly females contain more TBT than their male counterparts, e.g. in *Nucella lapillus* and *Hinia reticulata* (STROBEN et al., 1992a), but in *Ocenebra erinacea* the same distribution as described here is realized (OEHLMANN et al., 1992a).

For all populations of both *Trivia* species, the three imposex indices (VDS, uncubed RPS and average female penis length) were calculated and correlated with ambient TBT concentrations in water (Fig. 7 a - c) and with TBT body burden in *T. arctica* (Fig. 8 a - c). A correlation check between TBT body burden and imposex development was not possible for *T. monacha* because mostly not enough specimens for organotin analysis were found. As shown in Fig. 7 and 8, it is obvious that TBT indices increase with ambient TBT pollution. The threshold for imposex is comparable in *T. arctica* and *T. monacha* at concentrations of 1.5 to 1.8 ng TBT-Sn/l, that is the detection limit for this xenobiotic. *T. monacha* exhibits a slightly higher TBT sensitivity in comparison to *T. arctica*. The threshold for imposex development is a little lower and VDS development is equilibrated at values of 4.0 at lower concentrations (Fig. 7 a). In areas likely to be contaminated by TBT *T. monacha* attains higher values for the uncubed RPS (Fig. 7 b) and average female penis length (Fig. 7 c).

Because ambient TBT concentrations in coastal waters exhibit great seasonal and tidal changes (WALDOCK et al., 1987; OEHLMANN et al., 1992c) it is more reliable to correlate imposex expression with TBT body burden and not with TBT concentrations in water. As shown in Fig. 8 a - c a correlation analysis between TBT body burden in *T. arctica* and imposex indices is

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$r = 0.968$ ;  $p < 0.001$ ). (b) DBT fraction in males vs DBT fraction in females (-) and calculated linear correlation (continuous line):  $\mu\text{g DBT-Sn/kg in males} = 0.877 \cdot \mu\text{g DBT-Sn/kg in females}$  ( $n = 32$ ;  $r = 0.821$ ;  $p < 0.001$ ). (c) TBT body burden vs TBT concentration in water (-) and calculated logarithmic correlation (continuous line):  $\mu\text{g TBT-Sn/kg in } T. arctica = 20.94 + 425.3 \cdot [\ln(\text{ng TBT-Sn/l})]$  ( $n = 61$ ;  $r = 0.894$ ;  $p < 0.001$ ).

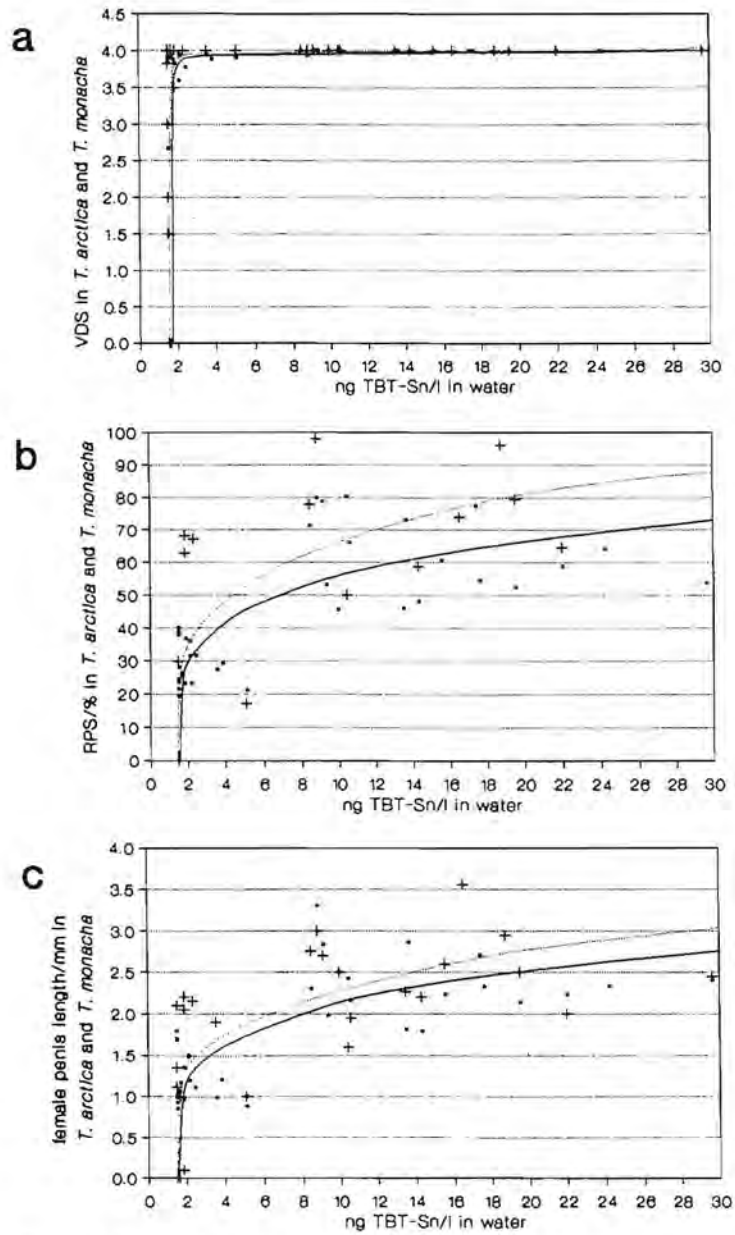


Fig. 7. Relationship between tributyltin (TBT) concentrations in ambient sea water and imposex indices in *Trivia arctica* (· and continuous line) and *T. monacha* (+ and dashed line). (a) VDS index. (b) Uncubed RPS index. (c) Average female penis length. Lines are eye-fitted.



highly significant in females. In males there is no dependence of average penis length and TBT body burden (male penis length/mm =  $4.20 - 0.0003 \cdot \mu\text{g TBT-Sn/kg}$  in males;  $n = 33$ ;  $r = 0.273$ ;  $p > 0.1$ ). In highly polluted areas VDS development comes into equilibration with a value of 4.0. The uncubed RPS or the female penis length allows in this case to discriminate TBT exposure of such severely contaminated populations. For TBT biomonitoring, the VDS plus uncubed RPS index or combined with the female penis length should be determined for *T. arctica* and *T. monacha* to allow a reliable monitoring of TBT exposure.

### 3.5 Comparison of organotin accumulation and TBT sensitivity in *Trivia arctica*, *T. monacha* and *Nucella lapillus*

The dogwhelk *Nucella lapillus* is one of the first imposex affected prosobranch species which was introduced for TBT biomonitoring. Because the data base concerning organotin accumulation and TBT sensitivity is very broad for dogwhelks, they are used as a reference species for comparison with potential indicators of TBT pollution (OEHLMANN et al., 1991, 1992 a, b; STROBEN et al., 1992 a, b). At slightly and moderately contaminated sites (Méan Mélen and Ile Verte) *Trivia arctica* and *N. lapillus* accumulate TBT and DBT at a comparable extent. At Roscoff harbor with its high TBT exposure *T. arctica* accumulates less TBT and DBT than dogwhelks (Fig. 9 a, b).

In order to compare the TBT sensitivity of both *Trivia* species with *Nucella lapillus* on the basis of imposex development, VDS, uncubed RPS and average female penis length of natural *T. arctica* and *T. monacha* populations were analysed and plotted against the corresponding values of *N. lapillus*. Logarithmic regressions were calculated (Fig. 10 a - c) and found to be highly significant ( $p < 0.001$ ).

Low TBT exposure results in a higher increase of all TBT biomonitoring parameters in *Nucella lapillus* compared to both *Trivia* species. But even at slightly TBT exposed areas, female *T. arctica* and *T. monacha* develop obvious imposex characteristics. At severely polluted regions, VDS development in both mesogastropod species is retarded and thus VDS, uncubed RPS and female penis length values in *N. lapillus* are higher. With the exception of VDS (Fig. 10 a) *T. monacha* attains higher values for uncubed RPS (Fig. 10 b) and female penis length (Fig. 10 c) than *T. arctica* if plotted against the corresponding values of *N. lapillus*. The VDS situation bases on a single sample of *T. arctica* at Méan Mélen with the value of 2.67, while *T. monacha* was found at this station more frequent exhibiting VDS values between 0.00 and 3.00 (mean 1.90). At moderately and severely polluted sites the VDS development of both *Trivia* species shows no significant dissimilarities (Fig. 10 a).

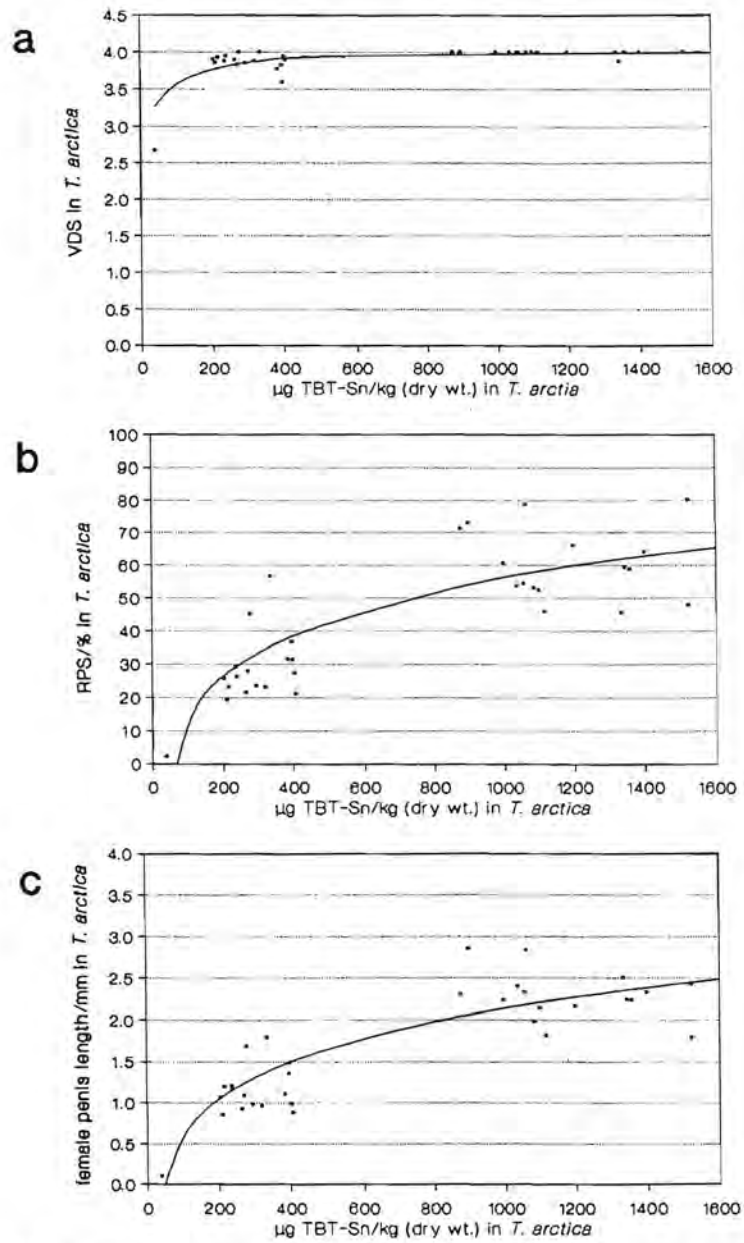


Fig. 8. *Trivia arctica*. Relationship between TBT body burden of females and imposex indices. (a) VDS index (·) with calculated logarithmic correlation (continuous line):

$$\text{VDS} = 2.70 + 0.190 \cdot [\ln(\mu\text{g TBT-Sn/kg in females})]; n = 33; r = 0.690; p < 0.001.$$

(b) Uncubed (RPS) index (·) with calculated logarithmic cor-

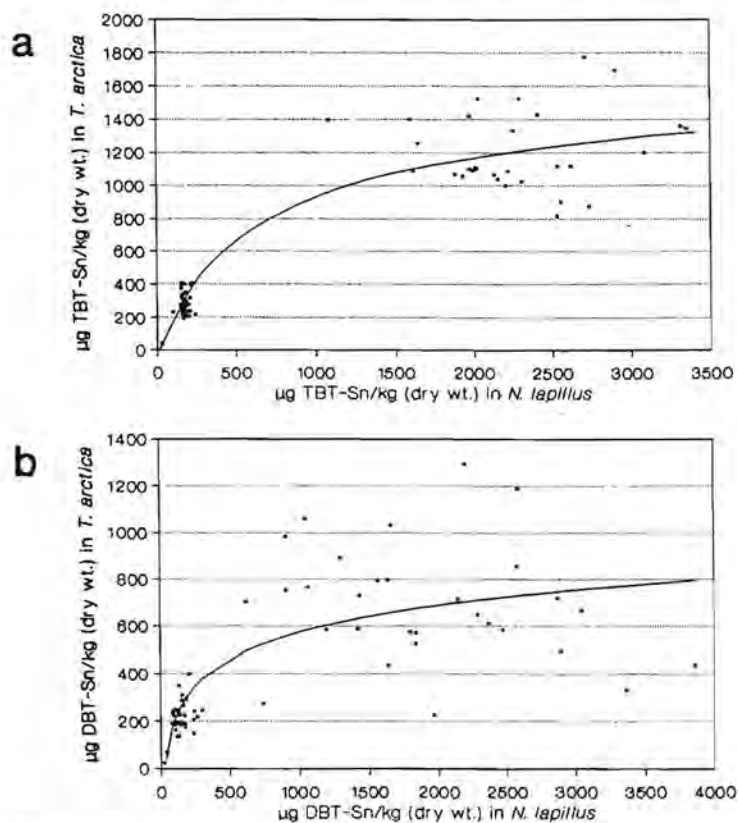


Fig. 9. *Trivia arctica* and *Nucella lapillus*. A time and site correlated statistical analysis of butyltin accumulation in both species. (a) TBT body burden in *T. arctica* vs *N. lapillus* (·) and calculated logarithmic correlation (continuous line):  $\mu\text{g TBT-Sn/kg in } T. arctica = -1368.7 + 331.3 \cdot [\ln(\mu\text{g TBT-Sn/kg in } N. lapillus)]$ ;  $n = 63$ ;  $r = 0.925$ ;  $p < 0.001$ . (b) DBT body burden in *T. arctica* vs *N. lapillus* (·) and calculated logarithmic correlation (continuous line):  $\mu\text{g DBT-Sn/kg in } T. arctica = -555.0 + 163.5 \cdot [\ln(\mu\text{g DBT-Sn/kg in } N. lapillus)]$ ;  $n = 63$ ;  $r = 0.761$ ;  $p < 0.001$ .

relation (continuous line): uncubed RPS =  $-77.38 + 19.233 \cdot [\ln(\mu\text{g TBT-Sn/kg in females})]$ ;  $n = 33$ ;  $r = 0.836$ ;  $p < 0.001$ . (c) Average female penis length (·) with calculated logarithmic correlation (continuous line): average female penis length in mm =  $-2.67 + 0.692 \cdot [\ln(\mu\text{g TBT-Sn/kg in females})]$ ;  $n = 33$ ;  $r = 0.875$ ;  $p < 0.001$ .

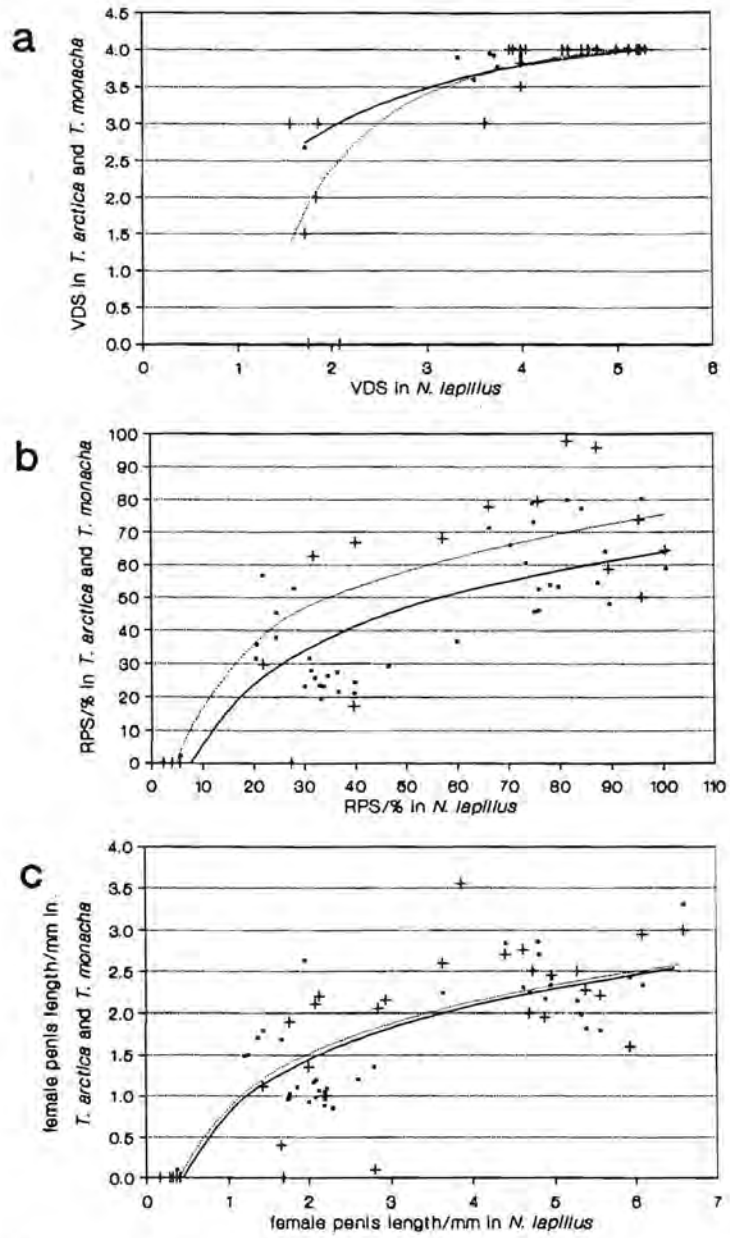


Fig. 10. *Trivia arctica*, *T. monacha* and *Nucella lapillus*. A time and site correlated statistical analysis of relative TBT sensitivity measured as increase of imposex parameters in both *T.* species vs *N. lapillus* with calculated logarithmic correlations. (a) VDS in *T. arctica* (•, continuous line) and *T. monacha* (+, dashed line) vs *N. lapillus*: VDS in *T. arctica* =

#### 4. Discussion

The differences of our descriptions of the genital systems to the results of FRETTER (1946) are already mentioned in chapter 3. It seems, that she has confused the spermtails in the seminal vesicle with cilia. The absence of amoebocytes in the organs of our analysed specimens may depend on morphological changes of the genital tract during the reproductive cycle. The species-specific differences of the penes were already mentioned by THIELE (1931) and LÉBOUR (1933). It is not clear why FRETTER (1946) has not detected the female gonopericardial duct, because this formation, also typical for the female genital system of different genera like *Littorina*, *Truncatella*, *Crepidula*, *Nucella*, *Hinia* etc. (FRETTER & GRAHAM, 1962), can be detected easily.

We interpret the median position of the female genital porus which differs from most of the meso- and neogastropods as relict of a former open system according to FRETTER (1946) and FRETTER & GRAHAM (1962). This porus has an analogue in the opening of the prostate. The South African species *Trivia ovulata*, *T. millardi* and *T. verhoefi* (GOSLINER & LILTVED, 1982) show the same phenomenon and furthermore a vas deferens which closes only secondarily. The diameter of the female opening is correlated with the size of the egg capsules (FRETTER & GRAHAM, 1962).

Against THIELE (1931), we consider the blind ending diverticula of the oviduct not as specific for the genus because they are lacking in *Trivia arctica*. Their presumed function as a disintegrating organ for superfluous spermatozoa seems to be a functional homology with the ingestion gland of *Nucella lapillus* (OEHLMANN et al., 1988) and other neogastropods (FRETTER, 1941; OEHLMANN et al., 1992a, b; STROBEN et al., 1992a).

This study is the first detailed description of imposex expression in mesogastropods. In the literature the mesogastropod genera *Hydrobia*, *Rissoina*, *Rissoa* and *Pterotrachea* are suspected to demonstrate imposex. For *Aporrhais pes-pelecani* this was clearly shown (FIORONI et al., 1991). Female

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2.38 + 1.041 · [ln(VDS in *N. lapillus*)]]; n = 39; r = 0.788; p < 0.001). VDS in *T. monacha* = 0.42 + 2.328 · [ln(VDS in *N. lapillus*)]]; n = 32; r = 0.805; p < 0.001). (b) Uncubed RPS in *T. arctica* (-, continuous line) and *T. monacha* (+, dashed line) vs *N. lapillus*: uncubed RPS in *T. arctica* = 51.8 + 24.92 · [ln(uncubed RPS in *N. lapillus*)]]; n = 39; r = 0.719; p < 0.001). Uncubed RPS in *T. monacha* = -34.74 + 23.60 · [ln(uncubed RPS in *N. lapillus*)]]; n = 17; r = 0.812; p < 0.001). (c) Average female penis length in *T. arctica* (-, continuous line) and *T. monacha* (+, dashed line) vs *N. lapillus*: female penis length/mm in *T. arctica* = 0.76 + 0.919 · [ln(female penis length/mm in *N. lapillus*)]]; n = 39; r = 0.747; p < 0.001). Female penis length/mm in *T. monacha* = 0.92 + 0.870 · [ln(female penis length/mm in *N. lapillus*)]]; n = 32; r = 0.820; p < 0.001).

Littorinidae have an enlarged ciliated groove with an ovipositor which is homologous to the pallial vas deferens and the penis base and can be interpreted as a natural case of imposex (FIORONI et al., 1991). But the females of different *Littorina* species never develop a penis additionally, even on extremely TBT polluted shores or after TBT exposure in laboratory studies and field experiments (U. DEUTSCH, pers. comm.). The reasons for missing imposex in Littorinidae are unknown. TBT caused changes in steroid titres are thought to be responsible for the imposex development in *Nucella lapillus* (SPOONER et al., 1991; STROBEN et al., 1991) and *Hinia reticulata* (STROBEN et al., 1991). *Trivia arctica* and *T. monacha* as exhibitors of imposex seem to demonstrate the same TBT-induced hormonal changes. Because the species-specific penis morphology occurs in *Trivia* females, TBT effects on the expression of the genome are probable, perhaps mediated by steroids.

The morphology of the imposex affected specimens described here for the first time could not be detected by FRETTER (1946). Imposex as a common phenomenon within neogastropods is only known since 1970, when TBT-based marine antifouling paints came into wide use, especially on pleasure and recreational crafts. FIORONI et al. (1990) mentioned for the first time imposex effects in *Trivia arctica* and *T. monacha*. GOSLINER & LILTVED (1982) described in *T. millardi* a "true case of hermaphroditism", which seems to be in reality the imposex stage 4.

The homology of the male parts of imposex with the corresponding organs of the male (neglected by BLABER, 1970) is not only valid for *Trivia*, but could be described also for other species as e.g. *Nucella lapillus*, *Ocenebra erinacea*, *Ocenebrina aciculata* and *Hinia reticulata* (STROBEN et al., 1989, 1992a; FIORONI et al., 1990, 1991; OEHLMANN et al., 1991, 1992a, b).

Most stages and types represented in Fig. 1 have been found in *Nucella lapillus*; fewer stages can be detected in *Trivia arctica* and *T. monacha*, as shown on Fig. 1. The final point of imposex development in both *Trivia* species is stage 4. The general scheme of imposex development in prosobranchs (first introduced by FIORONI et al., 1991) proved its validity also in describing imposex evolution in *T. arctica* and *T. monacha*.

Neither TBT-induced sex change nor sterilization was found in *Trivia arctica* and *T. monacha*, but occurs in muricid gastropods. A sex change from female to male is known for *Nucella lapillus* (GIBBS et al., 1988; OEHLMANN et al., 1991) and *Ocenebrina aciculata* (FIORONI et al., 1991; OEHLMANN et al., 1992b). Sterilization is either caused by a blockade of the pallial oviduct, as described for *N. lapillus* (GIBBS et al., 1987; OEHLMANN et al., 1991), *N. lima* (SHORT et al., 1989), *N. lamellosa* (BRIGHT & ELLIS, 1990), and *Thais haemastoma* (SPENCE et al., 1990) or by a splitted bursa copulatrix and capsule gland as in *Ocenebra erinacea* (GIBBS et al., 1990; OEHLMANN et al., 1992a)

and *Urosalpinx cinerea* (GIBBS et al., 1991). Thus either deposition of egg capsules or copulation and egg capsule formation in the oviduct are prevented. *Ocenebrina aciculata* is the only species which exhibits both forms of sterilization (OEHLMANN et al., 1992b).

The ovary, pallial glands, and female opening in *Trivia arctica* and *T. monacha* are unaffected. Copulation and deposition of egg capsules are possible, and consequently restrictions of fertility are not obvious. But it should be stressed that higher imposex stages of both *Trivia* species – as in *Ocenebra erinacea* (OEHLMANN et al., 1992a), *Ocenebrina aciculata* (OEHLMANN et al., 1992b) and *Hinia reticulata* (STROBEN et al., 1992a) – shows a tendency towards diminution of the female glands, which may reduce future reproductive success. Further work on this subject has to be done.

As for other imposex affected prosobranch species (FIORONI et al., 1991), the spatial distribution of imposex in relation to boating activity suggests that both *Trivia* species have potentials as bioindicators of TBT contamination. In *T. arctica* a significant correlation ( $p < 0.001$ ) between TBT body burden and the three imposex indices described (VDS, uncubed RPS, female penis length) was found (Fig. 8a - d). Imposex development is obviously correlated with TBT concentrations in ambient sea water as shown for *T. arctica* and *T. monacha* in Fig. 7a - c. We recommend the VDS index as the best index for TBT biomonitoring (OEHLMANN et al., 1991, 1992a; STROBEN et al., 1992a, b). Only in moderately and severely polluted areas, the uncubed RPS index or the female penis length may be used as secondary parameters because here the VDS index comes into equilibration with a value of 4.0. These indices discriminate TBT exposures of such highly contaminated sites.

For imposex affected neogastropods sex-related differences of TBT and DBT content are described. In *Nucella lapillus* and *Hinia reticulata* (STROBEN et al., 1992a) females accumulate significantly more TBT and DBT than males, perhaps as a consequence of greater reproductive effort in females, resulting in higher food consumption and thus of higher TBT and DBT uptake via contaminated food. In *Ocenebra erinacea* (OEHLMANN et al., 1992a) and *Trivia arctica*, females demonstrate a somewhat lesser TBT body burden compared to males, but a higher content of DBT. This might be a result of a more effective TBT metabolism (debutylation to DBT) in females.

The biological concentration factor (BCF) for TBT in *Trivia arctica* and *T. monacha* are comparable with values reported for *Ocenebra erinacea* (OEHLMANN et al., 1992a) and *Hinia reticulata* (STROBEN et al., 1992b), but lesser than TBT-BCFs in *Nucella lapillus* (STROBEN et al., 1992b) and especially *Ocenebrina aciculata* (OEHLMANN et al., 1992b). The dogwhelk *N. lapillus* accumulates more TBT and DBT than *T. arctica* (Fig. 9a, b).

Both *Trivia* species are suitable bioindicators, but *Nucella lapillus* as the best analysed reference indicator exhibits greater TBT sensitivity, particularly in highly polluted areas. Ambient water concentrations of 1.5 to 1.8 ng TBT-Sn/l are the threshold for imposex development in *T. arctica* and *T. monacha*. This value is slightly higher than in *N. lapillus*; GIBBS et al. (1987) report a threshold TBT concentration for penis development in female dogwhelks below 1 ng TBT-Sn/l. It has to be concluded that *T. arctica* and *T. monacha* are suitable bioindicators for TBT pollution, nevertheless *Hinia reticulata* and dogwhelks are more frequent and thus the established TBT biomonitoring species. In regions where dogwhelks are missing, e.g. the Mediterranean, *T. monacha* can replace *N. lapillus* in TBT survey programmes.

### Zusammenfassung

Die zwischen 1988 und 1992 an der bretonischen und normannischen Küste gesammelten Mesogastropoden *Trivia arctica* und *T. monacha* weisen Imposex (= Pseudohermaphroditismus) als Folge von Tributylzinn-(TBT-)Belastung auf; d.h. zusätzlich zum weiblichen Geschlechtssystem werden männliche Traktanteile ausgebildet. Neben wenigen normalen Weibchen (Stadium 0) konnten unterschiedliche Imposexstadien gemäß der Systematisierung von FIORONI et al. (1991) (Stadien 3a, 3b, 4 bei *T. arctica* und 3b, 4 bei *T. monacha*) (Abb. 1) nachgewiesen und erstmals rasterelektronenmikroskopisch dokumentiert werden. Zusätzliche Veränderungen im Genitaltrakt, wie z.B. Gewebshyperplasien des Vas deferens, ein gewundener Ovidukt, sowie die Ausbildung eines zwei- oder dreispitzigen Penis oder von 2 bis 5 Penes wurden beobachtet. Weder TBT-induzierte Sterilisierung, noch ein Geschlechtswechsel traten auf. Die TBT-Akkumulation im Weichkörper wird dargestellt und geschlechtsspezifische Unterschiede beschrieben. Der VDS (Vas deferens Sequenz) Index, der nicht kubizierte RPS (Relativer Penis-Größen) Index und die durchschnittliche weibliche Penislänge der Populationen wurden ermittelt; diese erwiesen sich als abhängig von der TBT-Belastung des Meerwassers und der TBT-Körperbelastung der Tiere. Eine statistische Analyse natürlicher Populationen von *T. arctica*, *T. monacha* und *Nucella lapillus* ermöglicht den Vergleich der spezifischen TBT-Sensitivität der drei TBT-Bioindikatoren. *N. lapillus* weist den niedrigsten Schwellenwert für die Imposexauslösung und die größte TBT-Sensitivität auf. Beide untersuchten *Trivia*-Arten erwiesen sich als geeignete TBT-Bioindikatoren.

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