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TBT effects on the female genital system of *Littorina littorea*: a possible indicator of tributyltin pollution

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Abstract

Specimens of the prosobranch *Littorina littorea* (L., 1758) collected along the East Frisian North Sea coast in summer 1993 exhibited alterations of the pallial oviduct termed as intersex in response to tributyltin (TBT) pollution. The range of TBT body burden was between 150.9 and 1289.5 μg as Sn kg^{-1} (dry wt.). Five stages of intersex development (0–4) could be distinguished and are documented with scanning electron micrographs. In stages 2–4, which can be found in the direct vicinity of harbours and marinas, the morphological malformations of the oviduct inhibit successful copulation and capsule formation, resulting in sterilization.

The intersex index (ISI, calculated as the average intersex stage of a population) and the average prostate length of females were used as parameters for the determination of intersex intensities in the populations. Both indices show significant and positive correlations to the TBT body burden of *L. littorea* and are promising parameters for TBT biomonitoring. A comparison of TBT bioconcentration factors with populations from England and France indicates that the threshold concentration for intersex development is in the range of 15 ng TBT as Sn/l.

Morphometric analyses of the midgut gland revealed no significant differences between sampling stations. In the ovary a retardation and blockage of maturation (atresia) was observed in populations close to harbours. Lytic processes in ovary follicles were observed not only at TBT exposed sites but also at reference stations.

Introduction

Tributyltin (TBT) compounds used as biocides in antifouling paints and in various other formulations are known to produce a variety of malformations in marine animals, with molluscs being one of the most TBT-sensitive groups of invertebrates (for review Bryan & Gibbs, 1991). TBT-induced detrimental effects in Great Britain and France, e.g. malformations of oyster shells and the imposex phenomenon of prosobranchs, became evident in the 1980s. Consequently, legislative restrictions were drawn up to reduce TBT contamination in coastal waters. The imposex phenomenon of prosobranchs has been successfully used as a biomonitoring system to determine the degree of environmental TBT pollution (e.g. Gibbs *et al.*, 1987; Oehlmann *et al.*, 1993).

The established European imposex species for TBT biomonitoring (e.g. *Nucella lapillus* (L.), *Hinia reticulata* (L.), *Ocenebra erinacea* (L.)) are absent on the German North Sea coast or can only be found in restricted areas. The periwinkle *Littorina littorea* (L.) is the only prosobranch which is very common and can be sampled in sufficient numbers. In the present study the potential of *L. littorea* was evaluated for TBT biomonitoring.

Littorina littorea is a shallow water species which lives on rocky and sandy shores. Though the bulk of the population occurs intertidally, some specimens can be found up to a depth of 15 m. Their geographical distribution ranges from Asturia to northern Norway in the

east Atlantic and from New Jersey to Greenland in the western Atlantic (Nordsieck, 1968; Graham, 1988). The species feeds largely on epilithic algae and vegetable detritus (Taylor & Miller, 1989), although it may occasionally feed on dead animal matter (Matthiessen *et al.*, 1991). The sexes are separate and, after reaching maturity at shell heights of 10–12 mm and at an age of 12–18 months, females produce about 500 planktonic egg capsules each containing 1–5 eggs. After 5–6 days the animals hatch as free swimming veliger larvae drifting with the plankton. Metamorphosis occurs after 4–7 weeks (Linke, 1933; Thorson, 1946; Matthiessen *et al.*, 1991). Adults can live for more than 9 years (Heller, 1990) and can reach a shell height of 40 mm (Nordsieck, 1968; Fretter & Graham, 1980).

Materials and methods

More than 500 *Littorina littorea* were collected at 11 stations in summer 1993 along the East Frisian North Sea coast between Emden and Cuxhaven (Fig. 1). Sample sites included marinas, ferry and fishing harbours as well as reference stations far away from shipping activities.

Snails were relaxed using 7% $MgCl_2$ in distilled water and shell and aperture height were measured to the nearest 0.1 mm. The shells were then crushed in a vice to remove the soft tissues and the specimens sexed. External dimensions of the genital tract, including pallial glands of females and males and penis length, were determined to 0.1 mm. *L. littorea* is often parasited by various trematodes such as *Cryptocotyle lingua*, *Himasthla elongata* and *Renicola roscovita* (Lauckner, 1980). Because parasitation causes suspicious modifications of the genital system, including gonad and midgut gland, infected animals were excluded from morphometric and histological analyses.

As a result of TBT exposure the winkle *L. littorea* exhibits malformations of the pallial oviduct which were termed as intersex in contrast to the imposex phenomenon of neogastropod species. Intersex is defined as any disturbance of the congruity between gonad and genital tract, while imposex is a superimposition of male sex organs (penis and/or vas deferens) on females (Smith, 1971). During intersex development no superimposition of male characters occurs but the organs of the pallial oviduct are modified towards a male morphological structure. For all populations two indices for the measurement of intersex intensity were employed: (1) Intersex index (ISI) is calculated as the average intersex stage (according to Fig. 2) of a population; (2) Average prostate length of females.

For serial sections and scanning electron microscopy, specimens were fixed in Bouin's fluid and then preserved in 70% ethanol. After embedding in paraplast, serial sections (7 μm) were made and stained with haemalun-chromotrope (Romeis, 1989). Specimens for scanning electron microscopy were critical-point dried.

For histopathological analyses, 10 specimens from every site were fixed in Bouin's fluid and embedded in Technovit (hydroxymethylmethacrylate). 5 μm sections were stained with H & E and PAS (Romeis, 1989). The areas of midgut gland tubules, lumina, crypt- and digestive cells were measured using a graphic tablet as described by Vega *et al.* (1989). Only tubules in the holding phase were used for morphometric measurement (Langton, 1975).

The determination of TBT was based largely on Ward *et al.* (1981), modified according to Stroben *et al.* (1992b). Five to seven snails were homogenized in stoppered tubes, and 10 ml of concentrated HCl (Merck 'suprapur') were added. After shaking for 30 min, the homogenate was extracted with 10 ml of hexane (pesticide grade) on an automatic shaker for 30 min and then centrifuged. TBT as Sn (TBT-Sn) was determined in the hexane extract after shaking with 3 ml 1N NaOH for 3 min using a Perkin-Elmer HGA-500 attached to a Perkin-Elmer 5000 AAS (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, National Research Council of Canada) was also analysed. Our own results were within the standard deviation of the certified values for the CRM. Recovery factors were 78.0±12.6%. The detection limit (3σ) in a single sample was 6.7 ng TBT-Sn.

Results

Genital system of Littorina littorea

The anatomy and histology of the normal female and male genital tract of *Littorina littorea* are described in detail by Linke (1933), Fretter & Graham (1962), Fretter (1980) and Reid (1986a, 1989) In both sexes gonadial, renal and pallial sections of the genital tract can be distinguished. The testis and ovary are large and diffuse organs which lay in the upper parts of the visceral mass, branching between the tubules of the

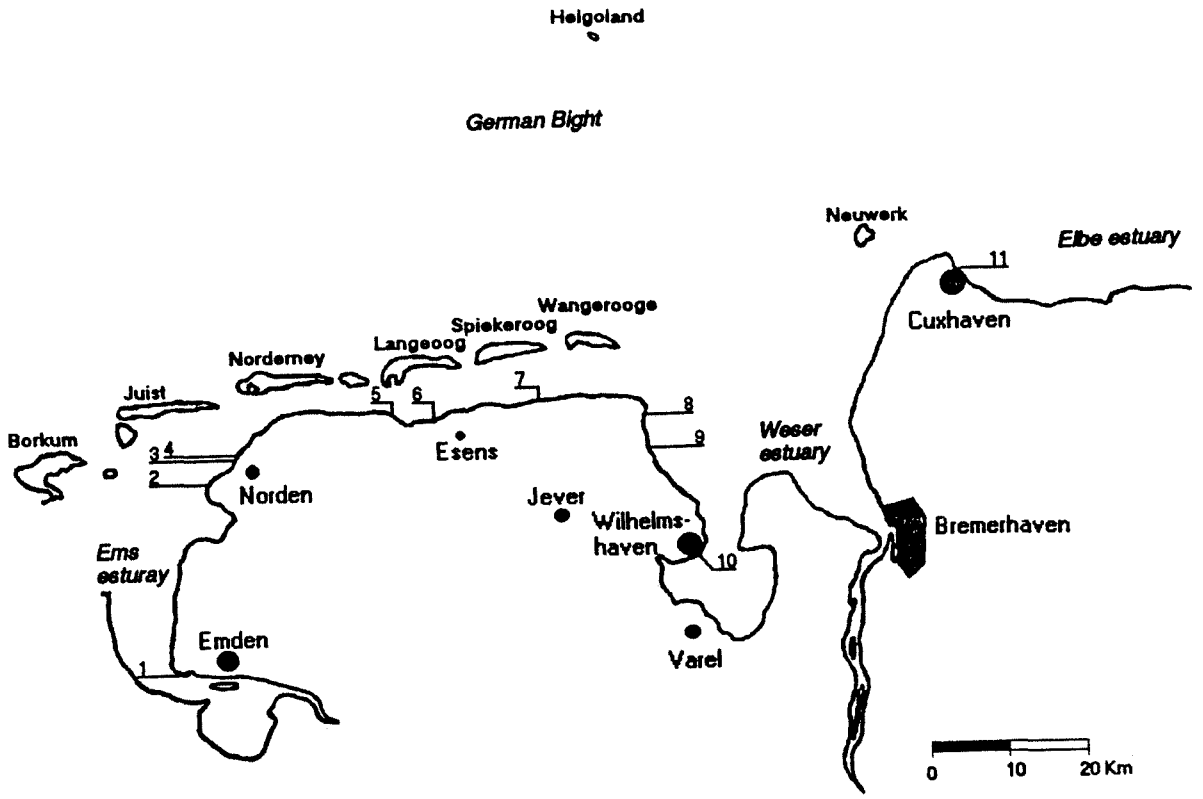

Fig. 1. Map of East Frisian coast showing stations sampled in summer 1993. (1) Knock, near Emden, (2) Westermarsch II, (3) Norddeich: outside harbour, (4) Norddeich: marina, (5) Dornumersiel: marina, (6) Bensersiel: marina, (7) Harlesiel: 800 m outside ferry harbour, (8) Horumersiel: entrance of harbour, (9) Hooksiel: entrance of outer harbour (the marina is separated from the sampling site by a sluice), (10) Wilhelmshaven: Nassauhafen, (11) Cuxhaven: ferry harbour.

midgut gland. The most proximal parts of the male and female genital tract are the gonadial sections of the vas deferens and oviduct. These run parallel to the posterior aorta at the columellar side of the visceral hump and continue in the rather short renal section.

The most elaborated part of the reproductive tract of *L. littorea* is the pallial section of males and females, which originates ontogenetically from an infolding of the mantle epithelium. The pallial section of males is an open sperm groove for its entire length. In the most proximal part the walls of the groove are tall and characterised by the development of folds of glandular tissue which form a well developed prostate gland. From the prostate the sperm groove runs forward on the floor of the mantle cavity to the base of the penis behind the right ocular tentacle. The copulatory organ is conical, flattened laterally and carries the sperm groove to its summit along the dorsal edge. On the ventral border of the penis numerous mamilliform penial glands can be found. Their secretions hold the penis in position during copulation.

The pallial section of females is highly developed in order to store sperm, to provide extraembryonic nourishment for the embryos within the capsule and to produce the planktonic egg capsules. It consists of a receptaculum seminis, and albumen, capsule and jelly glands, and is completed by the bursa copulatrix in a ventral position and the vagina (Fig. 2, stage 0). In adult females the pallial oviduct is a closed tube originating from an infolding of the mantle epithelium with a consecutive fusion of its flaps (Fig. 3b, c). The ovoid-shaped vaginal opening is the only aperture towards the mantle cavity. Distally from the vaginal opening an egg channel extends over the floor of the mantle cavity and runs down the right side towards the foot. It ends in the so-called ovipositor (Fig. 3a). The ovipositor is a muscular and glandular organ with the function of launching the egg capsules on their pelagic life phase. Its topographical position, and histologi-

cal and ultrastructural properties are identical with the base of the male penis which remains after the periodic shedding of the copulatory organ during the sexual repose phase. Consequently, the male penis base and the ovipositor were deemed to be homologous by Fioroni *et al.* (1991).

Intersex development and TBT

Especially in direct proximity to harbours and marinas we found malformations of the female genital tract which were termed as intersex (see above). The female specimens affected by intersex were either characterised by the development of male features on the female pallial organs (inhibition of the ontogenetic closure of the pallial oviduct) or female sex organs were supplanted by the corresponding male formations. The intersex phenomenon of *L. littorea* is a gradual transformation of the female pallial tract, which can be described by an evolutive scheme with five stages (0 to 4) (Fig. 2).

Stage 0. Normal female without intersex characteristics (Fig. 3a). The entire pallial oviduct is a closed tube (Fig. 3b, c). This stage was found in 200 specimens (= 77.4% of the 258 females analysed).

Stage 1. The bursa copulatrix is split ventrally, exposing its internal lobes (Fig. 3d). This malformation inhibits successful copulation because sperm can be spilled into the mantle cavity (Fig. 3e) (27 specimens = 10.5% of all analysed females).

Stage 2. The entire pallial oviduct (bursa copulatrix and jelly gland) is split ventrally (Fig. 3f, 4a). This open structure is a male characteristic because the prostate gland of males is also an open groove. (2 specimens = 0.8% of all analysed females).

Stage 3. The distal part of the pallial oviduct (capsule and jelly gland with bursa copulatrix and vagina) are supplanted by a prostate gland. In some specimens the free edges of the open prostate gland fuse to form a closed tube in order to establish a more female anatomical structure (Fig. 4b, c). The line of fusion of the prostate edges is detectable by its white coloration. The proximal female parts of the pallial oviduct (seminal receptacle and albumen gland) are conserved (28 specimens = 10.9% of all analysed females).

Stage 4. Additionally to the characteristics of stage 3, a penis with an open sperm groove is developed (Fig. 4d, e). This stage was found in a single specimen (= 0.4% of all analysed females).

All analysed intersex females have an ovary with no signs of the onset of spermiogenesis but with signs of a disturbance of oogenesis (c.f. below). Intersex development causes restrictions of the reproductive capability of females. In stage 1 a loss of sperm during copulation is possible and consequently the reproductive success is reduced. Females in stages 2–4 are definitively sterile because the capsular material is spilled into the mantle cavity (stage 2) or the glands responsible for the formation of the egg capsule are missing (stages 3 and 4). Due to female sterility, populations of *L. littorea* can be in decline but are not likely to become extinct because of the planktonic veliger larvae of the species. Veligers produced by populations with lower intersex intensities can guarantee a minimum abundance of periwinkles even at sites suffering from high TBT contamination and reproductive failure.

Two of the 258 analysed females (one in each of stages 1 and 3) were characterised by a coiled renal and gonadial oviduct (Fig. 4f). This condition was interpreted by Smith (1980, 1981a–d) as a mimic seminal vesicle in *Ilyanassa obsoleta* and can also be found in many other imposex-affected species as a result of high TBT exposure (Fioroni *et al.*, 1991).

Contrary to the imposex phenomenon of muricids, sterilization caused by intersex development in *L. littorea* provokes poor recruitment of juveniles but not high female mortality. In *Nucella lapillus* (Gibbs *et al.*, 1987; Oehlmann *et al.*, 1991), *Nucella lima* (Short *et al.*, 1989), *Nucella lamellosa* (Bright & Ellis, 1990) the final stages of imposex development are sterilized due to a blockage of the female opening by proliferating tissues. This leads to an accumulation of abortive egg capsules, to a distension and finally a rupture of the pallial oviduct, causing the death of the female. In *L. littorea*, as in the imposex stage 5 of *Ocenebra erinacea* (Gibbs *et al.*, 1990; Oehlmann *et al.*, 1992) and *Urosalpinx cinerea* (Gibbs *et al.*, 1991), the pallial oviduct is split ventrally. Consequently, these malformations inhibit the formation of egg capsules and prevent their accumulation in the pallial tract. Thus the sex ratio, even in highly polluted periwinkle populations, does not change towards male dominance (χ^2-Test; $p > 0.9$) (Table 1).

For the measurement of intersex intensities in the populations two indices were established. The intersex index (ISI) is the average intersex stage in a popula-

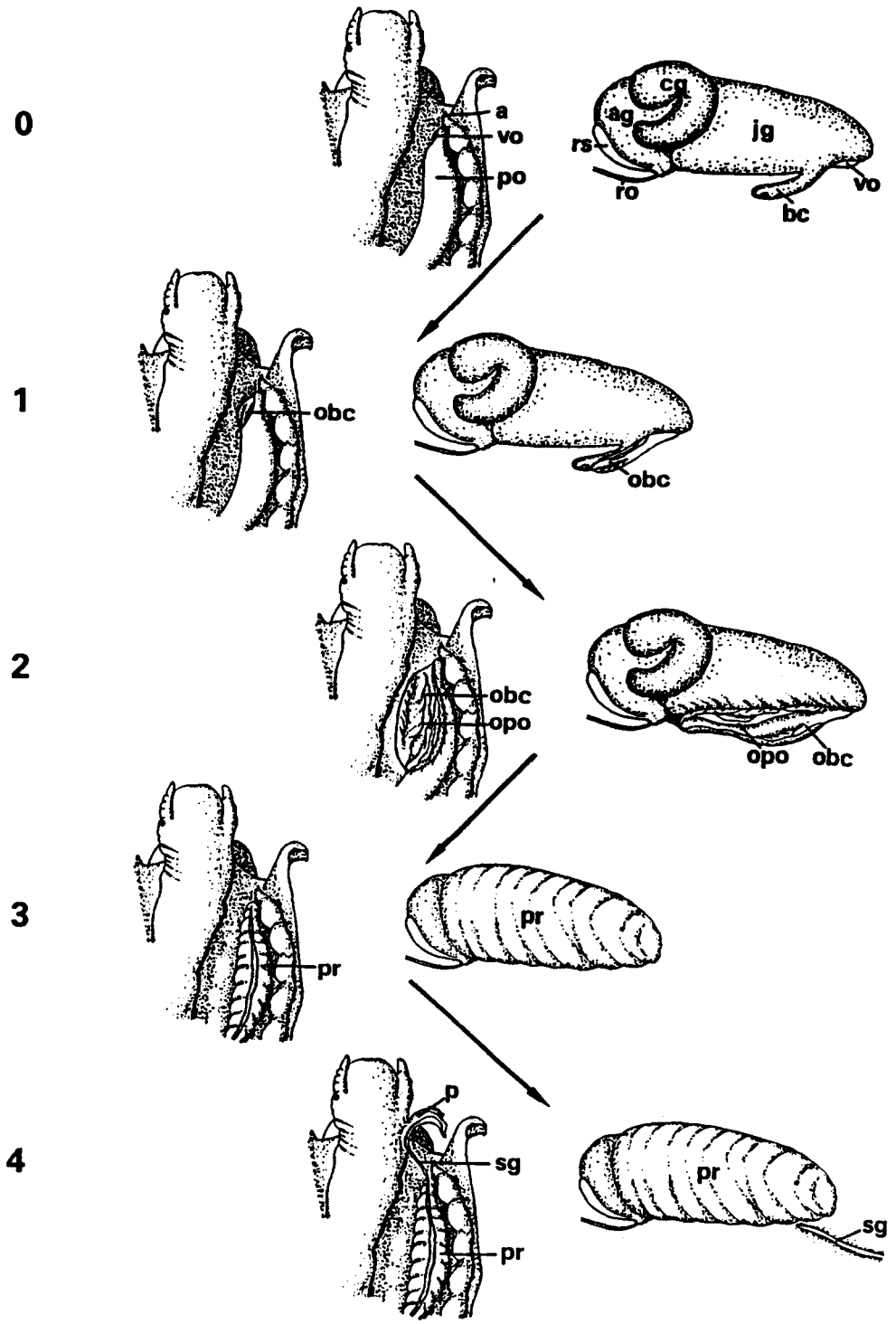

Fig. 2. Littorina littorea. Scheme of intersex development. Dorsal views with opened mantle cavity (left) and lateral views of pallial oviduct (right). Abbreviations: a, anus; ag, albumen gland; bc, bursa copulatrix; cg, capsule gland; jg, jelly gland; obc, open bursa copulatrix; opo, open pallial oviduct; p, penis; po, pallial oviduct; pr, prostate; ro, renal oviduct; rs, receptaculum seminis; sg, sperm groove; vo, vaginal opening.

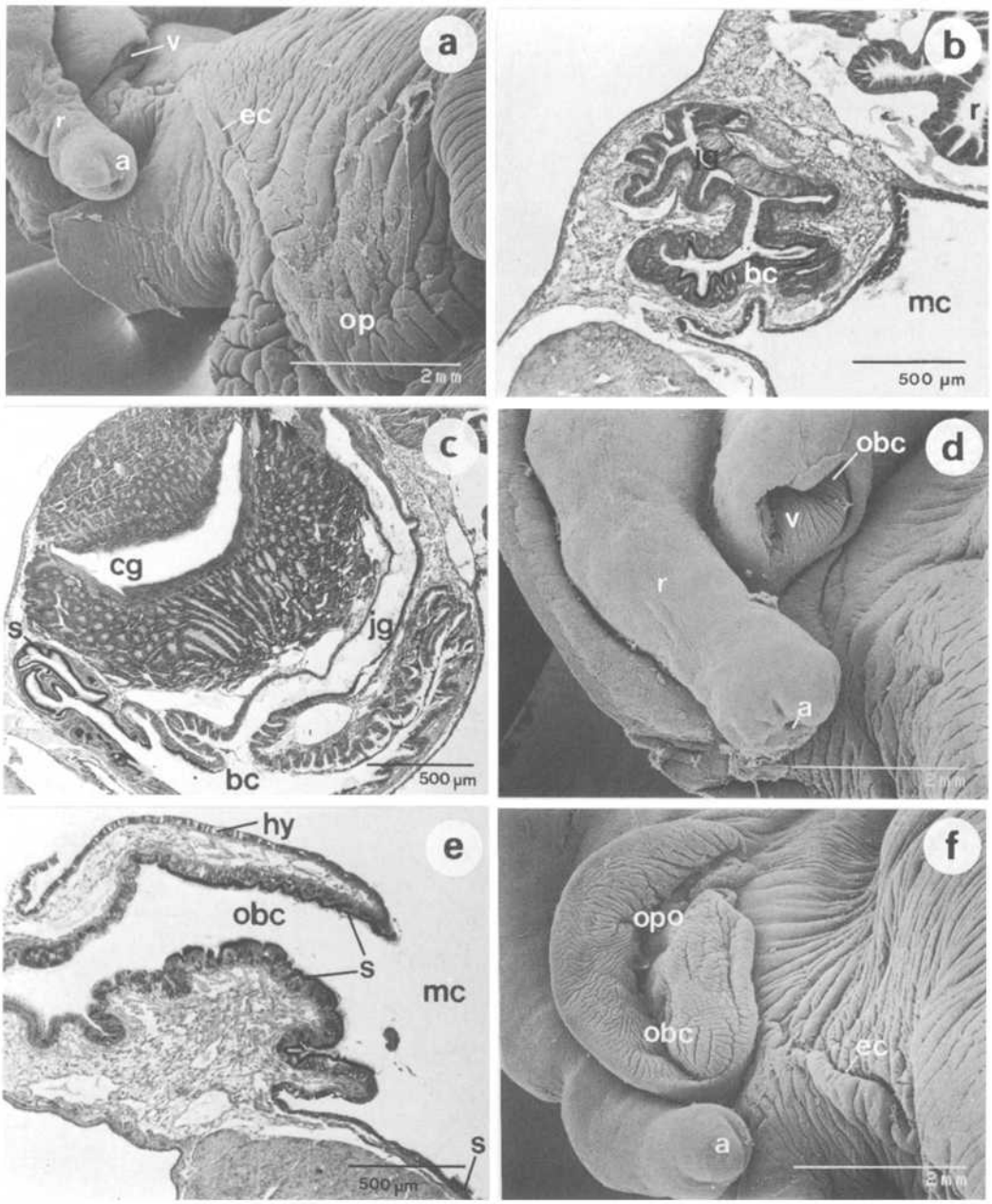

Fig. 3. Littorina littorea. Scanning electron micrographs of the mantle cavity and histological sections of intersex stages 0 to 2. (a) Stage 0 (normal female). (b) Stage 0: transverse section of bursa copulatrix and jelly gland. (c) Stage 0: transverse section of bursa copulatrix, jelly and capsule gland. (d) Stage 1. (e) Stage 1: transverse section of open bursa copulatrix. (f) Stage 2. Abbreviations: a, anus; bc, bursa copulatrix; cg, capsule gland; ec, egg channel; hy, hypobranchial gland; jg, jelly gland; mc, mantle cavity; obc, open bursa copulatrix; op, ovipositor; opo, open pallial oviduct; r, rectum; s, sperm; v, vaginal opening.

tion according to Fig. 2 (Σ intersex stages in a sample $\div$ number of analysed females). A value of 0.0 indicates that only normal females (stage 0) occur and no restrictions of the reproductive capability have to be expected. ISI values above 0 show that intersex-affected females can be found and that reproductive success may be reduced. An ISI of 1.0 indicates that all females exhibit stage 1 and an ISI above 2.0 that most females in a population are sterilized due to intersex development. The second parameter is the average

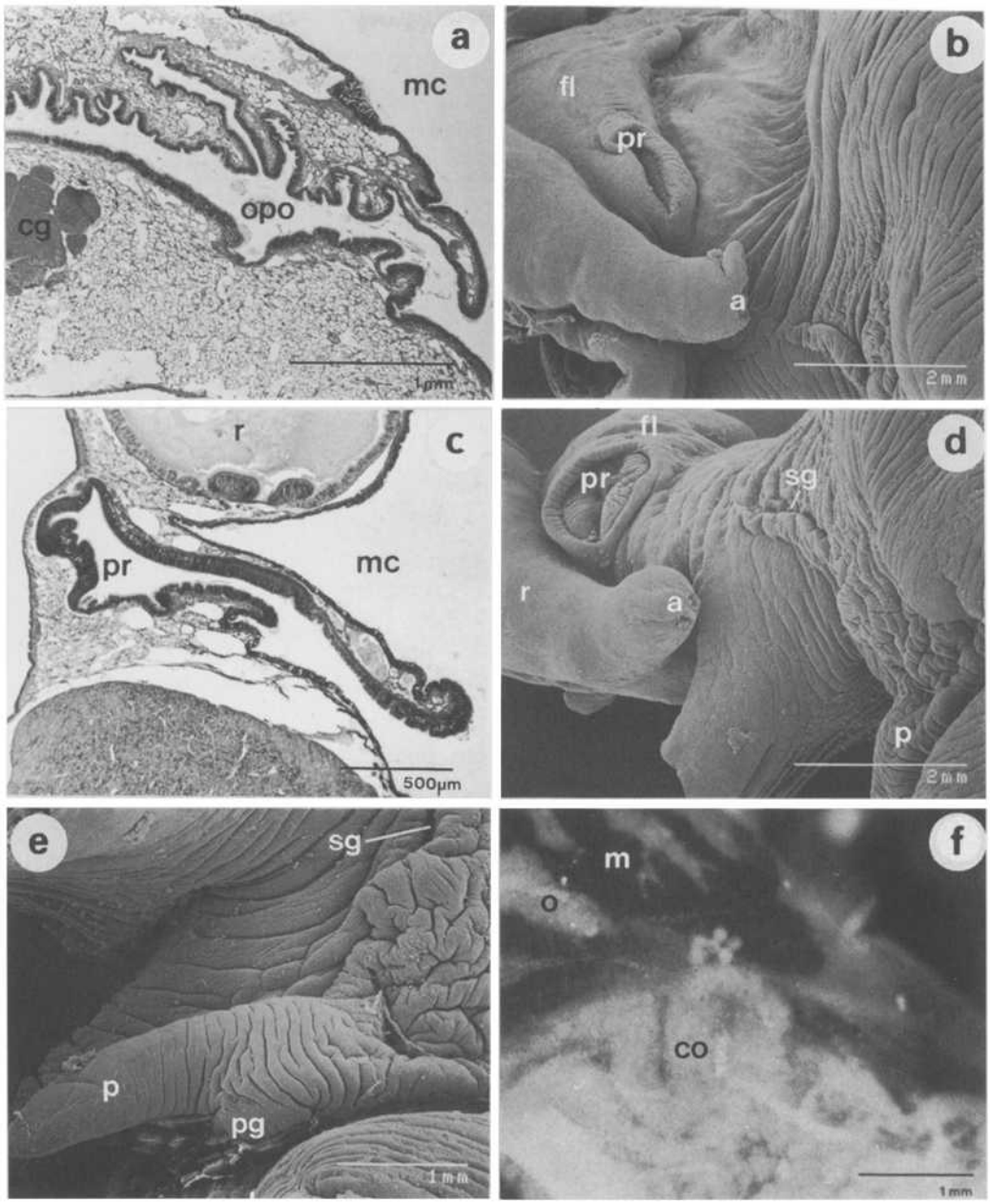

Fig. 4. Littorina littorea. Scanning electron micrographs of the mantle cavity and histological sections of intersex stages 2 to 4. (a) Stage 2: transverse section of open pallial oviduct. (b) Stage 3 with subsequent closed prostate gland. (c) Stage 3: transverse section of prostate gland. (d) Stage 4 with subsequent closed prostate gland. (e) Stage 4: detail of penis. (f) Detail of coiled oviduct. Abbreviations: a, anus; cg, capsule gland; co, coiled oviduct; fl, fusion line of free prostate edges; m, midgut gland; mc, mantle cavity; o, ovary; opo, open pallial oviduct; p, penis; pg, penial glands; pr, prostate gland; r, rectum; sg, sperm groove.

length of the prostate gland in females. This index has the disadvantage that it does not give authentic measurements of the proportion of sterile females in the population. Values above 0.0 are only attained if the sterile stage 3 occurs. But individuals already in stage 2 are not capable of producing egg capsules although females have no prostate, and consequently the length of the female prostate gland is 0.0 in such populations.

Table 1. Littorina littorea. Sampling stations (cf. Fig. 1; capitals indicate proximity to: D, dock yard; F, ferry harbour; M, marina; S, fishing harbour. R, reference, i.e. no apparent TBT sources) with TBT body burden (in μg as Sn kg^{-1} dry wt.), sex ratio, intersex index (ISI), average length of prostate gland in females in mm, proportion of females (of 4–7 analysed specimens) affected by atresia and lytical processes in follicles of the ovary. nd, not determined.

Sampling station	TBT body burden	Males ÷ females	ISI	Ø length of prostate	% of females with atresia	% of females with lysis
Knock near Emden (R)	202.2	0.54	0.08	0	0	33
Westermarsch II (R)	244.9	0.40	0.10	0	20	20
Norddeich (M)	978.9	0.86	1.50	3.44	40	40
Norddeich (F, S)	482.4	1.50	0.25	0.39	0	17
Dornumersiel (D, F, M)	1289.5	1.05	2.75	7.02	20	60
Bensersiel (F, M)	666.2	0.95	0.43	0.78	40	60
Harlesiel (R)	150.9	0.64	0	0	29	71
Horumersiel (M)	564.3	1.05	0.30	0	0	75
Hooksiel (R)	156.6	0.71	0	0	0	17
Wilhelmshaven (M)	380.4	1.41	0.12	0	0	40
Cuxhaven (F, M)	247.6	0.73	0.08	0	nd	nd

Table 2. Littorina littorea. Sampling stations with phases of midgut gland tubules. *n*, number of analysed specimens.

Sampling station	Holding phase	Absorption phase	Disintegration phase	Reconstitution phase	*n*
Knock near Emden (R)	7	36	58	0	9
Westermarsch II (R)	92	8	0	0	10
Norddeich (M)	24	23	43	8	9
Norddeich (F, S)	16	14	67	0	9
Domumersiel (D, F, M)	57	0	41	2	10
Bensersiel (F, M)	80	12	2	2	10
Harlesiel (R)	16	46	39	0	9
Horumersiel (M)	58	33	10	0	9
Hooksiel (R)	51	34	12	0	11
Wilhelmshaven (M)	78	21	0	1	9
Cuxhaven (F, M)	65	17	18	0	6

In this study TBT concentrations in the water column were not determined because the investigation period was too short to give reliable information of TBT pollution on the background of high season and tide related changes in coastal waters (Oehlmann *et al.*, 1993). However, stations were chosen on the background of former investigations on TBT concentrations in water and sediments (Kalbfus *et al.*, 1991). The range of TBT body burdens was between 150.9 (Harlesiel) and 1289.5 μg TBT as Sn kg^{-1} dry wt. (Dornumersiel) (Table 1). In a reference population of *L. littorea* from Roscoff harbour (Brittany, France; mean TBT concentration in sea water between 1989 and 1992: 15.4±5.84 ng TBT as Sn l^{-1}, $n = 19$) a TBT body burden of 406.6 to 534.5 μg TBT as Sn kg^{-1} dry wt. was determined. This shows, that at least at some sites of the German North Sea coast, aquatic TBT concentrations exceed the values measured at Roscoff harbour considerably.

There are highly significant sigmoid correlations between the TBT body burden and the ISI or the average female prostate length (Fig. 5a, b). This shows

that both parameters give not only an assessment of the intersex intensity in the populations but allow a determination of the TBT pollution in different populations. The ISI is characterized by a higher sensitivity, *i.e.*, this index increases at lower TBT body burdens compared to female prostate length. The threshold concentrations are 200 μg TBT as Sn kg^{-1} dry wt. for the ISI and 500 μg TBT as Sn kg^{-1} dry wt. for the prostate length.

Histopathological results

Parallel histological investigations were performed to elucidate if the observed morphological changes were accompanied by alterations at the level of cells and tissues. Morphometric measurements of the midgut gland revealed no significant differences between the sampling stations with regard to the area of the digestive epithelium and the luminal area in relation to the whole tubule (Fig. 6).

At Norddeich marina and Dornumersiel, tubules with a diminished area of the digestive epithelium were observed. These occurred at very low frequencies and did not affect the mean values. The synchrony in the phases of the tubules was pronounced at Westermarsch, Bensersiel and Wilhelmshaven with a dominating holding phase. At all other stations a distinct asynchrony was found (Table 2).

Tubules in disintegration and reconstitution phases occurred at the reference stations (Knock, Harlesiel) and in the harbours of Norddeich and Dornumersiel. Consequently, this characteristic was not useful for assessing pathological responses to TBT.

Most of the pathological disorders were observed in the female gonad. Retardation and interruption in the maturation of the oocytes (atresia) occurred mostly at stations in the vicinity of harbours. Lytic processes in follicles were observed at all stations and were frequent in harbours as well as at reference stations (Table 1). With increasing intensity of atresia in the follicles, glycogen was found to be accumulated in the connective tissue cells around the follicles, instead of stored in the yolk masses of the eggs. Additionally, diffuse haemocytic infiltrations were present in and around the gonads of female specimens. At Knock, near Emden one case of a granulocytoma was recorded.

Discussion

Despite the widespread distribution of *Littorina littorea* only a few TBT effects have been reported in this species. Matthiessen *et al.* (1991) reported a TBT-caused decline of periwinkle populations in southwest England and also a decrease in the abundance of veligers in the plankton until 1987. The situation has improved since the partial TBT ban in England in 1987. In the literature we have found no indication for the phenomenon described in this study as intersex. Only Fioroni *et al.* (1992) found a single penis-bearing female on the rocky shores of Helgoland but unfortunately the pallial oviduct of this female was not analysed in detail. Generally, littorinids are gonochoristic with the exception of the protandric species *Mainwaringia rhizophila* (Reid, 1986b). Penis-bearing females were also reported in *Bembicium auratum* and *Nodilittorina acutispira* (Muggeridge, 1979) but according to Reid (1986b) a trematode infection can be responsible for this malformation. In the present study parasited specimens were excluded from morphometric and histological analyses.

Only stage 4 of intersex development reported here, with an additional female penis, shows parallels to the imposex situation of other prosobranch species. Imposex is a superimposition of male sex organs (penis and/or vas deferens) on females (Smith, 1971). During intersex development no superimposition of male characters occurs but the organs of the pallial oviduct are modified towards a male morphological structure. Only in the final stages are female organs supplanted by the corresponding male formations. We want to stress that the main indices for intersex (ISI) and imposex measurement (vas deferens sequence (VDS) index according to Gibbs *et al.* (1987) and Fioroni *et al.* (1991)) are not comparable.

The imposex phenomenon has proven to be a reliable and widely used biomonitoring system for the detection and estimation of environmental TBT contamination (e.g. Gibbs *et al.*, 1987; Oehlmann *et al.*, 1992; Stroben *et al.*, 1992a, b). However, the established European imposex species for TBT biomonitoring are absent on the German North Sea coast or can only be found in restricted areas. For the TBT survey of the North Sea Task Force (Harding *et al.*, 1992) *Nucella lapillus* was transplanted in cages for three months to numerous sites on the Dutch, German and Danish North Sea coast. Because of the unnatural environmental conditions at these locations most of the

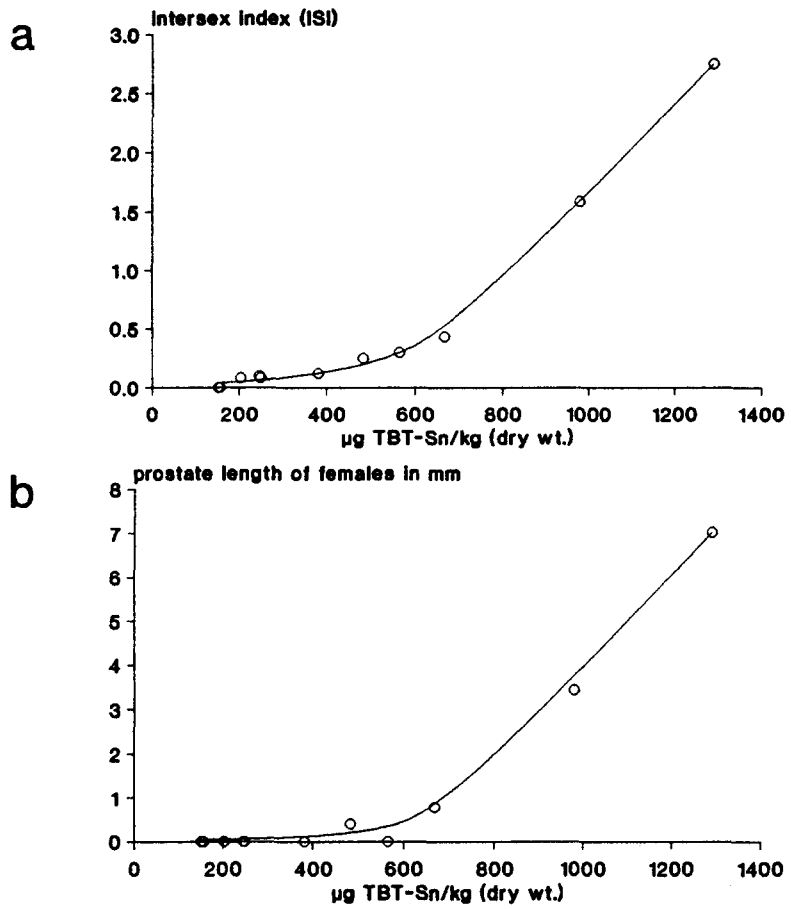

Fig. 5. Littorina littorea. Relationship between TBT body burden and intersex parameters ($n = 11$ populations with ≥ 40 specimens analysed from each). (a) Intersex index (ISI), with calculated sigmoid correlation: $y = -3.28 \div (1 + e^{0.0055(x-995)}) + 3.29$; $r = 0.997$; $p < 0.0005$. (b) Average prostate length of females, with calculated sigmoid correlation: $y = -7.76 \div (1 + e^{0.0075(x-995)}) + 7.79$; $r = 0.990$; $p < 0.0005$.

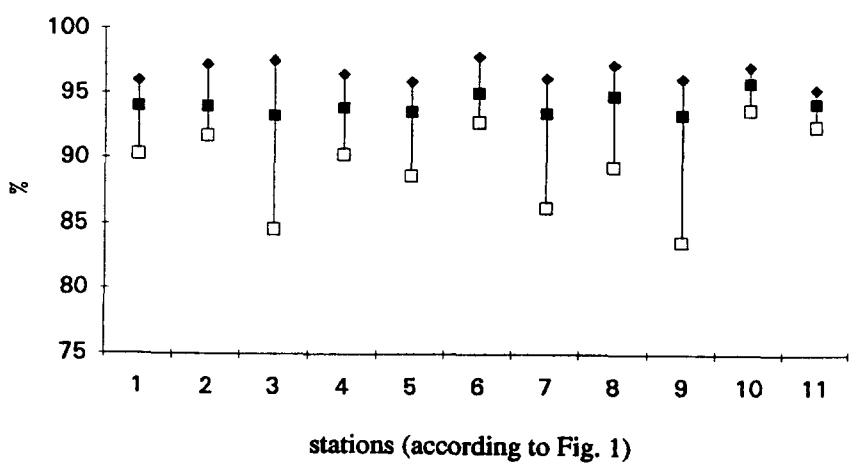

Fig. 6. Littorina littorea. Proportion of epithelial area from whole tubulus area. ■ mean value, □ minimum value, ♦ maximum value.

animals died and the results obtained are probably not reliable.

Because of intersex development *L. littorea* is a promising candidate for TBT biomonitoring at those sites where imposex-affected species are missing due to high TBT contamination or inappropriate habitats. The development scheme (Fig. 2) gives a very simple description of easily detectable alterations in the pallial oviduct. Furthermore, it is the basis of the intersex index (ISI). The present (and still preliminary) results indicate that the ISI is more suited for the description of intersex intensities and for the determination of TBT exposure than the average female prostate length. ISI values increase with the first occurrence of stage 1, whereas prostate length increases are detectable only after stage 3 is reached.

Although the correlation between TBT body burden and ISI or female prostate length (Fig. 5) is a strong indication that intersex is caused by TBT, this needs to be confirmed by laboratory experiments under controlled conditions. These investigations will have to answer the question whether or not these malformation can be induced by TBT in adult and sexually mature females or only during a earlier specific sensitive phase in the life cycle of *L. littorea*.

Bryan & Gibbs (1991) found TBT body burdens of 403.6±171.2 μg TBT as Sn kg^{-1} dry wt. in *L. littorea* from Northam Bridge (Itchen estuary; average aqueous concentration: 27.3±16.2 ng TBT as Sn l^{-1}; $n=8$). The calculated bioconcentration factor (bcf) is 1.48×10^4. Langston *et al.* (1987, 1990) reported values between 100–1120 μg TBT as Sn kg^{-1} dry wt. in periwinkles from Poole Harbour (south England) at ambient TBT concentrations of 2–139 ng as Sn l^{-1} (bcf: 8.1×10^3–5.0×10^4). The values determined by Langston *et al.* (1987, 1990) are in good accordance to the body burdens of periwinkles analysed here. Based on the reported bcf from England, the present TBT contamination in our investigation area has to be expected to be in the range of 2–150 ng TBT as Sn l^{-1}. This indicates that, in spite of the ban of TBT containing antifouling paints on boats <25 m overall length, there is still a considerable degree of TBT pollution in German coastal waters. The calculated bcf values at Roscoff harbour are 2.64×10^4–3.46×10^4. This result from France confirms the findings of Langston *et al.* (1987, 1990) and Bryan & Gibbs (1991) in England.

The analysed *Littorina* populations from Roscoff harbour allow an estimation of the threshold concentrations for intersex development. Calculated ISI indices in these samples were in the range of 0.0–0.06, *i.e.* only up to 6.1% of the females in the populations exhibited intersex. Consequently, the average aqueous TBT concentration of 15.4 ng as Sn l^{-1} at this station seems to reflect the most probable threshold concentration for intersex development.

Histological observations of the midgut gland did not reveal any severe lesions. Normally, the digestive tubules show a synchrony of the different phases in each specimen. This means that one phase is clearly dominant (Langton, 1975). Several stressors have been shown to upset the digestive rhythm. These include spawning activity, starvation, temperature and pollutants. If all biological factors can be separated, the mean height of digestive cells and the loss of synchrony are estimated as good indicators for the influence of anthropogenic stressors (Bayne *et al.*, 1984; Couch, 1985).

During our investigations, no severe pathological deviations could be found in the midgut gland. This might indicate that the general pollution load along the East Frisian coast (Koopmann *et al.*, 1993) is insufficient to induce persistent histopathological changes in *L. littorea*. This has to be proved in more detailed studies with seasonal sampling.

The pathological disorders in the female gonad, such as atresia and lysis, coincide only partially with TBT body burdens and intersex intensities. Probably additional stressing factors have to be taken into account. Beside TBT copper and other heavy metals can also induce a retardation in gonadal maturation (Myint & Tylor, 1982; Sindermann, 1985).

The glycogen accumulation around the follicles, in combination with lytic and atretic processes inside the follicles, indicates changes in the energy metabolism and the incorporation of glycogen into the yolk. This phenomenon is well-known to occur following starvation and under the influence of heavy metals (Thompson *et al.*, 1974; Bayne *et al.*, 1978). The combination of histopathological techniques with morphological parameters revealed dominant alterations in the female gonad at histological and morphological levels. TBT body burdens of *L. littorea* were comparable with those measured in periwinkles from other European coasts with shipping activities (Langston *et al.*, 1987, 1990). On the other hand, the accumulation of other pollutants, such as heavy metals, from the East Frisian cast revealed relatively low levels for periwinkles (Thiel *et al.*, 1992). The effects reported here are therefore likely to be mainly associated with TBT.

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