Intersex and sterility in the periwinkle *Littorina littorea* (Mollusca: Gastropoda) along the Western Scheldt estuary, the Netherlands

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Abstract

In this study we present the results of an intersex survey of *Littorina littorea* along the heavily polluted Western Scheldt estuary (the Netherlands), and record for the first time the intersex phenomenon in *L. littorea* from Dutch waters. Intersex differed significantly between localities and was the highest in the vicinity of the harbours of Antwerp and Vlissingen, as reflected by the ISI distribution along the estuary. In contrast, sterility did not differ significantly between the different sites, but nonetheless, followed the ISI distribution as well, ranging from 0 to 33%. Despite the current ISI levels are high (maximum = 1.26), they are still much lower than the ISI levels reported for *L. littorea* in some German harbour areas.

Keywords: Intersex; *Littorina littorea*; TBT; Western Scheldt estuary

1. Introduction

The phenomenon of imposex, or pseudohermaphroditism, firstly described in the neogastropod, *Nucella lapillus* (Blaber, 1970), is characterised by the development and superimposition of non-functional male sexual characteristics (i.e. vas deferens and/or penis) in female individuals (e.g. Bryan, Gibbs, Hummerstone, & Burt, 1986; Gibbs & Bryan, 1986; Oehlmann, Stroben, Schulte-Oehlmann, Bauer,
Fioroni, & Markert, 1996). As a result, females become sterile and affected populations may become locally extinct (Bryan et al., 1986). Organotins, such as tributyltin (TBT) compounds, mainly used in antifouling paints, have been shown to induce imposex at very low concentrations (as low as 1 ng l\(^{-1}\) as Sn) in various neogastropod species (Gibbs & Bryan, 1994). Besides causing imposex, TBT may produce another masculinising condition, called intersex. First described in the periwinkle *Littorina littorea* (Bauer et al., 1995), intersex is expressed at higher TBT concentrations (10 ng l\(^{-1}\) as Sn), and may yield sterile females as well (Bauer, Fioroni, & Schult-Oehl, 1997). Four distinct intersex stages can be recognized (Bauer et al., 1995). At stage 1, the female genital opening is enlarged by a proximal slit and the bursa copulatorix is split, while at stage 2, the rest of the palial oviduct is opened ventrally, exposing the internal lobes (Bauer et al., 1995). At stage 3 the palial oviduct glands are partially or totally supplanted by a prostate gland, while a penis and a seminal groove can be detected in stage 4 (Bauer et al., 1995). Stage 2, 3 or 4 females become sterile, either because the glands for the formation of the egg capsules are missing (stage 3 and 4) or because oocytes and capsular material leak into the mantle cavity (stage 2; Bauer et al., 1997; Minchin, Bauer, Oehlmann, Schulte-Oehlmann, & Duggan, 1997).

In contrast to *N. lapillus* populations affected by imposex, *L. littorea* populations affected by intersex are less likely to go extinct, given their planktonic developing nature. Larval input from populations with lower intersex intensities can namely increase the periwinkles' abundance at sites with high TBT contamination and high reproductive failure incidence (Oehlmann, Schulte-Oehlmann, Bauer, & Markert, 1997). In contrast, in a non-planktonic developing organism such as *N. lapillus*, abundance will only depend on local recruitment. Populations of the non-planktonic developing *N. lapillus* are declining or have become extinct near TBT point sources on many European coasts (Bryan et al., 1986; Nehring, 2000; Oehlmann et al., 1997). Hence, intersex in *L. littorea* can be used as an alternative monitor for TBT pollution at those sites where ambient TBT concentrations are high (> 2.0 ng as Sn l\(^{-1}\)), or where *N. lapillus* is absent or has become locally extinct (Oehlmann, Bauer, Minchin, Schulte-Oehlmann, Fioroni, & Markert, 1998).

Here we present the results of an intersex survey of *L. littorea* along the heavily polluted Western Scheldt estuary (the Netherlands), where *N. lapillus* is absent.

2. Material and methods

On 8 August 1998, *L. littorea* was collected at six sites along the Western Scheldt (Fig. 1). These sites included, in order of increasing salinity (i.e. from ±10 to 30%o): Waarde (1), Hansweert (2), Hoedekenskerke (3), Ellewoutsdijk (4), Borssele (5) and Vlissingen (6) (Fig. 1). At each site, 40 animals were collected at the mid-littoral zone on a rocky surface. Only those specimens were included that had reached sexual maturity (shell height above 12 mm). The shell height of each individual was measured to the nearest 0.05 mm, using a calliper. The shells were removed and the animals were sexed based on the presence of the vesicula seminalis. Intersex females
were categorised to either one of the four intersex stages. The intersex index (ISI), measuring the average intersex stage in that particular population (Bauer et al., 1995), and the sterility percentage (i.e. intersex stages 2, 3 and 4) was determined at each site.

To test whether (1) sex ratios, (2) intersex number and (3) sterile females differed from site to site, three 6x2 contingency tables were constructed, employing the Metropolis algorithm to obtain unbiased estimates of the exact P-value (Miller, 1997). Contingency table analysis was performed using the RxC package (Miller, 1997).

3. Results

Sex ratios did not differ between sites (P = 0.187) and are given in Fig. 2. In contrast, the intersex numbers differed significantly between populations (P = 0.005), as reflected in the ISI value distribution (Fig. 3). ISI values ranged namely from 0 at Hansweert to 1.26 at Borssele, indicating that contamination was greatest in the direct vicinity of the harbour of Vlissingen, decreasing towards the mainland and the North Sea (Figs. 1 and 3). One exception was found at Waarde, the site located furthest upstream, where contamination increased again, as indicated by the third highest ISI value (i.e. 0.47, Fig. 3). The number of sterile females did not differ significantly between sites (P = 0.238), but sterility percentages did follow the ISI value distribution (Fig. 3).

The previously reported shell size transition at the 20% salinity range (De Wolf, Blust, & Backeljau, 2001) was confirmed in the current study (Fig. 2). Specimens collected below the 15–20% salinity mark are on average smaller than animals collected above that salinity mark (Fig. 2).
4. Discussion

Despite the 1989 European Community ban on the use of TBT in antifouling paints on vessels of less than 25 m, TBT is still present in the marine environment (Davies, Bailey, & Harding, 1998). Based on the annual percentage of merchant shipping, entering each of the nine North Sea states, it is estimated that the Netherlands contribute 33% (i.e. 79.2 t year\(^{-1}\)) to the total maximum North Sea TBT input, placing them on top of the contributors list, well in front of the UK (i.e. 34.9 t year\(^{-1}\)) and France (32.5 t year\(^{-1}\); Davies et al., 1998). This estimated annual TBT input and subsequent ranking of the different North Sea areas for risk of biological impact, largely coincides with the North Sea distribution of imposex in *N. lapillus* (Davies et al., 1998).

In the Dutch Wadden sea and in the Eastern Scheldt estuary, populations of *N. lapillus* are indeed declining or have become locally extinct (Bakker et al., 1997; Mertens & Van Zwol, 1988). Likewise, populations of the common whelk *Buccinum undatum*, are nowadays restricted to the Eastern Scheldt estuary, whilst they were still abundant in the Wadden Sea and coastal areas of the North Sea 25 years ago (ten Hallers-Tjabbes, Boon, Lindeboom, & de Vooijs, 1993). In addition, imposex incidences in *B. undatum* collected in the Eastern Scheldt estuary were always above 90% (Mensink, Everaarts, Krait, ten Hallers-Tjabbes, & Boon, 1996).

In the Western Scheldt estuary, *N. lapillus* has only occasionally been found and is restricted to the estuaries mouth (Sips & Waardenburg, 1992; personal observations). Hence, despite its higher TBT sensitivity, *N. lapillus* cannot be used as a general TBT biomonitor in the Dutch coastal and estuarine waters. In contrast, *L.*
*littorea* is found along the Dutch coastal and estuarine waters, while the current intersex findings, suggest that this animal may be a good alternative TBT bio-monitor. The masculinisation effect in *L. littorea* is namely expected to be the most intense in the neighbourhood of harbours, given the elevated TBT input in those areas (Davies & Bailey, 1991; Minchin & Minchin, 1997; Morcillo, Borghi, & Porte, 1997; Oehlmann et al., 1996). This is indeed shown for both the harbours of Vlissingen and Antwerp (Figs. 1 and 3). Effects of TBT from the harbour of Antwerp are apparently still picked up in Waarde (Fig. 3), while the highest ISI value and the highest sterility percentage are found at the site which is located closest to the harbour of Vlissingen (i.e. Borssele), decreasing from there on towards the mainland (i.e. Ellewoutsdijk, Hoededefkenskerke) and the North Sea (i.e. Vlissingen; Fig. 3). The fact that sterility follows the ISI value distribution pattern, but does not differ significantly between sites, can be explained by the fact that it is a less sensitive parameter. Sterile females include only intersex stages above 1, while ISI values take all intersex stages into account (Bauer et al., 1995; Oehlmann et al., 1998).

Compared to literature, the highest ISI value found in the present study (i.e. Borssele; ISI 1.26) is higher than ISI values reported for *L. littorea* collected at Rio de Aveiro (0.50) (NW Portugal; Barroso, Mareira, & Gibbs, 2000), comparable to the highest ISI value found in the Irish Killybergs harbour (i.e. 1.30; Minchin et al., 1997) but is nonetheless considerably lower than ISI values reported at some German ports (> 3.0; Bauer et al., 1995). Bauer et al. (1997) have shown that there is a close relationship between TBT body burdens and ISI values. Based upon this relationship the extrapolated TBT concentration is about 300 μg TBT Sn kg⁻¹ dry weight at Borssele. This is in agreement with ISI values and TBT concentrations found in *L. littorea* from Killybergs harbour (Ireland; Minchin et al., 1997). The ISI
values reported in that study are comparable to the highest ISI levels found at Borssele (1.30 as opposed to 1.26 in the present study), corresponding to measured TBT tissue concentrations ranging between 200 and 500 µg TBT Sn kg\(^{-1}\) dry weight (Minchin et al., 1997).

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