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Short communication

Evaluation of the use of transplanted *Nassarius reticulatus* (Linnaeus, 1758), in monitoring TBT pollution, within the European Water Framework Directive

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ABSTRACT

The European Water Framework Directive (WFD) requires monitoring programmes, to establish a coherent and comprehensive overview of ecological and chemical water status. Recently and within this context, the use of biomarkers has been proposed for incorporation into WFD monitoring programmes. In the present study, cages have been used to transplant (ranging between 14 and 75 days) adults of the dogwhelk *Nassarius reticulatus* in relation to a gradient of tributyltin (TBT) pollution; this is in order to evaluate its usefulness within the WFD monitoring programmes. Only the most polluted site (close to a shipyard) shows evidence of TBT contamination effects. As such, it may be concluded that the use of caged transplants with *N. reticulatus* (adults) is useful only for areas of high TBT pollution. Thus, within the European WFD context, this approach is generally not useful for surveillance and operational monitoring, but has some potential in investigative monitoring.

1. Introduction

Tributyltin (TBT) compounds have been used widely as biocides in antifouling paints, being probably the most cost-effective compound preventing fouling on vessels (Terlizzi et al., 2001). However, TBT is considered as one of the most toxic xenobiotics ever produced (Goldberg, 1986). As such, the use of TBT in several industrialized countries started to be restricted, at least partly, in the 1980s (Bosselmann, 1996). In a more general way, following the approval (on 5 October 2001) of the International Convention on the Control of Harmful Antifouling Systems on Ships (IMO, 2005). Hence, some worldwide monitoring is necessary to determine if the TBT ban is effective (Sousa et al., 2007; Smith et al., 2008; Bigatti et al., 2009).

The evaluation of bioaccumulation and the responses of biomarkers on transplanted bivalves and gastropods has been studied widely, in ecotoxicological research focused on TBT pollution (see e.g., Bellas et al., 2007; Rank, 2009). Within this approach, the imposex phenomenon (*i.e.*, the superimposition of male organs onto female dioecious gastropods (Smith, 1971)) is among the most studied biomarkers; this is due to its high specificity and sensitivity to TBT, as found in some species. In fact, imposex is considered the best biological indicator of TBT pollution in marine waters (e.g., Svavarsson et al., 2001). Within this context, the most studied species, using the transplant approach, is *Nucella lapillus* (see e.g., Quintela et al., 2000; Giltrap et al., 2009); however, other species have also been used: *Thais orbita* (Foale, 1993; Gibson and Wilson, 2003); *Thais distinguenda* (Bech et al., 2002); *Thais clavigera* (Shim et al., 2000; Chan et al., 2008); *Nassarius obsoletus* (Jenner, 1979); *Lepsiella scobina* and *Lepsiella albomarginata* (Smith and McVeagh, 1991); *Hexaplex trunculus* (Lahbib et al., 2008); and *Buccinum undatum* (Svavarsson et al., 2001). Such studies have identified that the use of transplanted gastropods is useful in the biomonitoring of TBT pollution.

In relation to European monitoring, TBT is one of the priority substances (Directive 2008/105/EC; EC, 2008) which have to be taken into account, in assessing chemical pollution of marine waters within the Water Framework Directive (WFD) 2000/60/EC (EC, 2000). However, the main objective of the WFD is to assess the ecological status of European water bodies, using different biological quality elements (*i.e.*, phytoplankton, macroinvertebrates and fishes); these can be affected not only by chemical pollution, but also by other anthropogenic pressures (Borja, 2005). Hence, although the assessment is undertaken on an ecosystem basis, the use of single species could be justified, to investigate any rapid and evident biological effects of some pollutants, upon indicator species (van Strien et al., 2009). Due to the fact that the abovementioned species are absent along some European coasts, it

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is necessary to investigate if the transplant approach is useful with other species, with a wider latitudinal distribution. As such, *N. reticulatus* (L.) is a gastropod found commonly on the North Atlantic and Mediterranean coasts of Europe and Africa, including the Azores and the Canary Islands (Rolán and Luque, 1995). This species has been used widely for monitoring TBT contamination in Europe, including Portugal, Spain, France, Denmark and Sweden (see, e.g., Huet et al., 1995; Strand, 2003; Magnusson and Granmo, 2004; Sousa et al., 2007; Wirzinger et al., 2007; Rato et al., 2008; Couceiro et al., 2009; Rodríguez et al., 2009). Hence, the objective of this contribution is to evaluate the usefulness of the transplants of *N. reticulatus*, as a tool in WFD monitoring, to assess TBT pollution by means of imposex development.

2. Materials and methods

Netted dogwhelks, *N. reticulatus*, were collected (on May 2008) from a subtidal area off the Port of Pasaia (North of Spain), which had been surveyed previously and found to have no imposex (see data for Sampling Site 21, in Rodríguez et al., 2009). Toothed adults were transplanted to four sites in plastic fishing cages (1000 individuals per cage), with frozen fish (*Scomber scombrus*) as food. The implant sites were selected so as to follow a gradient of TBT pollution: (i) Site C (control), located 3 km off the Port of Pasaia; (ii) Site 28 (the open berth area of San Juan); (iii) Site 24 (the harbour of San Pedro); and (iv) Site 26 (the shipyard of Lezo) (Fig. 1a). The 'gradient of TBT pollution' (see above) has been based upon previously-collected data, in which TBT content in sediments (in 2007) was 203, 571, 3132 ng g^{-1} expressed as Sn, at Sites 28, 24 and 26, respectively (for methodological details, see Rodríguez et al., 2010). Moreover, imposex development in local populations of *N. reticulatus*, in 2007, was 47%, 68% and 92% (expressed as RPLI—Relative Penis Length Index), at the same sites (Rodríguez et al., 2009). At Site C, no previous data on TBT pollution are available. Nonetheless, taking into account the location of the TBT sources, together with the prevailing hydrodynamics, *a priori* it was expected to be the site with the least TBT pollution (among the four sites tested).

At each of the four sites, a cage was buried into underlying bed sediment to half of the height of the cage (*i.e.*, 20 cm) (Fig. 1b). Moreover, at Sites 28, 24 and 26, a cage was hung from the buoys, located at 2 m below the water surface. Sub-samples of 100 gastropods were collected at each cage after 14, 35, 56, and 75 days of exposure. Imposex on the gastropods was evaluated as detailed in Rodríguez et al. (2009). The Vas Deferens Sequence (VDS), in females, and the Vas Deferens Sequence Index (VDSI) were determined following the approach of Stroben et al. (1992).

A chi-squared test with Yates' correction was performed, to test if differences of proportions between time 0 and other sampling times were significant (at alpha = 0.05). A two-tailed Fisher's exact test was performed, to test if differences of proportions between hung and buried cages were significant (at alpha = 0.05).

3. Results

Females (68) from the donor site (21, Fig. 1a) were evaluated at time 0 (day); none were imposex-affected. At the control site (C), none of the females evaluated were imposex affected after 14, 35, 56, and 75 days of exposure. Survival rates were higher than 91%, in all of the cages.

After two weeks of exposure only one imposex-affected female was found, at the hung cage located at Site 28 (Fig. 2). After 35 days of exposure, no affected females were found at Site 28; however, they were at Sites 24 and 26 (Fig. 2). After 56 days of exposure, affected females were found in all of the exposed cages, except for the hung cage located at Site 28 (Fig. 2). After 75 days of exposure,

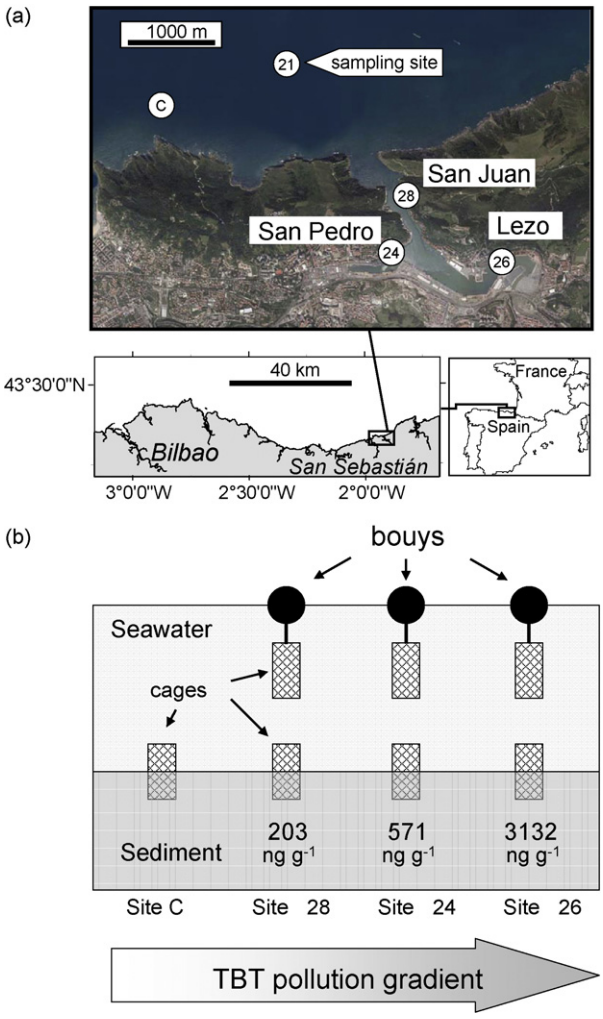

Fig. 1. (a) Location of sampling station (Site 21) and implant sites (C, 24, 26 and 28) in the Port of Pasaia, northern Spain; (b) schema of cages disposition. *Note:* the numerical values in (b) are concentrations of tributyltin in surficial sediments given as Sn, dry weight (data obtained one year before the experiment (Rodríguez et al., 2010)).

imposex-affected females were found in all of the exposed cages (Fig. 2). The percentage of affected females was not significantly different between the hung and the buried cages, at each of the sampling sites.

At Site 28, no-significant differences in the percentages of affected females compared to time 0 were found, at any of the sampled dates (Fig. 2). At Site 24, a significant difference was found after 75 days of exposure, in the hung cage (Fig. 2). In the hung cage of Site 26, significant differences were found after 35, 56 and 75 days of exposure; in the bottom cage, significant differences were found after 56 and 75 days of exposure (Fig. 2). At Sites 28 and 24, the imposex-affected females had VDS stages 1a, whereas at Site 26 VDS stages 1b, 2a and 2b were also found (Table 1). The highest Relative Penis Length Index (RPLI) and Vas Deferens Sequence Index (VDSI) values were found at Site 26 (Table 1).

4. Discussion

In spite of the fact that the species *N. reticulatus* has the advantage of having a wide geographical distribution and low mortality rates, the results obtained here show that its use for routine TBT biomonitoring, through transplants, is somewhat limited. Thus, as a proportional relationship between exposure