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Declines in TBT contamination in Irish coastal waters 1987–2011, using the dogwhelk (*Nucella lapillus*) as a biological indicator

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ABSTRACT

Using the vas deferens sequence index (VDSI) and relative penis size index (RPSI) in dogwhelks (*Nucella lapillus*), imposex levels were assessed at 63 sites within 11 sea inlets during 2010/2011 and compared these with levels gathered since 1987. Sterile females (VDS > 5.0) were found at 14 of the 63 sites and 47 sites (75%) met the EcoQO (VDSI < 2.0). The absence of imposex in 'control' areas on the west coast is due to the lack of vessel paint applications or net dips with TBT being used as an active anti-fouling ingredient. A significant decline was observed following 2005 when comparing VDSI levels which is consistent with the decline of TBT usage. Current levels are consistent with an overall improvement towards achieving Good Environmental Status according to the requirements under the Marine Strategy Framework Directive.

1. Introduction

Tri-butyl tin (TBT) is an effective anti-fouling agent used in marine paints for the wetted hull surfaces of ships and other marine structures. Its persistence and toxicity at low concentrations has caused many problems in the marine environment (Hall, 1988). Whilst TBT itself is considered as the most toxic compound, TBT ultimately degrades to DBT and MBT, which can be toxic to a different suite of organisms including bacteria (Cooney, 1995). Butyltin compounds in general are strongly bound to the particulate phase which makes them liable to enter the marine food chain via infaunal biota. The degradation rates of TBT in seawater vary according to pH, temperature, turbidity and light, with a compound half-life ranging from days to weeks (Díaz et al., 2007) whereas degradation rates in marine sediments are slow with a half-life of between 1 and 5 years (Hoch, 2001), posing risks from contaminated sediment which may take place from storm events or from dredging operations. The most well-known effects of TBT are shell malformations of oysters (Alzieu et al., 1982), imposex and intersex in marine snails (Oehlmann et al., 1998; OSPAR, 2000), reduced resistance to infections (e.g., flounder) (Grinwis et al., 2000), and effects on the human immune system (OSPAR Commission, 2011). TBT is sufficiently toxic to harm many marine organisms at very low concentrations.

TBT has been listed by OSPAR as a substance for priority action (OSPAR Commission, 2011) and considered a *priority hazardous substance* by the European Commission Directive 2008/105/EC (European Commission, 2008) with environmental quality standards (EQS) set for total water at 0.2 ng/L for annual average (AA)-EQS and 1.5 ng/L for maximum allowable concentration (MAC)-EQS. This is lower than the levels that can be routinely measured. Since the first reporting of its deleterious effects (Bryan et al., 1986; Alzieu et al., 1982; Minchin et al., 1987; Bryan and Gibbs, 1991), the use of TBT has been progressively restricted. In 1987, use of TBT based anti-fouling paints was prohibited from use on structures, including fish cages and vessels under 25 m length in Ireland (Anon, 1987), and EC Regulation (EC) No. 782/2003 implemented the provisions of the International Maritime Organisation's Antifouling Systems Convention (IMO, 2001) prohibiting application of TBT surface coatings to all vessels by 2003 and to eliminate TBT from ship hulls by 1st Jan 2008. A global ban on TBT entered into force from September 2008.

The low TBT detection limits in water, led to the worldwide usage of neogastropods which are sensitive to low levels of TBT causing impairment to sexual development. In northern Europe the dogwhelk provides a useful indicator for low levels in the environment and the periwinkle *Littorina littorea* has been used where TBT contamination is high (Oehlmann et al., 1998). Imposex is a condition resulting in the development of a penis, and other male features, in female marine snails and has been adopted as a biomarker under the OSPAR Joint Assessment and Monitoring Programme (JAMP) — a mandatory component of the Coordinated Environmental Monitoring Programme (CEMP) (OSPAR,

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2009). Imposex is measured using two indices — the Vas Deferens Sequence Index (VDSI) and the Relative Penis Size Index (RPSI). OSPAR produced guidelines for assessing imposex (OSPAR, 2008–2009) with the index having been established as a North Sea Ecological Quality Objective (EcoQO) for TBT-specific effects (OSPAR, 2005). This states that the average level of imposex in a sample, of not less than 10 female dog whelks, should be consistent with exposure to TBT concentrations below the OSPAR derived environmental assessment criterion (EAC) for TBT, of <2.0, as measured by VDSI. Imposex is a sensitive and specific marker that can occur at TBT water concentrations below the detection limits for routine chemical analysis. As a result this index is an effective monitoring tool (European Commission, 2008). The Commission Decision (477/2010), for setting criteria and methodological standards for Good Environmental Status (GES) of Marine Waters, requires that an indicator for GES descriptor 8 is established where levels of pollution effects on ecosystem components show a cause and effect relationship. Imposex is currently a clear example of a contaminant specific cause–effect relationship. Interpretations in the assessment of effects of TBT on *Nucella lapillus* imposex (OSPAR, 2005), are detailed in Table 1.

Guidance values for the assessment of RPSI are less straightforward. Spence et al. (1990) noted that at RPSI levels of <5%, sterile females were absent generally, whilst at RPSI >40% most, and sometimes all, females were sterile. Bryan et al. (1989) suggested there was a RPSI limit of 20–25% above which the reproduction of the female would be affected.

This investigation documents imposex data compiled from 1987 to 2010/2011 and reports on temporal trends at selected locations, establishing the current imposex status around the Irish coast.

2. Materials and methods

2.1. Sampling, site selection

Dogwhelks were collected from October 2010 to October 2011 at 63 sites from regions that have historically exhibited either high or low imposex levels (See Fig. 1 and Table 2). For temporal assessments, data was compiled from a combination of regular and one-off surveys from 1987 to 2011 (Minchin and Minchin, 1997; Minchin et al., 1995, 1996, 1997; Minchin, 2003, 2011; Giltrap et al., 2009) with temporal data being available from a total of 43 common sites (Table 3).

2.2. Biological measurement and quality assurance aspects

VDSI and RPSI measurements were made in accordance with OSPAR guidelines for TBT-specific biological effects monitoring (OSPAR, 2008). Those conducting analyses had satisfactorily completed QUASIMEME and BEQUALM biological effects inter-calibration exercises.

VDSI was calculated as;

$$(\text{sum of imposex stage values of all females sampled}) / (\text{number of females at each site}).$$

The measurement of the degree of male organ superimposition on the female *Nucella lapillus* has six stages ranging from A (the least affected) to F (the worst cases likely to be observed).

RPSI was calculated as;

$$(\text{female penis length})^3 / (\text{male penis length})^3 * 100.$$

The results were classified according to the ecological quality objective level laid down by OSPAR.

2.3. Methodology for trend analysis

All trend analyses were completed using R 2.14.2 (R Development Core Team, 2011), using the lme4 package (Bates, D., Maechler, M., Bolker, B.M., Walker, S. (2014). "lme4: Linear mixed-effects models using Eigen and S4." ArXiv e-print; submitted to _Journal of Statistical Software_, <URL:http://arxiv.org/abs/1406.5823>.). A three-phased assessment procedure was completed with available data, using: a) direct comparison of VDSI (and RPSI) to the relevant assessment criteria in order to derive the current status, b) an aggregated time classed assessment, and c) temporal assessment from selected locations.

For the purposes of current status, VDSI indices at each individual site were directly compared with OSPAR derived assessment classifications (See Table 2). RPSI data were compared to best practice guidance values (Spence et al., 1990; Bryan et al., 1989).

Aggregated temporal trend analysis was performed on time classified, pooled, VDSI data from 10 regions: Mulroy Bay, Killybegs Harbour, Ballinakill Bay (locations A to C respectively), Galway Bay, Shannon, Tralee Bay, Castletownbere, Cork Harbour, Waterford, Wexford, Dublin and Carlingford (locations D to L in Fig. 1 respectively). In addition to this, temporal sampling timeframes were divided into three categories: early (1987–1995), middle (1996–2004), and recent (2005–2011). Sampling site within each region was included as a random effect in the model to account for the repeated measures design of the sampling protocol (Bates et al., 2011).

In addition to the aggregated assessment, trends at the ports, within Dublin Bay, Killybegs Harbour and Cork Harbour were tested. A mixed-effects general linear model (Bates et al., 2011) was used to determine whether VDSI values changed for these regions between 1987 and 2011. An MCMC algorithm (10,000 iterations) was used (Baayen, 2011) to derive p-values, comparing both the middle and recent time categories, to the early time category. The same statistical procedure was used to investigate changes in VDSI values within the individual locations Dublin Bay, Killybegs and Cork harbours.

3. Results

3.1. Current status of VDSI and RPSI

The VDSI and RPSI values obtained in the 2010–11 survey are compared with the OSPAR assessment criteria (Table 2). Previous years' survey data for the 10 inlets and 43 sites are shown in Table 3.

For the current status assessment (2010/2011 survey), the majority (47/63) of the sites sampled met the EcoQO, i.e., a VDSI of < 2.0. Whilst the majority of the sites sampled met the EcoQO, A or B classes, with 12 sites at class A <0.3 i.e., below OSPAR's Background Assessment

Table 1
Interpretations of the assessment classes and criteria for *Nucella lapillus* (OSPAR, 2005).

OSPAR assessment class	EcoQO status	Nucella VDSI	Effects and impacts
A (Blue)	EcoQO met	VDSI = <0.3	Imposex (and hence TBT) close to zero (0–~30% of females have imposex)
B (Green)	EcoQO met	VDSI = 0.3–<2.0	Imposex below the EAC derived for TBT (~30–~100% of the females have imposex)
C (Red)	EcoQO not met	VDSI = 2.0–<4.0	Imposex higher than the EAC derived for TBT with a risk of adverse effects, such as reduced growth and recruitment
D (Red)	EcoQO not met	VDSI = 4.0–5.0	The reproductive capacity affected, some females sterile.
E (Red)	EcoQO not met	VDSI = > 5.0	Populations unable to reproduce; majority, if not all females sterile
F (Red)	EcoQO not met	VDSI = negative	Gastropod populations absent/expired

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