A decade of UK organo-tin-specific biological effects monitoring
- Trends, ecological quality assessment and future monitoring requirements.

MJ Gubbins, M O’Reilly, L McIlroy, J Thain, IM Davies

Abstract

The imposex / intersex response of marine gastropods to tributyltin (TBT) exposure is a well established and much studied phenomenon, the monitoring of which has contributed to the banning of TBT based antifoulants. Over the last 10 – 15 years, UK competent monitoring authorities have conducted three comprehensive spatial surveys of organo-tin effects on dogwhelks (*Nucella lapillus*) and periwinkles (*Littorina littorea*) around the UK coastline. In addition, the Scottish Environment Protection Agency annually surveys some 65 shoreline sites in Scotland and the Department of Environment in Northern Ireland has an on-going monitoring programme for imposex. A point source monitoring programme at the Sullom Voe oil terminal in Shetland has also provided a time series of imposex data from dogwhelks spanning some 15 years. Several monitoring authorities have collected data on offshore effects of TBT on common whelks (*Buccinum undatum*) at shipping lanes, anchorages and dredge spoil disposal sites. Further, in 2004, under the auspices of the UK National Marine Monitoring Programme, a survey of imposex / intersex in shoreline and offshore gastropod populations is being conducted in accordance with the OSPAR guidelines. This will provide a baseline against which to measure recovery following further limitations through IMO on TBT for large (>25 m) vessels.

All these data are compiled and assessed with respect to OSPAR assessment criteria to provide an overall picture of the current status of TBT-effects in the UK, inform ecological quality assessments and direct future monitoring efforts.

Keywords: Imposex, TBT, monitoring, gastropod
1 Introduction

Contamination of the marine environment by tri-butyltin (TBT) has long been known to cause the imposition of male sexual characteristics (imposex) on female neogastropods and the development of intersex in female Littorinid gastropods. Recognition of these effects has lead to a range of legislative actions in different countries to prohibit or restrict the use of the TBT-based antifoulants on the hulls of vessels and in aquaculture.

Monitoring for imposex / intersex in gastropods has been undertaken in the UK since the late 1980’s to assess the biological effects of TBT contamination. The majority of UK monitoring effort in this area has concentrated on the development of imposex in dogwhelks, *Nucella lapillus*. This species is the most sensitive to TBT exposure and is relatively abundant around the majority of UK rocky shorelines, except in the vicinity of some major point sources of TBT contamination such as ports and harbours where populations have expired. In such areas, and where *Nucella* populations are absent due to lack of rocky substrate, periwinkle (*Littorina littorea*) populations have been assessed for intersex. Offshore gastropod (*Buccinum undatum* and *Neptunea antiqua*) populations have also been monitored on an ad hoc basis for imposex.

The imposex / intersex response in marine gastropods is sensitive, contaminant specific and can be directly related to the reproductive potential of the population. For example, sterility in female *Nucella* can arise in severe cases of imposex by occlusion of the vagina by proliferation of *vas deferens* tissue, which prevents the release of egg capsules (Gibbs et al., 1987). Therefore, this biological effect measurement is of great use in ecological quality assessment. This has been recognised by bodies such as the Oslo and Paris Commission for the protection of the marine environment of the NE Atlantic (OSPAR), which has recently developed assessment criteria for imposex / intersex (OSPAR, 2004). OSPAR is also currently in the process of developing an Ecological Quality Objective based on imposex in *Nucella lapillus*, with input from the ICES Working Group on the Biological Effects of Contaminants (WGBEC) and the associated ICES Advisory Committee.

Particularly over the last decade, competent monitoring authorities (CMAs) in the UK have collected a large quantity of data on the biological effects of TBT contamination from a number of spatial and temporal surveys. Presented here for the first time is the scope of the UK TBT-effects monitoring programme, compiling data from the different CMAs to show geographical and temporal coverage over the last 10 years. These data are subjected to preliminary assessment using the recently developed OSPAR assessment criteria and some spatial and temporal trends are identified and future monitoring requirements highlighted.

2 Methods

Gastropods collected from the intertidal shoreline (*Nucella lapillus* and *Littorina littorea*) and from offshore areas (*Buccinum undatum* and *Neptunea antiqua*) were assessed for imposex (*Nucella* and *Neptunea*), intersex (*Littorina*) and penis classification index (*Buccinum*) by various UK competent monitoring authorities between 1992 and 2003. Methods used for assessment were as described by Gibbs et al. (1987), Oehlmann et al. (1991), Mensink et al. (1996a, 1996b) and were in accordance with the relevant OSPAR Guideline (OSPAR, 2002). External quality control was achieved through participation of all monitoring authorities in QUASIMEME Laboratory Performance Studies during this period.
The surveys:

National shoreline surveys

During 1992 /1993, 23 sites on the East and South coasts of the UK were surveyed for imposex in *Nucella* and intersex in *Littorina* (Harding et al, 1992). Twenty one of these sites were resurveyed in 1998 / 1999 (Harding et al, 2000). Stations sampled during these surveys were not associated with known point sources of TBT contamination. During 1997 / 1998, western coastlines (including Northern Ireland) were subject to a more extensive survey including sites expected to be subject to ‘background’ levels of contamination and groups of stations positioned either side of known TBT point sources (Davies et al, 1998). These sites included fishing harbours, industrial ports, marinas and aquaculture facilities, some of which had previously used TBT-based antifoulants on cages and other equipment. The geographical coverage achieved by stations from these surveys is shown in Figure 1. In addition, the Department of Environment Northern Ireland (DOENI) have conducted surveys of some 40 stations along the coast of Northern Ireland between 1994 and 2003 (at approximately 2 year intervals). The Scottish Environment Protection Agency (SEPA) have also surveyed a further 65 sites (approximately) around Scotland during annual surveys between 1998 and 2003. Stations from these monitoring programmes have been a combination of ‘background’ sites and sites associated with known TBT inputs.

Sullom Voe

The Sullom Voe oil terminal in Shetland has provided a unique opportunity to monitor the spatial and long-term temporal trends of effects of TBT from antifoulants on oil tankers visiting the terminal since 1987. Sullom Voe is a large fiordic inlet on the mainland of Shetland. The mouth of the Voe is approximately 5 km wide, and the Voe extends approximately 13 km southwards. A large oil terminal, situated near the mouth of the Voe, was opened in November 1978. The Shetland Oil Terminal Environmental Advisory Group (SOTEAG) have commissioned Fisheries Research Services to analyse *Nucella* for imposex at 20 sites around Sullom Voe and the nearby waters of Yell Sound approximately every two years since 1987, providing a valuable long-term monitoring data-set.

Offshore (sub-tidal) surveys

Imposex in whelks *Buccinum undatum* and *Neptunea antiqua* collected by trawl from offshore areas around England and Wales ('background' sites, anchorages and shipping lanes) has been assessed by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) on several occasions since the mid 1990s. In recent years (2002 – 2004), Fisheries Research Services (FRS) has also collected monitoring data on imposex in *Buccinum* from large vessel anchorages, shipping lanes and dredge spoil disposal sites in Scottish coastal waters. Analysis was undertaken according to the OSPAR guidelines, but monitoring has often been hampered by difficulties in collecting sufficient numbers of whelks for reliable analysis of the data.

Data Assessment

Assessment of imposex data was based on Vas Deferens Sequence Index (VDSI) in *Nucella*, Intersex Index (ISI) in *Littorina* and Penis Classification Index (PCI) in *Buccinum*. These determinands of imposex / intersex were chosen by OSPAR for assessment criteria. Vas deferens sequence and Intersex stage are directly related
to the reproductive ability of female gastropods and as such are relevant end-points at the population level. In addition, there are known relationships between VDSI, ISI and PCI described in the literature determined from concomitant field studies on sympatric populations of gastropods (e.g. Stroben et al., 1995). This has enabled assessment criteria to be developed for the most sensitive gastropod species *Nucella lapillus*, and equivalent VDSI / ISI values used for other species. This enables assessment across the whole OSPAR area, including regions where *Nucella* are not present. The OSPAR assessment criteria contain 6 assessment classes, A-F, for *Nucella lapillus*, where

- A: represents close to zero effects,
- B: the response caused by TBT concentrations below the ecotoxicological assessment criteria (OSPAR, 2004),
- C: a level of response where females are not expected to be sterile,
- D: sterile females are present in the population, but reproductively capable females remain,
- E: populations are unable to reproduce and
- F: populations of *Nucella* have expired.

These assessment classes and the VDSI levels that describe them are presented alongside equivalent VDSI / ISI values for sympatric species of gastropods in Table 1.

VDSI / ISI data from shoreline surveys of *Nucella* and *Littorina* have been used to assign each station surveyed between 1994 and 2003 to an assessment class, by comparison with OSPAR assessment criteria. Much of the monitoring data collected since 1997 has been submitted to the ICES database and are available for assessment by ICES or OSPAR.

3 Results and Discussion

National shoreline surveys

In general, the VDSI values in *Nucella* from open coastal sites not close to likely inputs of TBT surveyed between 1994 and 2003 were low and gave no cause for concern for the health of the populations or individuals (OSPAR assessment classes A – C). Data from more detailed surveys of *Nucella* imposex carried out in and immediately around ports, marinas, etc, which are recognised as potential sources of TBT, showed that the maximum intensity of imposex differed considerably between sampling areas. This is likely to be dependent on the types of vessel using the facility and the capacity of the receiving waters to dilute and disperse the TBT. In general, *Nucella* VDSI exceeded 4 (OSPAR assessment class D) only at sites within a few hundred metres of input areas.

While spatial coverage by monitoring efforts on the west coast of the UK has been comprehensive, relatively few sites have been subject to repeat sampling to determine temporal trends. Sites on the east and south of the UK, however, were surveyed in both 1992/3 and 1998/9. These data showed that populations from all sites showed some degree of imposex development, and in most cases all females were affected. Small numbers of sterile females were found at 8 of the sites sampled. The intensity of imposex development had declined between 1992 and 1998 at most of the sites sampled (to 1.78 – 4.28 VDSI), while only two sites showed increasing VDSI.
**Sullom Voe**

Data from the most recent survey (2001) is presented, according to OSPAR assessment class, in Fig. 2. VDSI values are typically high (4.0 - 4.42, OSPAR assessment class D) inside Sullom Voe and around the oil terminal jetties and low (0.17 - 1.88, class A & B) in the open waters of Yell Sound.

The VDSI values for the populations at sites in the Sullom Voe (stations 7 - 12) sites where VDSI values exceeded 4 in early surveys have generally decreased with time (Fig. 3). Other reductions can be recognised at stations 14 – 20, particularly between 1991 and 1993. Generally, the largest decreases in imposex occurred prior to the 1993 survey. Since this time, the degree of imposex in the toothed adults from the Voe has declined more slowly.

The tonnage and number of crude and gas tankers visiting the terminal was at a maximum in 1984. There was also a general movement in the shipping industry away from high leaching rate free-association TBT paints to copolymer paints around this period. The tonnage and numbers of tankers fell from then until 1990, and have since levelled out at about half the peak tonnage and number.

The gradual decrease in imposex at many sites during the period of the study is consistent with these changes. Further restrictions on the use of TBT paints in 2003-2008 should result in further improvements.

**Offshore (sub-tidal) surveys**

Imposex (PCI) data were determined from *Buccinum* sampled atanchorages, shipping lanes and disposal sites in the Firth of Clyde during 2003 (Fig. 4). These data demonstrate that the highest levels of imposex were observed at large vessel anchorages in the upper Firth area (eg A4, A5, A7, PCI = 0.7 -1.5), where shipping traffic is abundant, and at the Cloch Point harbour dredge spoil site (PCI = 0.7 – 0.9) where sediments from potentially TBT contaminated areas are dumped (Fig 4). The level of imposex in these areas is presented against the OSPAR assessment class boundaries (Fig. 5) and shows that these areas, with mean PCI values <2 are class C. (ie where contamination levels should not result in the sterility of the most sensitive gastropod species). *Buccinum* from other sites in the Firth of Clyde demonstrated much lower levels of imposex (<0.3, OSPAR assessment classes A-B).

Data from *Buccinum* and *Neptunea* sampled at offshore sites around England and Wales during 1998 and 1999 demonstrate that imposex is generally lower in offshore populations removed from obvious point sources of TBT contamination (Figs. 6 & 7). VDSI was highest at a site sampled off Rotterdam (Fig. 7), however care should be taken with interpretation of much of these data since low numbers of whelks at most sampling sites prevented sufficient sample sizes (>100) being obtained (OSPAR, 2002).

**Assessment of shoreline monitoring data**

All monitoring data from shoreline surveys between 1994 and 2003 are presented according to OSPAR assessment class in Fig. 8. This includes all data from UK National surveys, Sullom Voe surveys, DOENI surveys and SEPA surveys for both *Nucella* and *Littorina*. A total of 490 station samples are represented in this figure.
The results show that the majority of UK data has been obtained from *Nucella*, and that most sites surveyed fall into assessment classes B – D. Only data from *Littorina* provides information on assessment classes E or F. These data should not be used to determine temporal trends, since the data represented came from multiple surveys covering different geographical areas and undertaken in different years.

**Future monitoring work**

With the international Maritime Organisation (IMO) moves to ban new application of TBT-based antifoulants on large (>25 m) vessels in 2003 and a total ban in 2008, there is an urgent need for baseline monitoring data against which to assess future recovery of the imposex / intersex response in gastropod populations. While the geographical coverage of the UK TBT effects monitoring programme is very comprehensive, with the exception of Sullom Voe, sites on the East and South coasts and some sites in Northern Ireland (DOENI) and Scotland (SEPA), most sites have not been subject to repeat sampling. In addition, many sites have not been sampled for several years. This confounds temporal trend monitoring and would hamper assessment of recovery following the TBT ban.

Therefore the UK Competent Monitoring Authorities, with funding from Defra (Department of the Environment Food and Rural Affairs), are undertaking a survey of TBT effects in intertidal and sub-tidal gastropod populations during 2004. Monitoring work is being coordinated by the UK National Marine Monitoring Programme (NMMP) Ecotoxicology Analytical Quality Control Group (NMEAQC). The survey will repeat all ‘background’ shoreline stations previously sampled to confirm baseline conditions and will provide data against which to monitor for recovery of the imposex / intersex response following the banning of TBT. More extensive surveys of point sources will also be conducted, including harbours and ports on the East and South coasts of the UK, where previous monitoring of this kind has been lacking. Further monitoring of sub-tidal populations in the vicinity of anchorages and shipping lanes will also be undertaken to improve the spatial and temporal coverage of monitoring in these areas.

4 **Conclusions**

Over the last 10 years, competent monitoring authorities in the UK have collected a wealth of monitoring data on the effects of TBT on gastropod populations in a variety of coastal and offshore environments. These data will inform environmental assessments of the state of the marine environment to be conducted by OSPAR and the risk assessment of water bodies failing to achieve ‘good’ ecological status under the Water Framework Directive. Preliminary assessment of the last decade of UK TBT effects monitoring data demonstrates the utility of the new OSPAR assessment criteria and has highlighted future monitoring requirements which are being met in the short – term by a further UK-wide survey during 2004.
5 References


Table 1. OSPAR Biological effect assessment criteria for TBT (from OSPAR, 2004). Assessment criteria for imposex in *Nucella lapillus* are presented alongside equivalent VDSI / ISI / PCI values for sympatric populations of other relevant species.

<table>
<thead>
<tr>
<th>Assessment class</th>
<th><em>Nucella</em></th>
<th><em>Nassarius</em></th>
<th><em>Buccinum</em></th>
<th><em>Neptuna</em>#</th>
<th><em>Littorina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VDSI</td>
<td>VDSI</td>
<td>PCI</td>
<td>VDSI</td>
<td>ISI</td>
</tr>
<tr>
<td>A</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>B</td>
<td>0.3 - &lt;2.0</td>
<td>0.3 - 2.0</td>
<td>0.3 - 2.0</td>
<td>2.0 - 4.0</td>
<td>0.3 - &lt;2.0</td>
</tr>
<tr>
<td>C</td>
<td>2.0 - 4.0</td>
<td>0.3 - 2.0</td>
<td>0.3 - 2.0</td>
<td>2.0 - 4.0</td>
<td>0.3 - &lt;2.0</td>
</tr>
<tr>
<td>D</td>
<td>4.0 - 5.0</td>
<td>2.0 - 3.5</td>
<td>2.0 - 3.5</td>
<td>4.0 ^</td>
<td>0.3 - &lt; 0.5</td>
</tr>
<tr>
<td>E</td>
<td>&gt; 5.0</td>
<td>&gt; 3.5</td>
<td>&gt; 3.5</td>
<td></td>
<td>0.5 - 1.2</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>&gt; 1.2</td>
</tr>
</tbody>
</table>

*Stroben et al., 1995, and ~ No correlation established. # field evidence that *Neptuna* has similar sensitivity as *Nucella*. ^ highest value possible.*
Figure Legends

**Fig. 1.** Geographical coverage of stations sampled during national shoreline surveys 1992 – 1998. Additional stations in Northern Ireland and Scotland were sampled during this time period by Department of the Environment Northern Ireland and the Scottish Environment Protection Agency respectively.

**Fig. 2.** Assessment of 2001 VDSI data from *Nucella lapillus* sampled from sites around Sullom Voe, Shetland oil terminal. Data are presented in accordance with OSPAR assessment classes A – D (see Table 1 for description).

**Fig. 3.** Temporal trend of *vas deferens* sequence index (VDSI) in *Nucella lapillus* from sites in and around Sullom Voe, Shetland, 1987 – 2001.

**Fig. 4.** Location of *Buccinum undatum* sampling sites in the Firth of Clyde area, May and November, 2003. Mid-trawl locations shown. A4, 5 & 7 are large vessel anchorages. Cloch Point is a harbour dredge spoil disposal site, Garroch Head is a disused sewage sludge disposal site, and three sites in the main shipping channel were also sampled.

**Fig. 5.** Tri-butyl tin specific effects on *Buccinum undatum* at various sites in the Firth of Clyde, May and November, 2003. Penis classification indices (PCI) are given for large vessel anchorage (A7, A4&5), shipping channel (Upper, Mid and Lower Clyde channel) and dump sites (Cloch Point and Garroch Head). PCI data are presented as means ± SEM. Horizontal lines represent boundaries between OSPAR assessment classes based on PCI.

**Fig. 6.** Imposex in whelks sampled in 1998 (*Buccinum undatum* = blue bars; *Neptunea antiqua* = purple bars)

**Fig. 7.** Imposex in whelks sampled in 1999 (*Buccinum undatum* = blue bars; *Neptunea antiqua* = purple bars)

**Fig. 8.** UK organo-tin effects monitoring data obtained from shoreline surveys of imposex in *Nucella* and intersex in *Littorina* from 1994 – 2003. Data are presented as number of sites surveyed falling into OSPAR assessment classes A – F according to VDSI or ISI determinations (see Table 1 for description of OSPAR assessment classes).
Figure 1.
Figure 8. VDSI values for populations in the surveys from 1987-2001.
Figure 4.
Figure 5.

Penis Classification Index (PCI)

- Class D
- Class C
- Class A & B

Assessment class:
- November 2003
- May 2003
Figure 6.
Figure 7.
Figure 8.