DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS

Research and Development

Final Project Report

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Project title	The fate of TBT in spoil and feasibility of remediation to eliminate environmental impact
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Executive summary (maximum 2 sides A4)

Executive Summary

The purpose of this project was to determine impacts of TBT on benthic assemblages, biological harm of paint-derived TBT material, fate of TBT and remediation options for removing TBT in highly contaminated dredged material. The principal findings of the project were:

- TBT contamination in sediments is widespread around England and Wales. Some 2-12% of dredged material samples taken over the last decade contained elevated levels thought sufficient to cause widespread environmental impacts if disposed to sea.
- TBT in contaminated dredged material was found in the finer fraction (sediment bound) and light fractions (paint particles) the latter occurring in the samples containing the highest TBT levels.
- After resuspension of sediments contaminated with TBT, paint flakes settled out with highest TBT concentrations at the surface (0-2 cm). This is likely to occur offshore as well as under estuarine conditions during disturbance events and points to a complex environmental fate for paint material.
- Sediment bioassays used for licensing dredged material showed toxicity of TBT at 1 mg kg^{-1} in acute tests. When presented as particulate paint material the assays underestimated potential toxicity in short term exposures but endpoints were similar for sediment bound and TBT in paint in chronic tests.
- Paint-derived TBT has an adverse effect on the diversity and structure of meiofaunal assemblages living in highly contaminated sediments.
- Effects on nematodes are likely to occur by (a) the uptake of leached TBT from sediment pore water through the permeable cuticle and (b) direct ingestion of paint-particles with food. However, large mature nematodes such as *Cyatholaimus gracilis*, were shown to be more resilient to TBT contamination than smaller or juvenile animals.
- Caged dogwhelks deployed in the inner estuary of the R. Tyne showed high levels of imposex after 6 months exposure. Comparative data for animals caged at the disposal site are in preparation

- Separation techniques based on density and chemical differences in dredged material are promising methods to reduce TBT contamination. These techniques could be used operationally during dredging to provide cleaner sediment for disposal, or use.
- Current licensing conditions are restricting biological harm from TBT largely to within the boundaries of disposal sites.

Policy Relevance

In 1972, the UK government, in common with many other European governments, signed the Oslo Convention for the Prevention of Pollution by Dumping from Ships and Aircraft and the London Dumping Convention for the Prevention of Marine Pollution by Dumping of Wastes and other Matter (de Silva & Thomas, 1994; Bray *et al*., 1997). The UK government enacted legislation to comply with these Conventions, firstly, the Dumping at Sea (1974) Act, and later the Food and Environment Protection Act (FEPA) (1985). The main provisions of this last Act are designed to prevent the pollution of the sea by substances that are liable to create hazards to human health, to harm living resources and marine life, to damage amenities or to interfere with other legitimate uses of the sea.

Under the OSPAR Convention, the UK plans to continuously reduce discharges, emissions and losses of hazardous substances to enable the protection and conservation of ecosystems and biological diversity of the maritime area. The UK has also agreed to maintain biodiversity and restore, where practicable, marine areas which have been adversely affected as a result of human activities. This project has been driven by both UK legislation and increasing UK government commitment in Europe. The research presented here has focused on: the fate and behaviour of TBT in dredged material; an assessment of biological effects found in marine/estuarine species continuously exposed to TBT in dredged material in docks, ports, rivers and at a dredged material disposal ground and an investigation of remediation options to reduce TBT contamination load in dredged material prior to disposal to sea. This project meets many of the aims of OSPAR and provides information to assist with decision making (i.e. licensing dredged material for sea disposal) under FEPA.

Scientific report (maximum 20 sides A4)

1. Introduction

Despite the huge amount of research and monitoring that has been carried out during the last 20 years on tributyltin (TBT) (Alzieu *et al*., 1982; Gibbs *et al*., 1988; Waldock & Thain, 1983; Waldock *et al*., 1990, 1992), there are still gaps in our knowledge when addressing the issue of environmental harm of TBT (in the form of waste paint material entrained in dockyard sediments), environmental impact (in terms of its fate and behaviour) and options to remove TBT in sediments especially when highly contaminated material is refused a licence to dispose of dredged material to sea. The fate of TBT in sediments and in dredged material when disposed within environmental systems is unknown. Assessing TBT contamination in sediments is often difficult, as there are no agreed UK quality criteria in sediments (Langston *et al*., 1990), although there is an EQS for TBT in water. Guidance is therefore sought on how to manage sites where TBT remains a problem, particularly where TBT exists as paint flakes and where sea disposal of sediments from the site has been refused.

TBT has entered the aquatic environment from the use of triorganotins as biocides and diorganotins as catalysts, timber preservatives and stabilisers in the plastics industry. Today, it is widely used as the biocidal component in boat antifouling preparations on vessels over 25m, although it will be banned from 2003 by IMO, the legacy will remain for some time. Past shipyard practices have caused local contamination problems and there is concern for aquatic life, especially in some estuaries around England and Wales. The risks posed by TBT adsorbed to sediments has been well documented (Waldock & Thain, 1983; Valkirs *et al*., 1987; Davies *et al*., 1988; Champ & Seligman, 1996; ORTEPA, 1997), however the implications of paint-derived material from historic TBT inputs has not been fully assessed. TBT occurs in sediments in three broad categories (i) large paint chippings (ii) small paint particles derived from high pressure hosing of copolymer formulations and (iii) TBT adsorbed to the sediments. Possible biological effects of TBT as paint-derived material include endocrine disruption, e.g. imposex (Matthiessen & Gibbs, 1998); shell thickening in Pacific oysters (*Crassostera gigas*) (Waldock & Thain, 1983); benthic community change (Rees *et al*., 1999; Waldock *et al*., 1999; Schratzberger *et al*., 2002), acute mortality e.g. larval mussels (*Mytilus edulis*) (Bryan & Gibbs, 1991; Lee, 1991) and water quality problems such as local exceedance of water EQS (Douglas, E., pers. Comm. 2002).

Gaps in the current risk assessments of TBT include (1) investigation of the fate and behaviour of paint-derived TBT (2) an assessment of biological effects of paint-derived material and (3) improved guideline values derived to assist the licensing assessment of dredged material. Specific areas of current concern have been developed into key research objectives:

1. To provide a list of priority sites of concern with regard to TBT contamination;
2. To measure TBT contamination at disposal sites and to relate the contaminants to the bulk properties of the sediment;
3. To evaluate methods presently available for remediation;
4. To examine the nature of the sediment material to establish the viability of physical methods of remediation;
5. To investigate the fate of paint-derived TBT within dredged material;
6. To assess bioavailability of paint-derived TBT from contaminated sediment;
7. To assess biological impact on sensitive species; and
8. To investigate benthic community change related to TBT.

At the start of the research programme, there were no proven remediation techniques aimed specifically at addressing TBT contamination. To address this issue, this study was commissioned to investigate sediment-TBT contamination in England and Wales, and to investigate a methodology to identify and remove paint-derived TBT from dockyard material.

The complexity of the current TBT problem has drawn on expertise from several science disciplines. The format of this report focuses on the key elements, that is: the distribution and fate of TBT in dredged material; identifying areas of concern; an assessment of remediation options; biological responses, impact and community changes related to TBT exposure and development of sediment quality criteria for TBT. This report, for clarity, will address the specific milestones independently.

2. To provide a list of priority sites of concern with regard to TBT contamination.

Monitoring of TBT concentrations and assessments of TBT load in sediments around England and Wales takes place under various monitoring and assessment programmes (e.g. NMMP, FEPA) (CEFAS, 1998). Investigations of TBT data held at CEFAS have shown that sites closest to dockyards, marinas and shipping activity cause the greatest concern (CEFAS, 2000). As such, detailed analysis has focused on dredged material assessments since 1992.

Over half of the dredged material samples analysed for TBT since 1992 have contained <0.1 mg kg^{-1} TBT and have been considered to be acceptable for sea disposal (Table 1). A third of all dredged material samples contain TBT levels between 0.1 and 1.0 mg kg^{-1} and have required more detailed consideration, and on a number of occasions samples have been highly contaminated (>10 mg kg^{-1}). Although sampling under FEPA is spatially limited due to costs, samples taken over the last few years have shown that in general TBT concentrations are lower than in previous years.

TBT is a hazardous substance because it is toxic, bioaccumulates and is persistent in the environment. The half-life of TBT is ½ - 1½ years in aerobic sediments and between 1 and >20 years in anaerobic sediments (Waldock *et al*., 1993). Consequently, some sites are still contaminated with TBT to an unacceptable degree, for example, sites within Falmouth and the River Orwell in 1993, Lyme Bay, River Humber and River Mersey in 1994, River Mersey in 1995, River Tyne in 1996, River Mersey, Swansea and River Tyne in 1997, River Tyne and Solent in 1998/9 and River Tyne and South Wales in 2000. The highest concentrations of TBT occurred in samples taken close to shipyards or adjacent to areas where ships were historically put into dry docks to be hosed down and re-painted with TBT-based antifoulant (Dowson *et al*., 1993). For this reason, the River Tyne is a particular hotspot of TBT contamination (Posford Duvivier, 1999). Other high TBT concentrations are found in marinas where small boats were historically re-painted with TBT-based paints.

In summary, sites of concern (defined as > 1 mg kg^{-1}) are within the R. Tyne and Southampton Water (1998-2000), R. Tees (1998-9), R. Mersey (1998 & 2000), R. Humber and Newport (1999-2000), Barry (2000) and Cardiff (2000-2001).

2.1 The River Tyne.

The River Tyne poses perhaps the most widespread problem of TBT contamination in the UK. Any option for remediation must be suited to the range of sediment types and contamination levels in the Tyne estuary. In order to provide baseline information a survey was conducted April 1999 with the Port of Tyne and Posford Duvivier of the most contaminated harbour frontages in the Tyne estuary. The survey was the most intensive ever conducted in the UK to assess the extent of TBT contamination in dredged material. Over 150 samples were analysed for TBT and the distribution of particle size was determined. The large proportion of sites (72%) contained TBT concentrations > 1 mg kg^{-1} suggesting that contamination may be due to paint particles within the sediment. The sediments were sampled at the surface, 0.5 m and 1.0 m depth to determine the spatial distribution of TBT contamination and distributions at depth.

The results from this survey do not show a clear pattern of TBT in surface sediments or at depth. The results also demonstrate that contamination is associated with sites containing fine material (see Figure 1). This rules out hydrocycloning alone as a useful method of remediation since the amount of coarse heavy material that can be removed readily from the bulk sediment is a very small proportion of the total. It may be possible to use physical separation by exploiting the properties of the paint particles to float them away from the sediment.

3. Measure TBT contamination at disposal sites and relate to bulk properties of the sediment.

3.1 Offshore Disposal Grounds

The capacity of the receiving environments to accept dredged material from ports and harbours identified above has been investigated at five disposal grounds: Tyne Dock (Souter); Falmouth; Liverpool Bay (site Z); Nab Tower and Swansea Bay (outer). Sediment cores were taken using a Rheineck corer and four subsamples were taken at 6cm depths and analysed for TBT. The main finding from this survey was that the Tyne Dock disposal ground contained TBT at an order of magnitude higher than other sites. Further investigations at the Tyne Dock disposal ground showed that contamination of marine sediments by TBT generally reflected the known contamination profiles of licensed materials. TBT:DBT ratios were up to 12:1 in the disposal site, suggesting 'fresh' inputs of TBT with a layering of material of high and low concentrations. Hazardous concentrations of TBT were largely confined to the disposal ground, although concentrations were elevated at the western edge of the disposal ground indicating dumping operations are concentrated on the landward margins of the disposal site or localised migration of sediments. The profiles of TBT concentrations in the sites north and south of the Tyne disposal grounds showed low concentrations of TBT and a decrease with depth indicating degradation of TBT. This is also confirmed by low TBT:DBT ratios (3 or 2). The other disposal grounds show similar degradation patterns although TBT levels were considerably lower. Results suggest that TBT is degraded in the receiving environment.

3.2 TBT and Bulk properties

Many organic chemicals tend to bind to sediments in the < 63 µm fraction (OSPAR, 1993) which suggested that TBT would also be associated with the finer size fractions. Understanding where TBT (sediment-bound or paint particles) resides within dredged material can improve our knowledge of the chemical's fate, behaviour and potential toxicity as well as assist with identifying remediation options if sediments are highly contaminated.

To determine whether TBT was contained in different size fractions, samples of sediments were taken from the River Tyne. The sediments contained a range of TBT concentrations (0.16 – 125 mg kg^{-1}). Sediments were separated into four sized fractions and analysed separately for TBT (<63µm, 63-120µm, 120µm-2mm, >2mm). The results showed that although TBT concentrations were present in all fractions, the majority of TBT (in this sediment sample) was associated with the finer fractions; as expected if TBT is bound to organic material.

Additional sediment samples were taken in the River Tyne to investigate very high levels of TBT (33 - 421 mg kg^{-1}) associated with the presence of paint particles rather than sediment-bound TBT. Both the size and density of particles were evaluated. The results (Table 2) showed that the highest concentrations of TBT (i.e., paint particles) were attributed to the light fractions and the lowest TBT levels were the heavy fractions confined to sizes between 63 - 180 µm. Elevated TBT was confined, in the light fraction, to the > 63 µm size fraction suggesting that Tyne sediments contain larger paint particles than previously suspected.

4. Evaluation of remediation methods.

Recent reviews have investigated the feasibility of decontaminating dredged material (Burt & Fletcher, 1999; Posford Duvivier, 1999; Sullivan, 2000), but few techniques have addressed the specific issue of removing TBT contamination. The most promising approach combines physical separation techniques (to remove TBT adsorbed to small particles), density separation (removing paint particles) and thermal degradation. Several initiatives have been undertaken in Europe (e.g. bioremediation; Sullivan, 2000) and under this programme, research has focused on developing remediation methods to mitigate environmental harm. The aim of this workpackage was to remediate sediments to increase the amount of the sediment that is available for conventional disposal whilst isolating contaminated material which can either be subjected to further remediation (i.e., biological or chemical methods), sent to landfill or placed in a contained site.

Research has focused on rapid methods for remediation and a number of different physical and chemical separation techniques have been evaluated (Reed *et al*., 2001). Experiments have been conducted on sediments spiked at two concentrations (1 and 10 mg kg^{-1}) of TBT paint particles and samples taken from contaminated environments (4 mg kg^{-1} TBT). Results are encouraging and it has proven possible to isolate a heavily contaminated component of the sediment leading to a considerable reduction in the volume of grossly contaminated material (up to ~80%). Samples were fractionated by size and density using froth flotation and density separation techniques and it was possible to identify paint particles in the large/light samples (250 - 500 µm). The results generally show that the highest concentrations of TBT (i.e., paint particles) are associated with the light fractions. Initial results suggest that it may be possible to enhance isolation of contaminated material by these two techniques although the percentage of TBT removed from the sediment would have to be increased for material to be approved by the regulatory assessment procedure and to prevent detrimental biological effects. Further method development is required to increase the proportion of TBT-enhanced material removed from the bulk sediment by these specific methods. Options for remediating contaminated material have been assessed by Posford Duvivier (1999) and Burt & Fletcher (1999) and are collated in Table 3.

CEFAS has worked with several organisations and companies to conduct research to treat TBT contaminated sediments. Sediment samples from the Port of Tyne are being remediated using a thermal desorption system (AMR, 2002) but concerns are raised as to the costs of implementing this scheme. This method has been successfully used in soil studies and has been reported to reduce TBT by >90%. A new remediation technique developed in Germany using a steam stripping process (Eschenbach *et al*., 2001) claims to be able to remediate TBT contaminated material by >98%. The method pre-treats the sediments by initially sieving them to <500 µm and <180 µm fractions. The latter fraction (containing the highest concentration of TBT) is used in the steam stripping procedure. Results have shown that a sediment sample (collected from the River Tyne) contaminated with TBT at 14 mg kg^{-1} can be remediated to 0.097 mg kg^{-1}. Dibutyltin values increased post-remediation suggesting a breakdown of TBT to DBT. A large scale system for use on a dredger could be an option here if treated material is cleaned and disposed to sea.

In summary, the TBT contamination in dredged material from the Port of Tyne is present both as paint flakes and as diffuse contamination adsorbed to fine sediment particles. Given the expense and infrastructure required for many of the treatment processes and the difficulty of handling the waste products, it is probable that separation processes are the best option for managing TBT contaminated dredged material (Sullivan, 2000). It is probable that hydrocyclones and density separators can be used to reduce the volume of grossly contaminated material and allow the clean fractions to be disposed of at sea. The only other suitable process is soil washing (Posford Duvivier, 1999) because other methods lack research and experienced operators, are costly and have problems with their remediation effectiveness.

4.1 Analysis of remediated and TBT enhanced sediment fractions.

Investigations were conducted to identify TBT-based paint particles removed from dredged material by different remediation methods to demonstrate conclusively that paint flakes were the source of the TBT contamination. Scanning electron microscopy (SEM) energy dispersive x-ray microanalysis (EDX) was employed to illustrate the presence of paint flakes within TBT-paint enhanced fractions.

Energy dispersive x-ray microanalysis (EDX) using scanning electron microscopy (SEM) allows for the collection and identification of x-rays emitted by a sample during it's bombardment with electrons. It allows rapid, qualitative identification of the elements present within a sample and provides a map of the spatial distribution of these elements in relation to its morphology and structure. EDX has been employed in a number of environmental applications. These include the detection of trace metals in otoliths (Pontual *et al*., 1997), the differentiation of fish stocks by elemental composition of the otoliths (Mulligan, 1985; Panfili, 1991) and the detection of contaminant particles in melanomacrophages (Pulsford *et al.*, 1992).

This technique was adopted here to demonstrate the presence of antifouling elements in samples and successfully used copper, zinc and titanium as surrogates for paint. Tin concentrations were too low to identify a TBT signal but paint flakes in sediment fractions were isolated (see Figure 2). Furthermore, EM was employed to determine the extent of mechanical degradation of the flakes over time and to identify the possible effects that such breakdown may have on the distribution of TBT paint contamination within sediments.

Identification of flakes and elemental mapping was successfully achieved for positive control sediments spiked with high paint flake loads (20 % w/w), although paint flakes were not detected in any of the TBT-paint enhanced remediate fractions, probably due to the relatively low concentration of particles in these samples (CEFAS, 2002a).

5. Investigate the fate of paint-derived TBT within harbour dredged material.

The fate and behaviour of paint-derived TBT within dredged material after sea disposal and during sediment removal from the river bed was evaluated using laboratory tests to simulate both marine and estuarine conditions. UK offshore conditions were simulated using a U-Tube to produce wave forces typical of such environments and estuarine conditions were simulated using a flume that mimics continuous flow in a river (Thomas *et al*., 1999; Thomas *et al*., 2001). The distribution of paint-derived TBT within sediments was measured at the sediment surface and at depth.

Results produced from the U-Tube experiment indicated that there was a tendency for paint-derived TBT to be drawn to the sediment surface (0-2 cm) under offshore conditions (James, 2001). TBT concentrations (1 mg kg^{-1} and 10 mg kg^{-1}) at depth are shown in Figure 3. Similarly, the results from the flume study also showed paint-derived TBT at the sediment surface. Highest concentrations of TBT were measured at the surface (0-4 cm) during short term tests (3hrs) and longer term tests (24 hrs) (Figure 4). During resuspension events paint particles are therefore likely to accumulate in the surface layers of sediment and this is likely to provide a different fate for TBT particles compared with the rest of the sediment-bound TBT. This corroborates with the results from the remediation work (e.g. density separation) and can explain the presence of high TBT levels found in surface sediments from the River Tyne survey (4.2 above). The latter case study illustrates the importance of disturbance on the remobilisation of historic inputs of TBT either by natural processes and/or by ship movements (e.g. propeller action from vessels or ship manoeuvring).

6. Assess the bioavailability of paint-derived TBT from contaminated sediment.

The assessment of dredged material, prior to licensing material for sea disposal, has in the past relied upon chemical analysis of sediment samples. More recently, biological testing of dredged material has been integrated into the assessment procedure. A battery of biological tests have been validated and are now used to determine both acute and sublethal effects of a suite of chemicals. These standard licensing bioassays were performed to determine the bioavailability of TBT from paint chippings,. Both acute and sublethal toxicity tests were conducted using sediments spiked with paint ground to particle size <63 µm to mimic waste material from ships and in sediment dosed with TBTO. Three concentrations were prepared (0.1, 1.0 and 10.0 mg kg^{-1}) which represented environmental values measured at a range of sites around England and Wales.

Initial results from the paint-derived TBT showed that the marine polychaete *A. marina* was able to survive exposure at all concentrations over the short term (10 days) but feeding was reduced and continued to reduce over the longer term (30 days) (see Figure 5). The casting rate of *A. marina* in sediments containing paint-derived TBT at >2.2 mg kg^{-1} was statistically significant from controls indicating an adverse effect on feeding in highly contaminated sediments.

Results of the sublethal assay (a 21 day whole sediment test) using *C. volutator* showed that there was no or low mortality at the lowest paint-derived TBT concentrations (0.1 and 1.0 mg kg^{-1}) whilst higher mortality (80%) occurred at 10 mg kg^{-1}. The level of mortality continued to increase to 100% over 42 days suggesting paint-derived TBT has an adverse effect on the survival of *C. volutator* over longer timescales and at the highest concentration tested. The increase in mortality with time supports the theory that paint particles leach TBT slowly and therefore continuously exposes the animal causing a delayed toxic response compared with the expected result based on analysis of sediments.

Toxicity data held by CEFAS for sediment-bound TBT suggests a lower value causes toxic responses, for example, a value of 0.16 mg kg^{-1}, the LC50 value, was found for *A. marina* using the same methodology as above (CEFAS, 2002b). The NOEC for *A. marina* in a mesocosm experiment was 0.03 ppm (Matthiessen & Thain, 1989). These results suggest that this form of TBT is more toxic than paint-derived TBT in the short term.

It has been clearly demonstrated that paint-derived TBT is not considered to be as toxic to *A. marina* as free TBT and is an order of magnitude different in its toxic response. Clearly, in the case of *A. marina* a much higher concentration of paint-derived TBT can be tolerated before the dose becomes lethal. Such results suggest that paint particles continue to emit TBT over longer periods which eventually affect the exposure and thus bioavailability of the TBT over this period. As can be seen from the results of these experiments, long term exposure to paint -derived TBT at concentrations >1 mg kg^{-1} can cause adverse biological effects.

7. Assess biological impact of TBT on sensitive species.

The development of male sexual characteristics on female prosobranchs exposed to TBT based paints is a well reported phenomena, particularly in the dogwhelk *Nucella lapillus* (Bryan *et al*., 1986; Gibbs & Bryan, 1986; Thain & Waldock, 1986; Bryan *et al*., 1988; Oehlmann *et al*., 1991; Minchin & Minchin 1997; Matthiessen & Gibbs 1998; Oehlmann *et al*., 1998; Barriero *et al*., 2001; Law & Evers in prep). To assess biological impacts of TBT on sensitive species, investigations of imposex development in the dogwhelk *Nucella lapillus* were conducted at a known TBT contaminated estuary, the River Tyne. The purpose of this research was to establish biological impacts within the River Tyne estuary and compare these with conditions of whelks placed at the Tyne dredged material disposal ground

7.1 River Tyne Survey - caged study

Four sites were initially selected on the Tyne to deploy caged dogwhelks for 6 months, and two further areas, one north and one south of the mouth of the Tyne, were selected as local control sites (i.e. non-impacted) and control were also held in the laboratory. Imposex was measured in *Nucella lapillus* at all sites. Whelks were exposed directly through the water column and through their diet (i.e. mussels deposited in the cages which were also exposed to water containing, on average > 25 ng l^{-1}, up to 100 ng l^{-1} TBT; Douglas, E., pers. comm. 2002). Controls in the laboratory were not subjected to TBT in either seawater or through their diet. Chemical analyses were conducted on whelk and mussel tissue to determine TBT concentrations at the start and end of the field trials.

Imposex was measured using the OSPAR JAMP guidelines (Gibbs *et al*., 1987; Oehlmann *et al*., 1991) and results are shown in Figure 6. Laboratory control and south of Tynemouth control were clean but imposex was recorded at the north site. Highest incidence of imposex (100 and 90%, respectively) was recorded at a busy dock (11-12 km upstream of the River mouth) refurbishing ships >25m in length and a site by a small pontoon roughly 30 metres out from the South bank approx. 2 km from the river mouth. Highest measured TBT concentrations (0.03 - 0.05 mg kg^{-1}) in female whelks corresponded with highest incidence of imposex.

Concentrations of TBT in mussels were low (0.007 µg g^{-1}) in the outer estuary whilst in the inner estuary, higher concentrations were recorded (0.2-0.5 µg g^{-1}). At the dock site, TBT levels were 0.5 µg g^{-1} TBT at the end of 6 months. Evidence suggests that whelks were being exposed through their diet intake as well as continuously exposed in the water column. Results showed that TBT accumulated over time in mussels and therefore increased the overall TBT burden in whelks. Biological impact at the end of the 6 month study was high imposex induction in the inner estuary.

The high index scores for both vas deferens and relative penis size show very high levels of imposex, especially considering that the animals were only exposed for 6 months. The dock site had the highest imposex score and many of the females had produced a vas deferens which ran complete from the penis to the vagina, just stopping short of blocking the vagina. This can eventually cause sterilisation of the females and cause death due to impaction of aborted capsules, or rupturing of the oviduct. The evidence suggests that it is possible that current TBT contamination in the Tyne estuary is likely to mitigate against the successful breeding of whelks in the vicinity of sites sampled.

In summary, it has been demonstrated that rapid induction of high levels of imposex in the dogwhelk *Nucella lapillus* occurs at sites in the inner estuary and low values for imposex were found in the immediate outer estuary. To confirm these results, a repeat survey with more sites both in the inner and outer estuary would be beneficial to gain (1) a clear understanding of the bioavailable TBT gradients in the estuary (2) determine the effect of the proposed 2003 ban on TBT antifouling (3) measure the expected decrease in TBT levels over five years as large ships gradually have to re-paint with newer alternatives and (3) establish improvements in the immediate environment and subsequent recovery in the whelk population.

7.2 Tyne disposal ground - caged study

A similar study was conducted at the Tyne Dock disposal ground. Caged whelks, *Nucella lapillus*, were deployed at five sites along a transect at the west side of the disposal ground. Previous sampling had shown this area to contain the highest sediment TBT levels at the site (Reed *et al*., 2001). Cages were exposed for six months. Chemical analysis and results are being processed and will be reported under DEFRA project C1035.

7.3 Tyne disposal ground - offshore sampling: whelks survey

Because TBT levels in sediments at the Tyne disposal ground were over an order of magnitude higher than elsewhere offshore, a survey was conducted at the disposal ground to assess biological effects (e.g. imposex) in wild (i.e. non-caged) whelks. Although sample numbers were small, *Buccinum undatum* and *Neptunia antiqua* were collected from the Tyne disposal ground and assayed for imposex. *N. antiqua* exhibited the same type of imposex characteristics as *B. undatum* but tended to be more sensitive to TBT levels (Strand, 1999). The presence of level two imposex in *B. undatum* on the Mensink Index (Mensink *et al*., 1996) indicates the formation of a small penis bump but no vas deferens development. No firm conclusions can be drawn from this due to limited sample numbers.

In the same survey, higher levels of imposex development, indicated by the ISI (Imposex Stage Index, which is an average for all females) of 2.5 out of a maximum of 3 occurred in *N. antiqua* compared to *B. undatum.* All the females showed vas deferens development as well as penis development. Fifty percent of females sampled had a well developed penis, although markedly smaller than an adult male penis. The Relative Penis Size Index (RPSI) in *N. antiqua* was a very high figure of 5.9, compared to 0.7 in *B. undatum*. A comparison with another study in the literature suggests that females (in this example they were dogwhelks) suffering from similar levels of imposex as *N. antiqua* found in this study could start to die due to the blockage of the pallial oviduct by the vas deferens. This does not appear to have been reported so far for *B. undatum* or *N. antiqua*.

The occurrence of imposex may be a function of continuous exposure to contaminated material both through the their food and sediment. Exposure to animals in the vicinity of this site may also occur through shipping activity. The latter explanation is supported by CEFAS data (NMMP and other DEFRA R&D projects). Data from the 1998-1999 surveys have been made available and results are shown in Figure 7 and 8, respectively. Low imposex was found throughout many sites sampled in England and Wales but highest imposex was recorded around shipping lanes near Rotterdam (0* - 3+*).

8. Assess benthic community change related to TBT

8.1 Laboratory trials.

To date, little information exists on the effects of TBT on meiofauna assemblages (Austen and McEvoy, 1997; Gustafsson *et al*., 2000). The aim of this workpackage was to address the potential confounding effects of burial (i.e. mimicking sediment disposed to sea) and contamination with TBT: as paint-derived contamination rather than TBT adsorbed onto sediments. A microcosm experiment was designed in which meiofauna were exposed to two types of treatments, involving uncontaminated sediment and sediment spiked with paint-derived TBT at 1 and 10 mg kg^{-1}. These concentrations are environmentally realistic in-shore and were expected to affect benthic communities (see, Matthiessen & Thain, 1989) as opposed to lower levels (<10 ng l^{-1}) affecting molluscs (*Nucella lapillus*) (Bryan & Gibbs, 1990). The main objectives were to (1) assess the survival rate of nematode species at different levels of TBT under ecologically realistic conditions and (2) to assess migration and survival rates of nematode species in different types of deposit, exposing meiofauna assemblages to clean and TBT-contaminated sediment. Experimental treatments and microcosm set-up is shown in Figure 9 and is also described in Schratzberger *et al*., 2002.

Nematode abundance, diversity, evenness, species richness and biomass were measured as well as environmental parameters such as particle size, OC and TBT concentrations. Both univariate and multivariate analyses were performed (Schratzberger *et al*., 2002). The effects of paint-derived TBT on species diversity and richness were statistically significant from controls suggesting an adverse effect on nematode assemblages living in highly contaminated sediments (>10 mg kg^{-1}). As experiments were run over two time periods (4 and 8 weeks), there was a clear delay in TBT exposure in the early stages. This might have been due to slow but continuous leaching of TBT from the paint particles into the sediment pore water and the subsequent uptake by nematodes through their permeable cuticle. After 8 weeks, changes to nematode assemblages showed a clear effect suggesting exposure time as well as level of contamination is important.

Because sediments were spiked with paint particles containing TBT, toxic effects on nematodes were also likely to occur by direct ingestion of paint particles with food, resulting in a species-specific response to contamination depending on feeding habit. Species feeding on large particles (i.e. non-selective deposit feeders) were most affected by high TBT concentrations. It is possible that those species ingested whole paint chips together with the food resulting in an adverse biological effect.

Results of the deposition experiment indicated an immediate and dominant effect of burial on most nematode species compared to the longer-term effect of TBT contamination. The abundance of species that were able to successfully migrate into the new deposit were high (e.g. *Cyatholaimus gracilis* and *Sphaerolaimus balticus*), whereas poor abundance was recorded for species that were not able to withstand burial (e.g. *Viscosia viscosa*). Such species selectivity not only reflects deposition but also contamination. For those nematodes that were successfully able to cope with the deposition and were also present in TBT contaminated sediment (e.g. *Cyatholaimus gracilis*), did suffer by experiencing a change in individual biomass. Results suggested that juveniles had a low survival rate and/or adult reproduction was reduced in the contaminated treatments. Size distribution of the surviving species, *Cyatholaimus gracilis*, exposed to the deposition of highly contaminated sediment (10 mg TBT kg^{-1}) were also affected. Results suggested that the survival rate of nematode species in the top layer of the sediment depends on their ability to migrate, survive and reproduce in the deposit treatment in addition to their ability to withstand TBT contamination. Also, species response is attributed to differences in the rates of uptake and elimination of TBT.

Additions of contaminated material to sediments have demonstrated, in the microcosm experiment, that nematode assemblages are altered and that changes depend on the duration and mode of exposure to the contaminated sediment. Also, it is suspected that TBT could bioaccumulate to high levels in benthic fauna. In general, nematode diversity decreased and changes to the assemblage structure increased with increasing level of TBT contamination. This is consistent with results from field studies on the effects of sediment adsorbed TBT on macrobenthic populations and communities (Langston *et al*., 1987; Waldock *et al*., 1999).

8.2 Field survey (TBT transect at Tyne disposal ground).

A meiobenthic field survey was conducted along a transect from the outer Tyne estuary to the dredged material disposal ground (Figure 10). Sediment samples were collected for the analysis of meiofauna, TBT, trace metals and particle size distribution. Inside the disposal ground, TBT concentrations of 0.121 ± 0.162 mg kg^{-1} (station 32) and 0.043 ± 0.027 mg kg^{-1} (station 33) were recorded whereas no TBT was detected at the two reference locations (stations 37 and 39) south of the disposal site.

In order to compare nematode assemblages from different stations along the transect, total abundance, number of species, diversity, richness and evenness indices were calculated. Results showed there were no significant differences in nematode assemblages collected along the transect in terms of univariate indices. Assemblages, however, differed significantly in terms of species composition. Nematode communities collected inside the disposal ground at stations 32 and 33 cluster separately from those collected at the reference sites. In addition, nematode distribution patterns at station 33 inside the disposal site were less different from those encountered at both reference sites than that at the other station inside the disposal ground (station 32). This suggests that other factors, such as physical disturbance (e.g. deposition), form of TBT contamination and length of exposure are determining nematode assemblage structure. These factors were clearly demonstrated by the laboratory study above.

Rank correlations were highest between nematode assemblage structure and TBT concentration in the sediment, indicating that TBT might be responsible for differences in the distribution patterns of nematode assemblages, however, contributions from deposition would also explain why there were changes to nematode assemblages inside the disposal ground.

To conclude, the changes observed in the laboratory mixture treatments were solely due to the effects of TBT. Nematode assemblages displayed a clear dose response. Diversity decreased and changes in assemblage structure increased with increasing level of TBT contamination. This is a clear indication that TBT caused the observed changes since we controlled for all other factors that might also affect community structure. We conclude that nematode assemblages can serve as good indicators of TBT contamination.

Also, there were confounding effects of the physical disturbance associated with the deposition and chemical disturbance associated with TBT contamination of the deposited material. Results indicated an immediate and dominant effect of burial on most nematode species compared to the longer-term effect of TBT contamination itself. The response of nematode species depended not only on the level of TBT contamination but also on the duration and mode of exposure to contaminated sediment which should be taken into account when assessing the effects of the deposition of TBT-contaminated sediment on aquatic communities.

9. Conclusion

This project has brought together many science disciplines and research groups to help address the problem of TBT contaminated material. Importantly, there are several areas in England and Wales that have a problem with TBT contaminated sediments. Because TBT exists in sediments in several forms, the exposure of TBT to animals can produce a wide range of effects. Research here has shown that the biological effects of paint-derived TBT are not seen as quickly as those shown for TBTO. However, long term impacts of paint-derived TBT are a problem, especially at some sites around the UK (e.g. Tyne) where TBT levels are high (>10 mg kg^{-1}), and where TBT leaches from paint particles in the sediment. Biological effects are seen at different scales both within macro- and meiofaunal groups. Both laboratory trials and field tests have shown that there are changes to animal populations e.g. survival, feeding and imposex; and benthic community changes such as species distribution, diversity, richness and biomass.

Effective management of TBT in dredged material is critical to prevent environmental damage and one viable approach in dealing with the most contaminated material is remediation. Several options were investigated above and could be an effective management tool to remove TBT from the environment. Although considerable progress has been made in this area, costs are high.

With a proposed ban on TBT based antifouling paints due to begin in 2003 (Thomas *et al.*, 2000), the primary source of TBT in the environment will be eradicated slowly. With no further inputs from primary sources, the effect of secondary inputs such as sediments will become more important (Harris *et al.*, 1996). There still remain several sites with localised hotspots especially in areas of intense shipping activity (Hartl *et al.*, 2001). Potential harmful effects to aquatic organisms especially benthic fauna (infauna and epifauna) and those living and feeding in the water column need to be monitored in the future after the proposed ban.

Monitoring at dredged disposal grounds has been informative and should be continued to ensure that the licensing procedure is meeting FEPA (1985) Part II objectives. The overall purpose of this project has been: to provide scientific evidence to aid the protection of the marine environment, the living resources which it supports and human health from TBT contamination; to consider other alternative methods of dealing with TBT contaminated sediments and to review current procedures to better manage sediments in the future.

11. Recommendations for future work

1. Survey on R. Tyne to establish TBT impacts on sensitive species pre- and post 2003 ban. Assess long term exposure and recovery of whelks in estuary and offshore.
2. Develop sediment quality criteria for TBT in marine sediments with further field validation.
3. Continue to assess site specific problems in the Tyne, developing comparative costings for methods such as e-clays, geotextile coverings and within estuary solutions.
4. Further work on the fate of particulate paint material – is it likely to accumulate in quiescent areas of estuaries?

Collaborative Institutes and projects

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Table 1. TBT in Sediments (mg/kg) between 1992-2001.

TBT Levels (mg/kg)	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**Total levels 1992-2001**
0 - 0.1	14% (1)	47% (81)	50% (116)	38% (78)	51% (110)	44% (106)	52% (231)	46% (253)	60% (244)	65% (291)	52% (1511)
0.11 - 1	43% (3)	43% (76)	42% (95)	51% (104)	32% (69)	38% (92)	38% (167)	31% (171)	32% (130)	32% (142)	36% (1049)
1.1 - 10	43% (3)	8% (14)	7% (17)	10% (20)	15% (33)	12% (29)	9% (39)	17% (94)	7% (30)	3% (14)	10% (293)
>10.1	0% (0)	2% (4)	1% (3)	1% (3)	2% (4)	6% (14)	1% (4)	6% (36)	0% (0)	0% (0)	2% (68)
										Total No. of sites	**2921**

N.B. (Number of sites in brackets)

Table 2: TBT concentrations in River Tyne sediment (4 mg kg^{-1})

density ↓   Size →

	38µm	63µm	125µm	250µm	500µm
light	0.73	0.97	3.25	11.48	8.1
medium	0.44	0.22	2.05	1.85	4.01
heavy	0.21	0.32	0.2	1.84	3.46

Table 3. Costs of the different remediation techniques (Burt & Fletcher, 1999).

	Technique	Costs US $ per tonne	£/m^3
Mechanical	Separation	5 - 44	5 - 35
	Sediment Washing	28	25
Physico-chemical	Extraction	40 - 268	30 – 210
	Wet air oxidation, base catalysed decomposition	34 - 945	30 – 735
Biological	Microbial degradation	39 - 181	30 - 140
Thermal	Thermal desorption	70 - 257	55 - 250
	Immobilisation	33 – 158	30 – 125
	Incineration		1000 – 2000

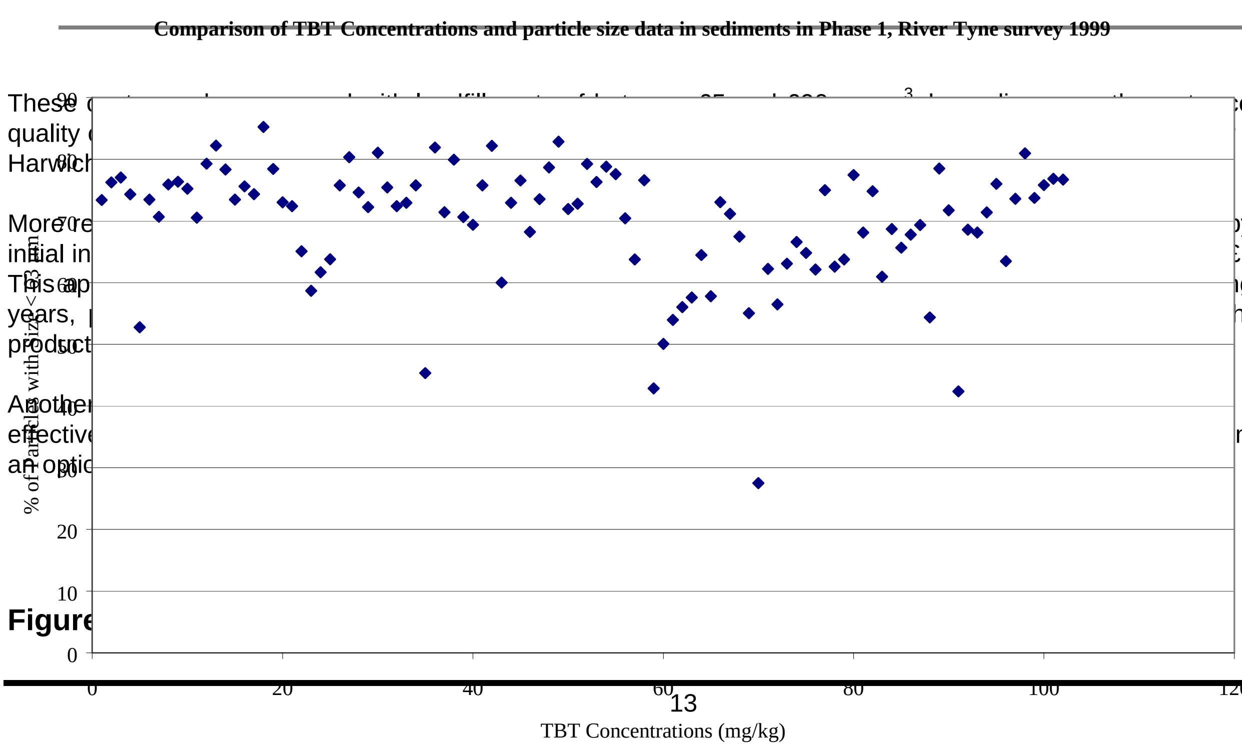

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Figure 2. Paint particle analysed by Electron microscopy SEM EDX

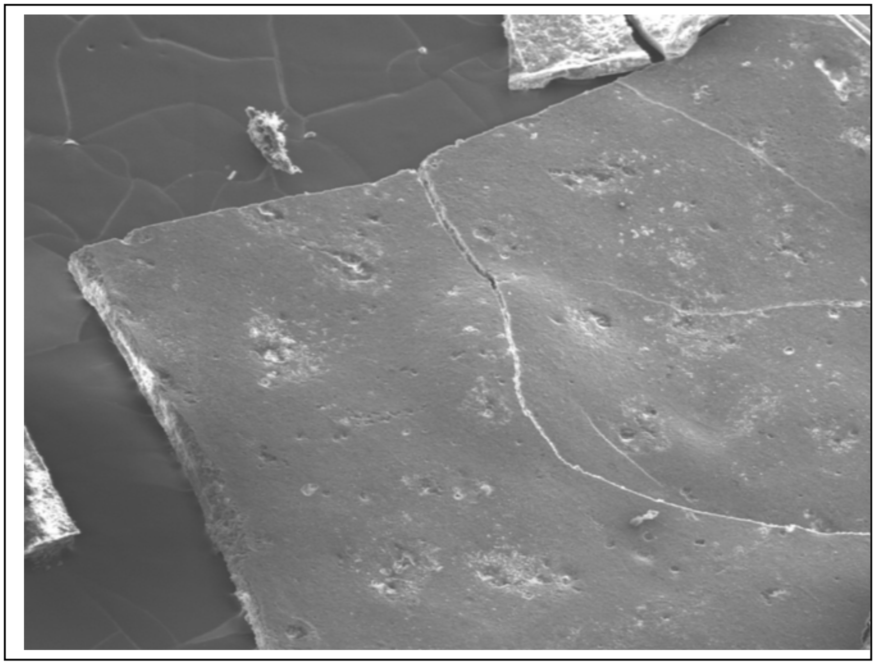
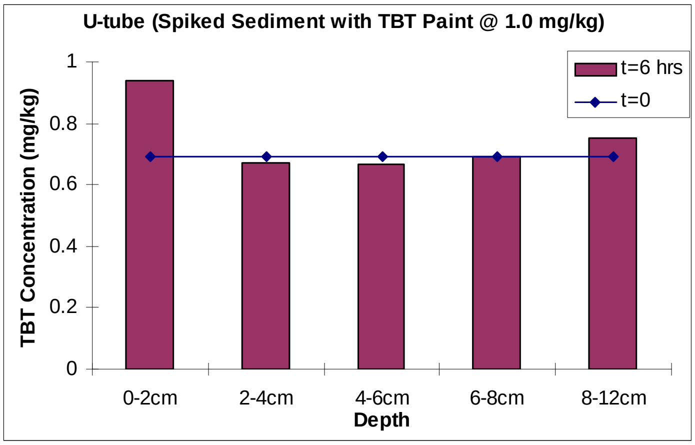


Figure 3. U-Tube experiment run for 6 hrs using sediments spiked with paint-derived TBT at 1 mg kg^{-1} and 10 mg kg^{-1}



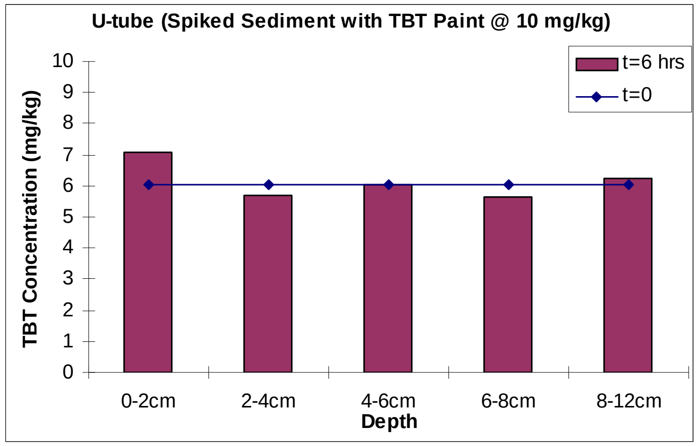
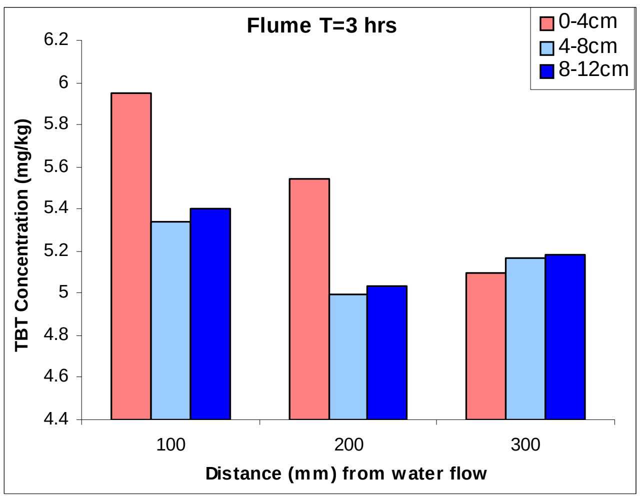


Figure 4. Flume experiment run for (a) 3 hrs and (b) 24 hrs using sediments spiked with paint-derived TBT at 10 mg kg^{-1}.



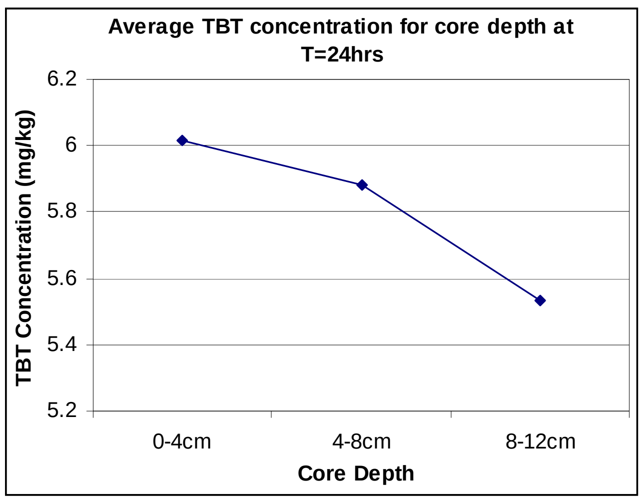
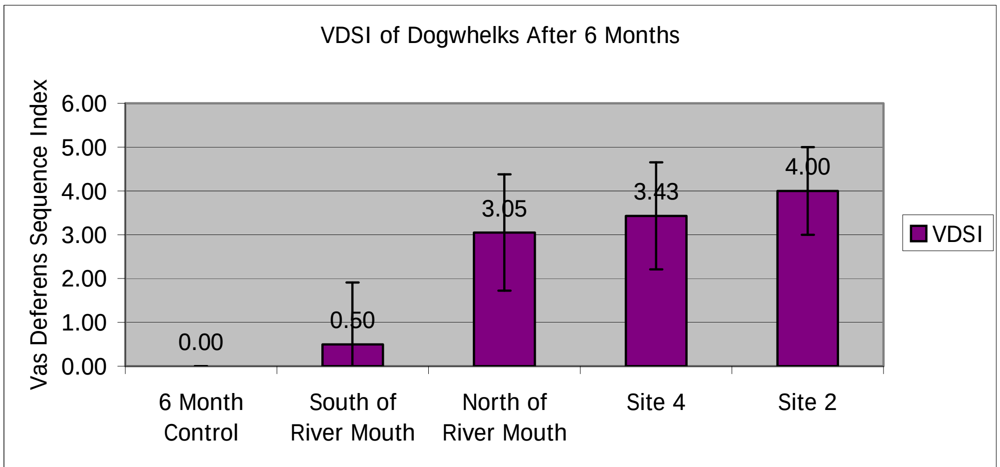
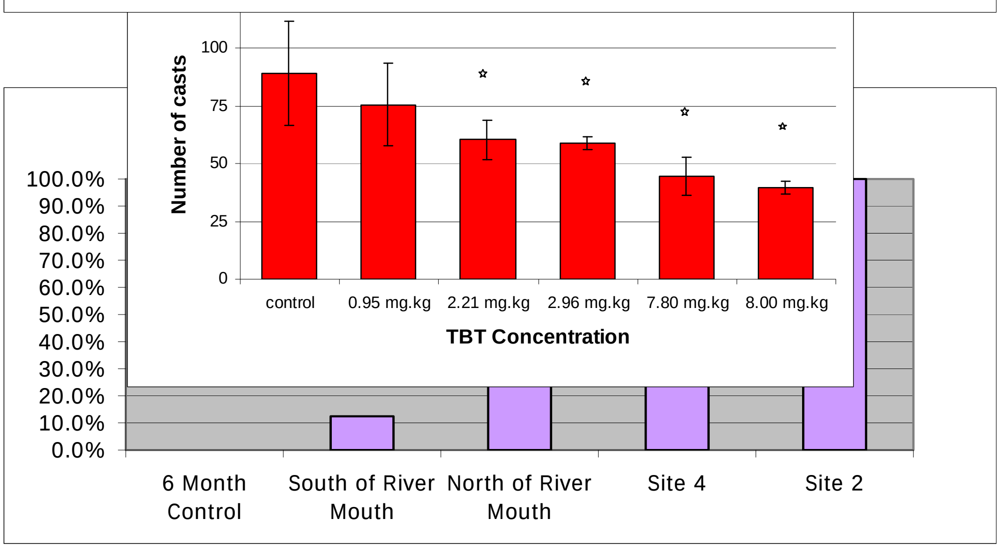
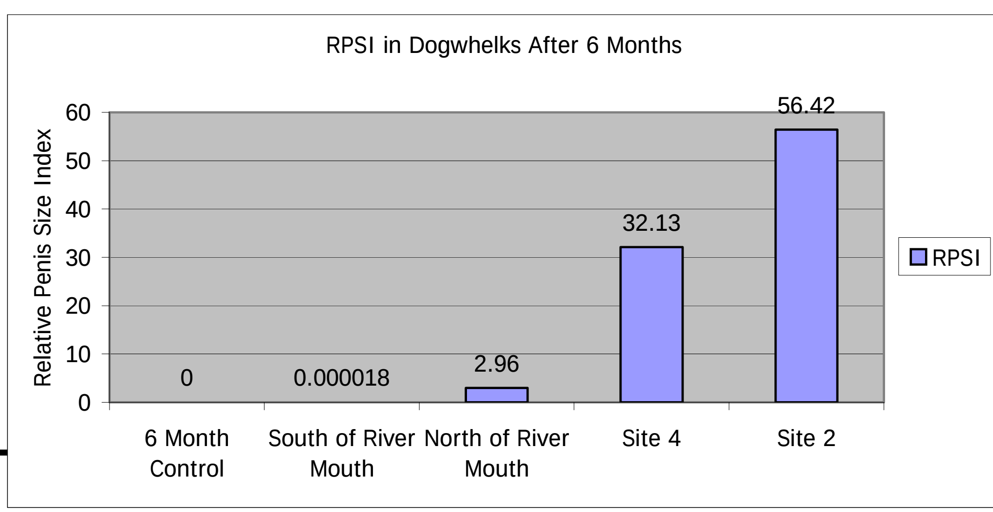


Figure 5. Sublethal effect of paint-derived TBT to *Arenicola marina* using feeding as endpoint.

Figure 6. VDSI, Incidence of Imposex and RPSI in populations of caged dogwhelks







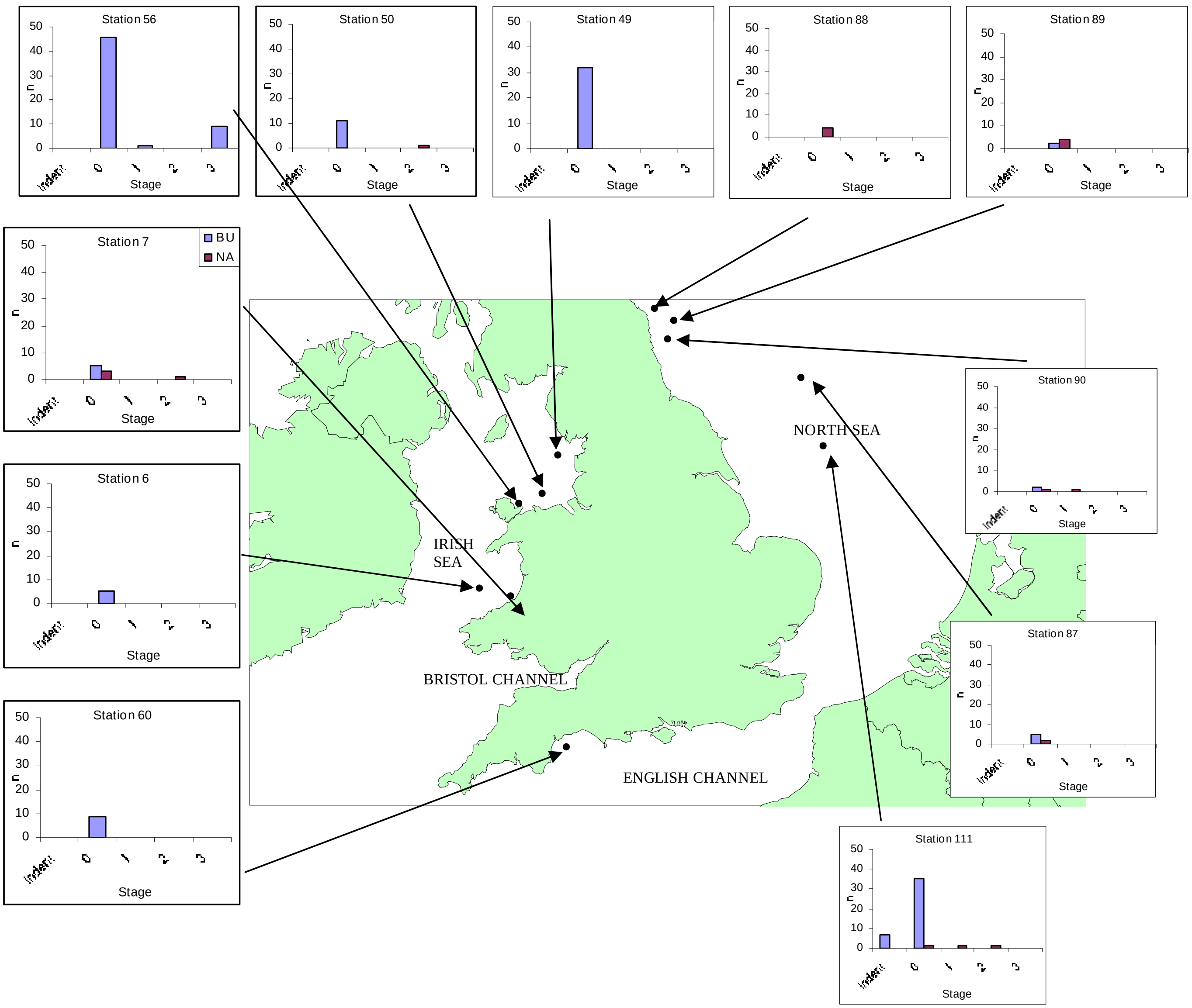


Figure 7. Imposex in whelks sampled in 1998 (*Buccinum undatum* = blue bars; *Neptunea antiqua* = purple bars)

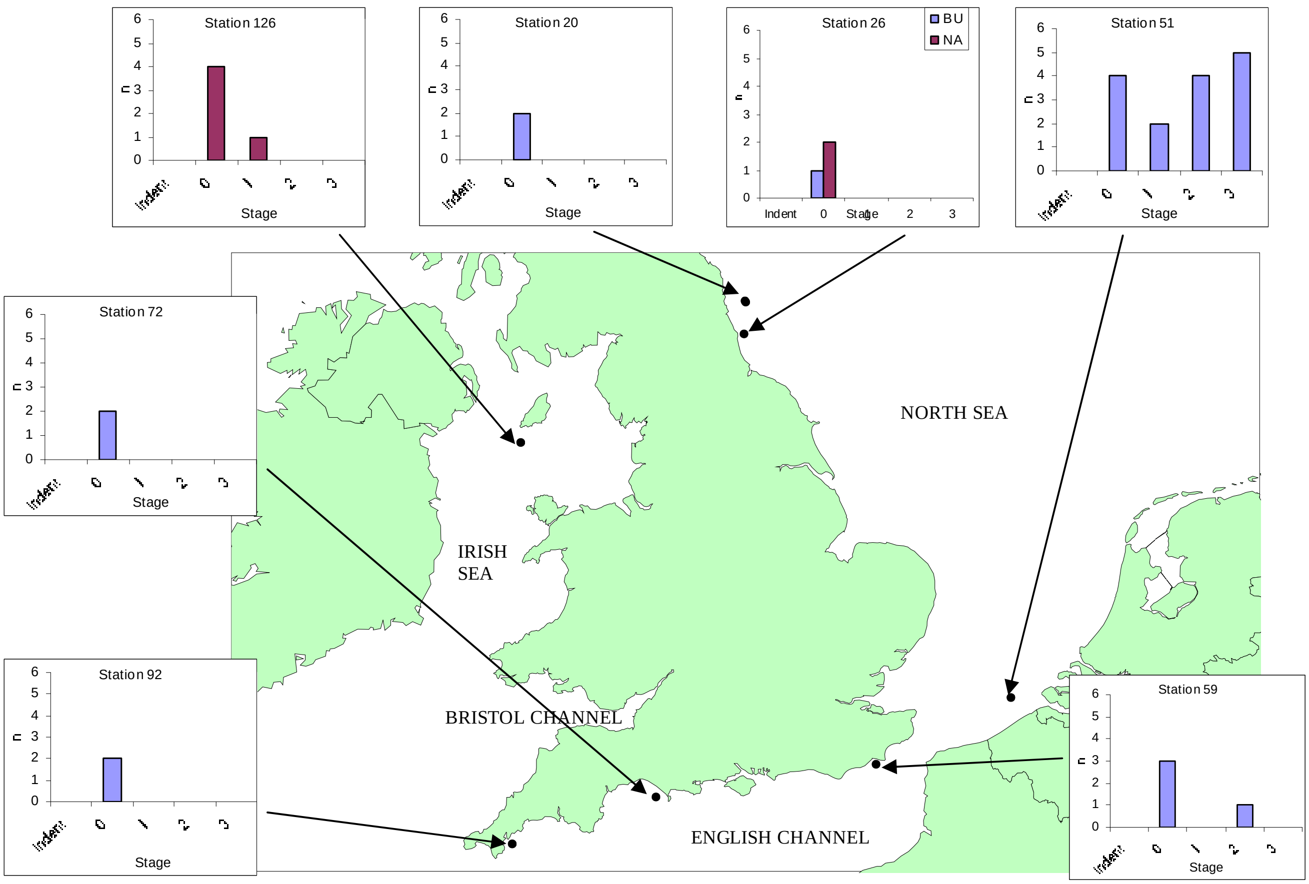


Figure 8. Imposex in whelks sampled in 1999 (*Buccinum undatum* = blue bars; *Neptunea antiqua* = purple bars)

Figure 9. Schematic diagram of experimental treatments.

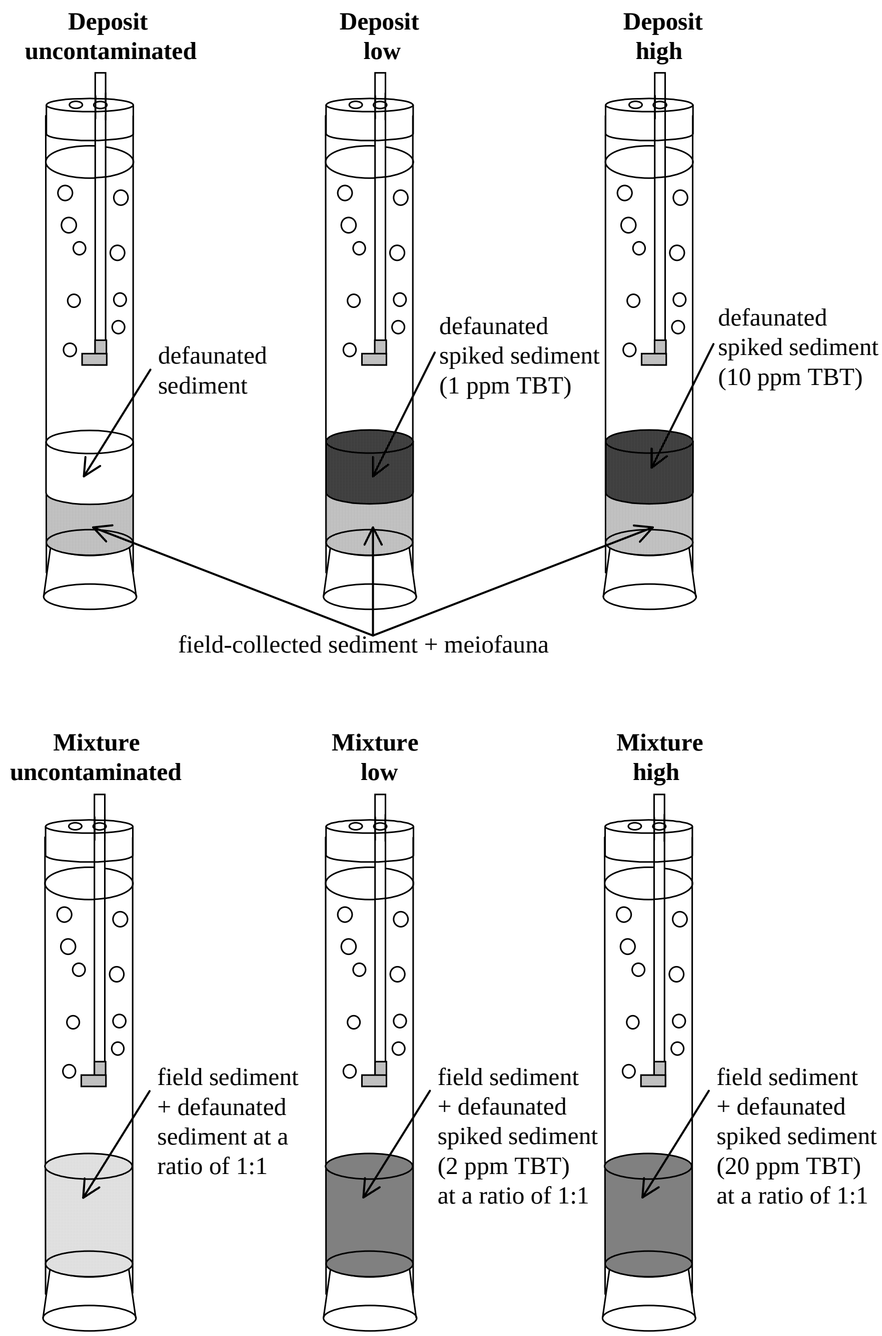
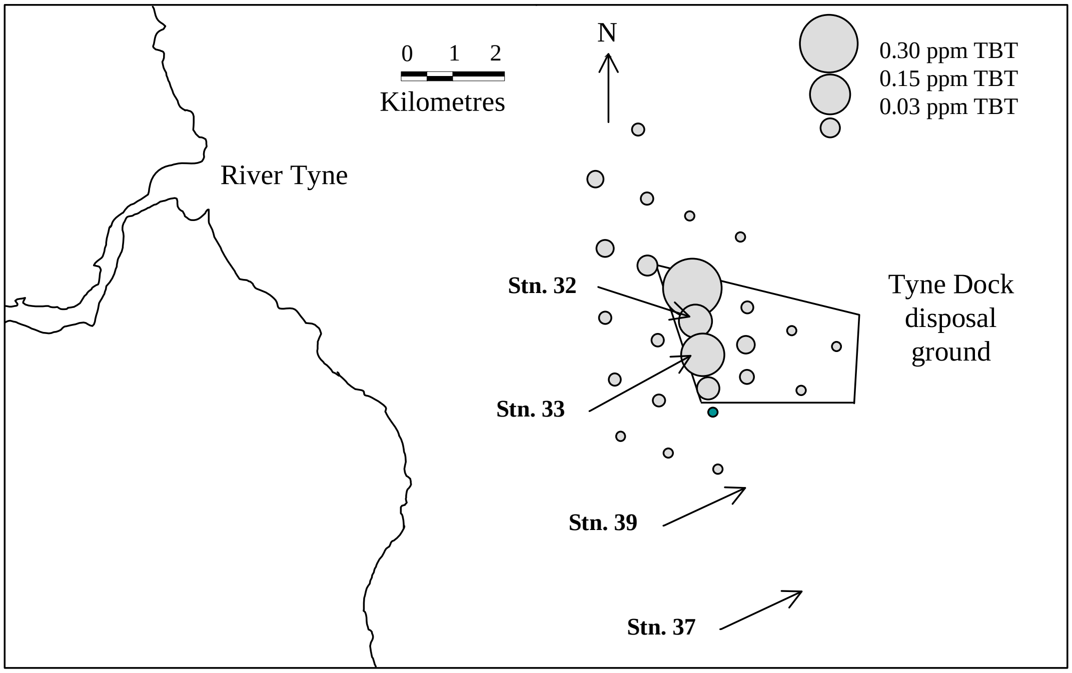


Figure 10. Locations of meiofauna sampling stations along the TBT-transect. Bubbles indicate TBT concentrations in 1999.



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