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Organotin levels in the Ria Formosa lagoon, Portugal

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Organotin concentrations were measured in water, sediments and clams (*Ruditapes decussatus*) from 11 sites in the Ria Formosa lagoon, Portugal, in 1992–93. Results showed a marked spatial pattern of tributyltin (TBT) and dibutyltin concentrations. The highest organotin concentrations were observed at Olhão (site 5), where the most important fishing harbour of the Southern coast of Portugal is located.

Results indicated that fishing vessels, moored in the harbour at Olhão (site 5), were the major source of organotin contamination to the lagoon. No significant seasonal trend was observed, suggesting a continuous input of organotin compounds throughout the year. In several areas of the lagoon the TBT burdens in *R. decussatus* could have deleterious developmental effects. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: organotin; TBT; DBT; Ria Formosa; *Ruditapes decussatus*

INTRODUCTION

Organotin compounds have been used in marine antifouling paints since the mid-1960s and high levels of tributyltin (TBT) have been recorded in coastal waters worldwide, mainly near dockyards, harbours and marinas. The leachates of these paints are known to have deleterious effects on many non-target organisms.[1] Molluscs, especially bivalves, are known to accumulate relatively high levels of TBT in their soft tissues.[2]

Extensive assessments of TBT contamination in bivalves have been performed in several coastal areas worldwide; however, data on TBT burdens in Portugal are scarce. Therefore, a survey on TBT burdens seemed crucial to assessing the potential hazards and implications for human consumption in a commercially important species, such as the suspension-feeding clam *Ruditapes decussatus*.

The Ria Formosa lagoon is a shallow coastal lagoon located in the south of Portugal (Fig. 1). Seaward, it is limited by a non-continuous belt of sandy dunes formed by two peninsulas and five barrier islands that separate the lagoon from the Atlantic Ocean. Six inlets allow exchange of the water with the sea. The mesotidal lagoon extends for about 55 km (E–W), is about 6 km at its widest point and has an area of approximately 84 km^2. The entire water-body is sheltered, with an average depth of 3 m. It includes different habitats, such as salt marshes, mud flats, sand banks and dunes interspersed by a branched system of channels, some of which are navigable.[3]

Only a small fraction (14%) of the lagoon is permanently immersed and approximately 80% of the total area is uncovered during spring tides. The tides are semidiurnal, with amplitudes that range from about 0.7 m (neaps) to about 3.5 m (springs). Daily, in the outer regions of the Ria Formosa, 50 to 75% of the water is exchanged between the lagoon and the ocean. The lagoon does not receive any significant freshwater input, and salinities range between 35.5 to 36.9 psu all year round.[4]

Owing to its significance as a wetland, conservation area and ornithological importance, the Ria was designated as a Portuguese Natural Park in 1987.[3] Its high nutrient concentrations and productivity[4,5] give rise to an important diversity and abundance of flora and fauna. For many aquatic species it constitutes an important spawning and nursery ground owing to its sheltered conditions. Moreover, the combination of hydrographic factors and the nature of the substrate (predominantly sand and silt) constitute ideal conditions for the development of benthic communities.[6]

Fisheries (bivalves and fish) are the main activities in the lagoon, which has great potential for aquaculture development. The Ria is a nursery and breeding ground for many

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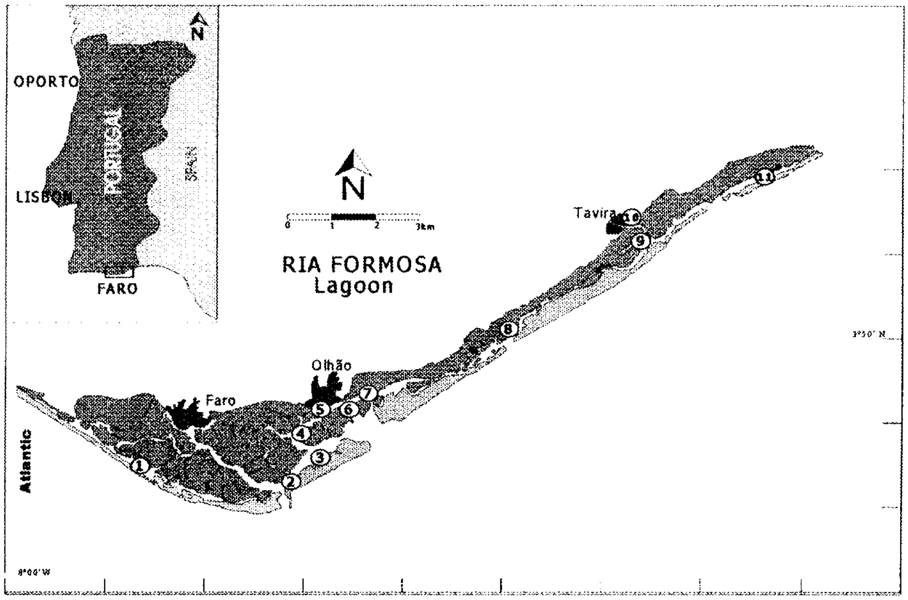

Figure 1. Map of Ria Formosa (southern Portugal).

aquatic species. The productivity of the Ria is evident from the abundance and diversity of the flora and fauna, and the yields from fisheries. The Ria Formosa lagoon has a long tradition of bivalve harvesting, especially of *R. decussatus* (80% of Portugal's mollusc fishery is harvested here). Other species of significance include *Ruditapes romboides*, the thick trough shell *Spisula solida*, the common cockle *Cerastoderma edule*, and oysters *Crassostrea angulata* and *Ostrea edulis*.[7]

Around 20% of the total area of Ria Formosa is occupied by on-growing banks of *R. decussatus* that are cultured throughout the entire lagoon and are the most important commercial species in the area.

In 1891 a total area of 44 m^2 of the lagoon was licensed for clam exploitation, and in 1899 clam exploitation was legislated. By 1996, a total of 1587 clam plots were identified, occupying around 47 km^2. The annual harvest of this bivalve approached 8000 tons in 1993. However, in recent years there has been a decrease in production, to about 3000 tons per year. Average bivalve mortality in the last 10 years has been estimated at ~50%, although reliable mortality data are not available. The mean bivalve production is currently estimated at 0.5 kg m^{-2}, whereas some years ago it reached ~3 to 4 kg m^{-2} and even 7 kg m^{-2} in places.[3]

Water quality in the lagoon has deteriorated during the last few years, due mainly to uncontrolled economic development. Untreated sewage (from a population of ~150 000 people), industrial discharges, agricultural drainage and aquaculture effluents are the major direct pollution inputs along the lagoon. The high number of boats present also has an important contribution to the poor water quality. Boat traffic is largely dominated by small leisure and fishing boats. However, large commercial and fishing vessels also call into the main harbours (Olhão and Faro) (Fig. 1). In fact, the main channels between the mouth of the lagoon and these two ports are the only navigable channels for large vessels.

Considering the high boating activity in the lagoon, an assessment of the contamination of organotin compounds leached from antifouling paints and the associated risks for bivalves seemed important. *R. decussatus* is a benthic suspension feeder, and thus is a potential bioaccumulator of pollutants, especially lipophilic compounds such as TBT. As an important edible species, these clams may also constitute a potential risk for human consumption. Thus, as part of the assessment, we report here the results of a survey of organotin compounds in water, sediments and clams (*R. decussatus*), carried out in the Ria Formosa during 1992–93.

MATERIALS AND METHODS

Sampling

Eleven sites in the Ria Formosa lagoon (Fig. 1) were sampled on each of four occasions: in winter (1992), spring, summer and winter (1993). Most of the sampling sites were located in clam culturing plots. Sites 1, 3, 9 and 11 are sheltered areas where a considerable number of small leisure vessels are usually moored. Site 5 (Olhão) was located next to the most important fishing harbour in the region, in terms of the number of boats and fishing products delivered (approximately 13 000 tons per year). Although there are a considerable number of fishing boats, the vast majority are small (<25 m in length) vessels.

Collection and treatment of samples

Samples of water, sediment and molluscs (where available) were collected for TBT and dibutyltin (DBT) analysis on low spring tides at each location. The methods used for sample processing and analysis are described elsewhere.[8,9]

All the glassware used during extractions and analysis was previously decontaminated with detergent (Decon-90) left in *aqua regia* for, at least, 24 h, to avoid TBT contamination, rinsed with distilled water and dried.

Water samples were collected in 1 l glass stoppered bottles, immediately acidified with 5 ml of concentrated HCl (BDH–Aristar) (to pH ~1) and kept in the dark to prevent decomposition of TBT by light. All samples were analysed within 48 h of collection. At each site an *in situ* measurement of the water temperature and salinity was also performed. Concentrations of organotin compounds were determined on unfiltered seawater samples, in order to include particles normally available to suspension-feeding organisms.

Water samples were divided into two aliquots: 500 ml for the determination of TBT in the sample and the remaining 500 ml for the standard addition (50 ng TBT l^{-1}, as tin). In each aliquot, organotins were extracted, with 5 ml of hexane (Sigma–HPLC grade) by shaking, for 4 min, in 1 l glass separating funnels. The phases were left to separate and the hexane extracts transferred to 20 ml glass vials and kept in the freezer (−20 °C) until analysed by graphite furnace atomic absorption spectrophotometry (GFAAS; see below). Prior to analysis, hexane extracts were treated with 1 M NaOH (Primar) to remove DBT from the extracts. For each set of extractions a blank was run in parallel, using distilled water and hexane.

Surface sediment samples were collected and kept frozen (−18 °C) until processed. In order to achieve better comparability of data, only the <100 μm fraction was analysed. The sediment sample was sieved through a 100 μm polypropylene mesh with 50% sea water and left to settle for 24 h before decanting the overlying water. Three aliquots of approximately 0.5 g (wet weight) each were put in previously weighed stoppered glass tubes and extracted as described below for clam tissues. A further aliquot was taken for wet:dry weight ratios.

Samples of approximately 20 clams were transported alive to the laboratory in ice boxes. Bivalves were then depurated in filtered seawater, for 48 h, to empty their gut contents and avoid interference from sediment contamination. Three replicates of samples of six pooled animals were selected and frozen (−18 °C) until further analysis.

After measuring and dissecting clams, soft tissues were homogenized (Ultra-turrax T25) and three aliquots of 0.5 g (equivalent to 0.2 g dry weight) placed in weighed, glass stoppered tubes. The first tube contained only sample homogenate; the second and third tubes were spiked with standard additions of TBT (0.2 μg TBTO (as tin)) and DBT (0.2 μg DBTCl (as tin)) respectively. A further aliquot was used for wet:dry weight determination before extraction.

All samples were extracted with 5 ml concentrated HCl (BDH–Aristar) (1 h) followed by 5 ml of hexane (Sigma–HPLC grade) (15 min). After centrifugation (3000 rpm, for 4 min), the hexane extracts were kept in vials at −20 °C until analysis.

Analysis of TBT and DBT

Organotin compounds (as tin), were analysed, in the hexane extracts, by GFAAS.[8–10] TBT and DBT concentrations (reported as tin) in sediments and bivalve tissues are expressed on a dry weight basis.

The tin detection limits were 1 ng l^{-1} for water samples, 0.01 μg g^{-1} (dry weight) for tissue samples and 0.005 μg g^{-1} (dry weight) for sediment samples. Validation of the technique is described elsewhere.[10]

Statistical analysis

Several statistical tests were applied to the data, including: a Student t test for paired means, a non-parametric Kruskal–Wallis Anova test and linear regression analysis. All tests were performed with a confidence level of 95%.

RESULTS

TBT in water

The TBT concentrations in water samples from the Ria Formosa (1992–93) are shown in Fig. 2.

A distinct spatial pattern in TBT concentrations in water was evident in winter and summer. Maximum TBT concentrations were detected at site 5 (33.8 ng l^{-1}) and site 6 (12.1 ng l^{-1}). These relatively high TBT levels are probably related to the presence of a fishing port and a dockyard at Olhão (site 5), and thus to a higher density of small vessels in the area.

TBT concentrations in water were not significantly different ($p < 0.05$) between the seasons. When data from all the samples were combined, the highest mean TBT concentration in water was at site 5 (13.7 ng l^{-1}). More than 98% of the water samples presented TBT concentrations in excess of the UK Environmental Quality Standard (EQS): 2 ng $l^{-1} \equiv 0.8$ ng l^{-1} as tin). Despite the localized contamination, results indicate that the concentrations of TBT in the water of the Ria Formosa, although exceeding the EQS, were generally low, with 93% of the samples having TBT concentrations lower than 10 ng l^{-1} (as tin).

TBT in sediments

TBT and DBT concentrations in the sediments in summer and winter (1993) are presented in Fig. 3 and display some variation, which may reflect sediment characteristics. Sediment–water partition coefficients K_d, the ratio between TBT concentrations in sediments and the overlying water, calculated for all samples in the Ria Formosa ranged from

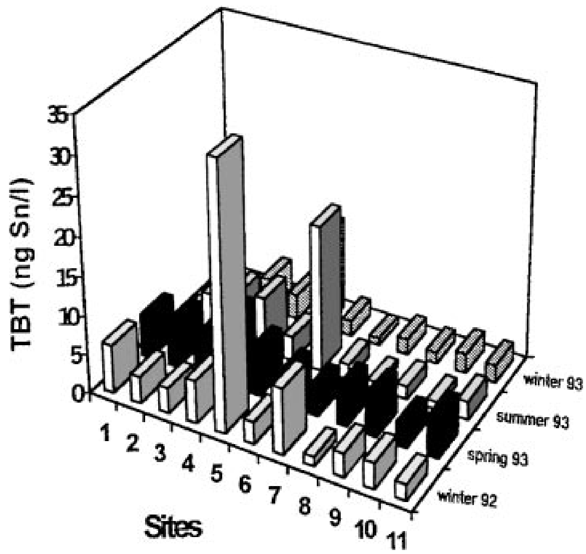

Figure 2. TBT concentrations in water samples collected during a 1 year period, in Ria Formosa.

328 to 39×10^3, perhaps reflecting these differences in sediment properties (see Discussion).

In summer 1993 the highest TBT and DBT levels (both 0.034 μg Sn/g) were observed at site 1. The means and ranges for TBT and DBT (as tin) were 0.010 ± 0.009 μg g^{-1} and 0.010 ± 0.010 μg g^{-1} respectively.

TBT concentrations in sediments in winter (Fig. 3B) showed similar TBT levels at most stations (0.007 ± 0.004 μg g^{-1}) (as tin), with the exception of site 3, where higher TBT concentrations were observed (0.17 μg g^{-1} (as tin)). The spatial pattern for DBT was similar to that of TBT,

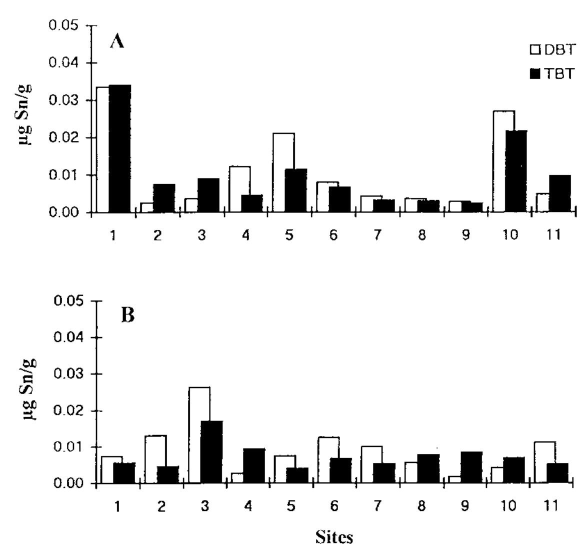

Figure 3. TBT and DBT concentrations (dry weight basis) in surface sediments sampled in summer (A) and winter (B) in the Ria Formosa lagoon.

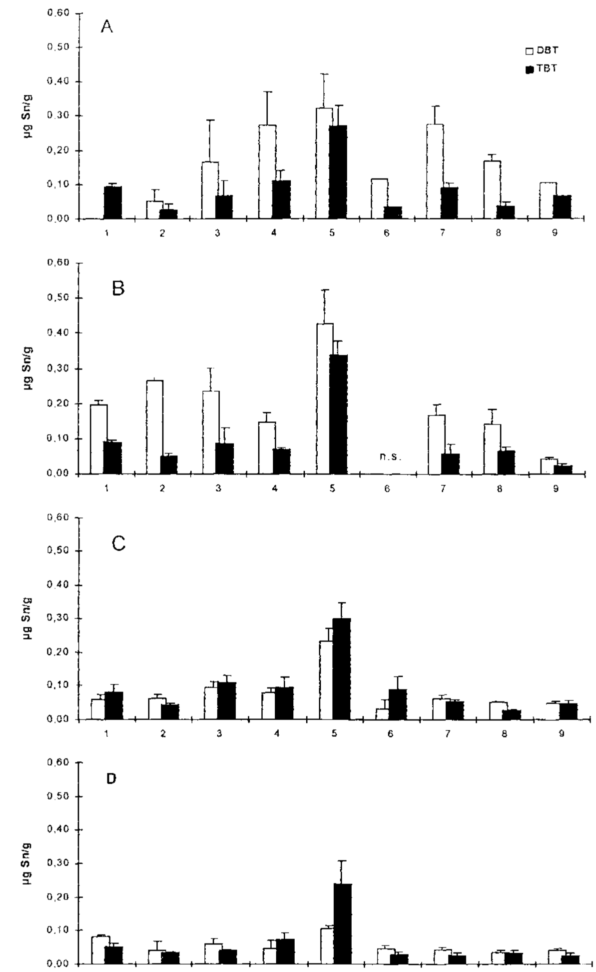

Figure 4. *R. decussatus* TBT and DBT concentrations (dry weight basis) in the whole soft tissues of clams collected in winter (1992) spring, summer and winter. Vertical bars are mean ± standard deviation.

with a mean value (as tin) of 0.009 ± 0.007 μg g^{-1} and a maximum at site 3 (0.26 μg g^{-1}).

The average proportion of extractable butyltin ($\sum$ TBT + DBT, expressed as tin) in sediments present as DBT was 51 ± 17%. A significant linear relationship was observed between DBT and TBT concentrations in surface sediments ([DBT] = 1.024[TBT] + 0.001, $r = 0.826$; $p < 0.01$).

TBT in *R. decussatus*

Seasonal TBT and DBT concentrations (mean ± SD) in the whole soft tissues of the clam *R. decussatus* collected from the different sites in the Ria Formosa lagoon, are shown in Fig. 4.

Organotin concentrations in clams sampled in winter 1992 (Fig. 4A), exhibited a marked spatial variation, similar to that observed for the water, with the highest levels at site 5

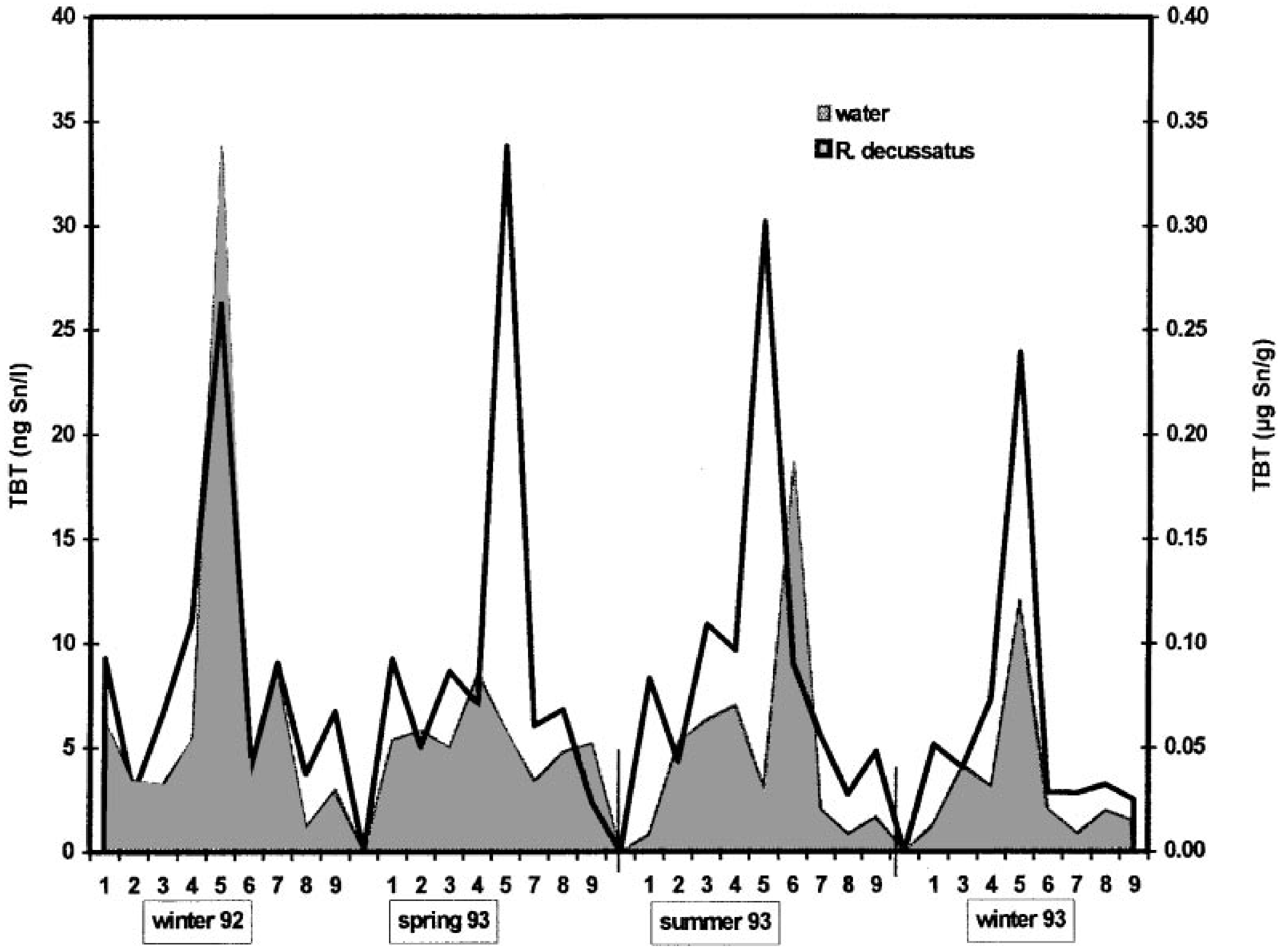

Figure 5. Relationship between TBT concentrations in water (left-hand axis) and in the whole soft tissues of *R. decussatus* (right-hand axis, dry weight basis) ($r = 0.506$, $p < 0.01$).

(0.271 µg g^{-1} and 0.324 µg g^{-1} as tin for TBT and DBT respectively). A comparable pattern was detected in clams sampled in spring, summer and winter 1993 (Figure 4(B)–(D)), with only small variations in maximum values (consistently found at site 5).

These results are consistent with data reported for water (Fig. 2), confirming that the fishing harbour at site 5 is an important source of TBT contamination in the lagoon.

No evident seasonal patterns were observed in TBT and DBT burdens in the whole soft tissues of *R. decussatus* during the sampling period (Fig. 4), suggesting a uniform input of organotin compounds throughout the whole year in the lagoon.

Mean DBT concentrations (as tin) in the whole soft tissues of *R. decussatus* ranged from not detected to 0.43 µg g^{-1}. The proportion of extractable butyltin ($\sum$ TBT + DBT) in *R. decussatus* that was present as DBT varied between 0 and 84% (mean: 57 ± 17%).

By combining all the results for TBT concentrations in these surveys, a significant linear relationship was obtained between TBT concentrations in water and those in the clams *R. decussatus* whole soft tissues ($[Sn_{water}]$(ng l^{-1}) = 0.07 $[Sn_{clams}]$(µg g^{-1}) + 0.05; $r = 0.506$; $p < 0.01$) (Fig. 5). However, TBT and DBT burdens in the clams were not significantly correlated with the organotin compounds present in the sediments. This is consistent with laboratory studies which imply that the major vector for TBT uptake in these suspension-feeding clams is the water column.[11,12]

DISCUSSION

Organotin concentrations in water, sediments and biota of the Ria Formosa lagoon showed marked spatial patterns throughout the year. Generally, higher organotin concentrations were observed at site 5, where the most important fishing harbour of the southern coast of Portugal is located. Results indicate that fishing vessels, mainly moored in the harbour at Olhão (site 5), are the major source of TBT contamination to the lagoon.

According to Portuguese and European legislation, fishing vessels (the great majority of which are smaller than 25 m in length) are nowadays forbidden to use TBT-based antifouling paints. Restrictions in Portugal only started in 1993, and thus were not fully effective at the time of the present survey. However, field studies carried out in the same location, more recently, showed little evidence of reduction in TBT burdens at site 5.[13] TBT contamination in this area of Ria Formosa is unlikely to change for a considerable period of time. Although organotin levels are not excessively high, in view of the importance of the shellfish industry, continued surveillance of TBT contamination should be carried out to ensure risks do not increase.

Since data obtained during the survey did not indicate a significant temporal trend in organotin contamination, a constant input of TBT throughout the year seems likely. The fact that fishing vessels are probably the major source of TBT contamination in the area may explain a permanent input of TBT, since fishing activity spans the whole year. The influence of TBT contamination from small fishing vessels was also reported for other sites on the Portuguese coast, particularly for Sines harbour.[13]

TBT concentrations measured in water from the Ria Formosa are within the range of those reported for estuarine and mariculture waters at other locations worldwide.[13–19] However, TBT levels at several sites in the Ria exceed the EQS for TBT ([TBT] = 2 ng $l^{-1} \equiv 0.8$ ng l^{-1} as tin) adopted in other European countries such as the UK. Furthermore, the chronic toxicity thresholds of TBT for most bivalve species, and particularly for *R. decussatus* and *Ruditapes semidecussatus*[20] are of the same order of magnitude of those found in the Ria Formosa. Possible adverse effects on this important bivalve fishery cannot, therefore, be ruled out.

TBT concentrations at (as tin) which no deleterious effects are observed (NOEC) are in the range of 0.8–10 ng l^{-1} for bivalves, generally.[21,22] Early life stages are particularly susceptible. Results obtained in laboratory experiments with planktonic larvae of *R. decussatus* have confirmed that TBT concentrations of $\geq$25 ng l^{-1} (as tin) cause a reduction in growth and development.[23] Thus, organotin concentrations detected at site 5 (34 ng l^{-1} (as tin)) are within the range that might influence planktonic larvae in the lagoon, where failure in recruitment could have important economic consequences.

Sediment concentrations (and sediment–water partition coefficient) for TBT are consistent with those reported for similar habitats elsewhere.[13,16–18,24–30] Localized contamination of sediments was observed (at site 1) in summer, perhaps due to the existence of a high number of boats moored at this time of the year in the area. Despite the localized contamination, generally the TBT burdens in sediments from the Ria Formosa suggest a moderate to low-level contamination.

TBT concentrations in *R. decussatus* from the Ria are of the same order of magnitude as those reported for harbours in the south of Spain (0.420 to 0.710 μg g^{-1}).[31] Comparable organotin levels have been detected in the oysters *Crassostrea gigas* from the Japanese coastal zone and an Australian estuary ([TBT] = 0.05–0.300 μg g^{-1} and 0.439 μg g^{-1} dry weight respectively)[19,32] and in mussels *Mytilus galloprovincialis* collected from the Portuguese Sado estuary.[25] Levels in *R. decussatus* from the Ria were, however, generally lower than those in clams *Scrobicularia plana* (up to 0.730 μg g^{-1} dry weight) collected from the Portuguese Tagus estuary.[13]

Although organotin levels are not excessively high in most of the Ria Formosa, contamination at low levels is fairly widespread, and, as indicated, is of potential significance to early life stages of bivalves.

Because of the importance of the lagoon as a shellfishery, its restricted flushing characteristics, and the longevity of TBT contamination in sediments, it would seem important to continue surveillance to ensure that the risks to this fragile ecosystem do not increase. In fact, a recent survey carried out on the same area during 2000 and 2001 has shown the TBT levels have actually increased and not decreased (Lobo and Bobianno, unpublished data), revealing that there is a constant souce of organotin compounds. On the other hand, imposex levels screened in gastropods, also for the same area, revealed that females have 100% imposex and in some sites female sterilization has been observed, thus supporting the presence of organotin contamination. Therefore, levels have not shown any significant decrease.

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