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Bioassaying the toxicity of tributyltin-(TBT)-polluted sediment to spat of the bivalve *Scrobicularia plana*

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ABSTRACT: A solid-phase sediment toxicity bioassay with small spat (2 to 3 mm in length) of the estuarine bivalve *Scrobicularia plana* (da Costa) was run for 36 d in 1992: lethal and sublethal effects were investigated. While 2 negative control sediments allowed juvenile weight gain in excess of 140% of initial weight, a control sediment contaminated with non-tributyltin (TBT) compounds resulted in mortalities >80% in 12 d. A TBT-polluted sediment (0.27 µg Sn g^{-1} dry wt) did not produce any mortality or avoidance response, but both growth and burying activity of clams at the end of the trial were significantly reduced with respect to those of spat in control treatments. Although frozen storage of sediment samples renders this experiment somewhat inconclusive, results confirm suspicion of field TBT-sediment deleterious effects at ~0.3 µg Sn g^{-1} dry wt. The environmental implications of findings are discussed in relation to the disappearance of *S. plana* populations throughout Atlantic Europe concurrent with TBT build-up in deposits; in addition, arguments are presented to emphasize the ecological relevance of sublethal stress affecting parameters such as the differential capability of benthic bivalves to avoid predation. Finally, the suitability of *S. plana* spat for widespread sediment toxicity bioassays is assessed.

KEY WORDS: Sediment toxicity bioassay · TBT pollution · Bivalve juvenile · *Scrobicularia plana*

INTRODUCTION

Over the last few years, a body of literature reporting on an unexplained decline of populations of the estuarine bivalve *Scrobicularia plana* (da Costa) has grown: the compilation by Essink et al. (1991) is a paramount reference on this decline for coasts from Germany to France, and wonders particularly about the observed non-winter disappearance of spat. A similar impoverishment of intertidal bivalve populations, notably *S. plana*, has also been recorded in the UK since the mid 1980s; in certain areas (e.g. Poole Harbour, Southampton Water) documented concurrent pollution by tributyltin (TBT) from antifouling paints was suspected of being the cause of this demise (Langston et al. 1987, 1990, Langston & Burt 1991). A correlation between TBT in sediments at ~0.3 µg Sn g^{-1} dry wt and disruption of the continuous structure of size-frequency histograms characteristic of healthy populations was established; it led to the hypothesis that spat are excluded from a long-lasting settlement by accumulation of toxic burdens through active deposit-feeding (Langston et al. 1990) (see also companion paper, Ruiz et al. 1994, this issue, for a more detailed introduction).

While it has been recently shown that environmentally realistic levels of TBT prevent a major proportion of the successive early stages (embryonic, larval) of *Scrobicularia plana* from undergoing normal development (Ruiz 1993), the companion paper proves that exposure of clam spat to low levels of dissolved TBT results in some adverse biological effects of particular relevance to the field situation described above. However, the holding of clams in artificial uncontaminated sand ignored a critical factor in the exposure of the juvenile stage of a deposit-feeding species, the sediment itself; this represents the primary source of food and, consequently, heavy metals (Luoma & Bryan

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1982) and TBT (Langston & Burt 1991). To overcome this deficiency, the solid-phase sediment toxicity bioassay described below was specifically designed to assess the suitability of field sediments for the development of *S. plana* juveniles. Of the approaches addressing sediment quality (see for instance Power & Chapman 1992), assessment of overall toxicity was selected because it integrates the effects of real-world substrata. Although examination of selected biological end-points (survival, growth and burying activity) was the main concern of the test, chemical analyses were also conducted in an effort to identify the cause of detrimental effects.

MATERIALS AND METHODS

Bioassay sediments and chemical analyses. All 4 bioassay samples were collected from the top few mm of the surface sediment layer at mid-intertidal level of selected mudflats and kept frozen in polythene bags up to 5 d before the beginning of the test (Day -5). There were 2 negative (i.e. relatively uncontaminated) control sediments, collected at sites in the Torridge (North Devon, England, July 1991) and Guernica (Cantabrian coast of Spain, December 1991) estuaries; both localities support abundant and apparently healthy populations of *Scrobicularia plana* (Ruiz 1993). A sample from Bilbao estuary (Cantabrian coast of Spain, December 1991) was included in the bioassay as a sediment contaminated with compounds other than TBT which, despite sustaining a clam population (with juvenile recruits; Ruiz 1993), might not be totally innocuous to non-native individuals. Finally, sediments were collected at Cracknore (Southampton Water, England), a site at which superficial layers had been found to contain considerable levels of TBT (above 0.2 µg Sn g^{-1} dry wt) but no *S. plana* spat since 1986 (at the latest). This mudflat is close to a dry dock facility and formerly supported a consistent clam population up to 1978; after this year, the *S. plana* population declined, the last remaining individuals (a few old specimens) contained around 3 µg Sn g^{-1} dry wt (own data). Since highest organotin concentrations usually occur over the summer months (see for instance Langston et al. 1987) and this is the season of most active deposit-feeding in *S. plana* (Hughes 1969, Zwarts & Wanink 1989), Cracknore TBT-contaminated sediments for bioassay were collected in July 1991.

Chemical extraction and analyses for 10 heavy metals and butyltins were performed on independently frozen aliquots of the fine portion of sediments (< 100 µm) as detailed by Bryan et al. (1985 and 1986, respectively); procedures for organotins included solvent rinsing with sodium hydroxide to separate the dibutyltin (DBT) from the TBT fraction. Using these methods trimethyltin might be present in the TBT fraction of natural sediments, although routine analyses of a small number of samples by GC-MS revealed that this was rarely the case. Tin was measured in a Perkin-Elmer 603 AA coupled with 76B graphite furnace; detection limit was about 5 ng Sn g^{-1} on a dry weight basis ($[TBT^+] \approx 2.5\,[Sn]$). The organic content of samples was also estimated from the loss in weight of dry (85 °C) aliquots heated at 400 °C for 6 h. Although no attempt was made to determine metallic content of interstitial water, it was deemed desirable to have an estimation of the concentration of selected heavy metals (Cd, Cu and Zn) and TBT desorbed from sediments and leached into overlying water. To this end, volumes renewed in replicated containers on Day 18 were kept and pooled per treatment. The resulting ~500 ml (see Table 1) were divided and analysed as follows: (1) 400 ml per treatment was acidified (5 ml concentrated HCl l^{-1}) and stored in the dark for TBT determination carried out as detailed in Bryan et al. (1986), rendering a detection limit of about 1 ng Sn l^{-1}; (2) heavy metal levels were directly analysed on small acidified volumes of the remaining sample in a Varian SpectrAA-20 flame (Zn) or a Varian SpectrAA-300 Zeeman graphite furnace (Cd, Cu).

Bioassay specimens and experimental set-up. Bioassay individuals were collected from the Torridge estuary in February 1992 (for details see companion paper) because winter is the season in UK waters when small clams occur at a density high enough to permit collection of a sufficient number of specimens. Juveniles of *Scrobicularia plana* were distributed into 8 sets (40 clams each, 2 sets per sediment treatment) of identical size class composition (mean length 2.14 ± 0.20 mm); weight of initial sets and of size class individuals was also that estimated for the low-TBT experiment described in the companion paper, but initial burying time in sand is not pertinent for the present case.

On Day -5, frozen sediment was taken to the constant temperature room (15 ± 1 °C, 12 h illumination d^{-1}) where the bioassay was conducted for 36 d. On Day -4, thawed sediments were sieved through a 500 µm pore mesh to retain large masses and allowed to settle overnight in plastic beakers with 24 ± 2‰ sea water. On Day -3 the overlying water was decanted off and filtered (0.45 µm) 24 ± 2‰ sea water (hereafter referred to as FSW) added to settled sediments in beakers to make up a stock slurry ~2:1 (sediment to FSW, by volume); aliquots (5 ml) of these stock slurries were taken to estimate dry weight of sediment samples used in each bioassay. Experimental chambers were cylindrical Pyrex bowls (9 cm diameter) which had been previously cleaned with detergent, acid and twice rinsed in distilled water; there were 4 bowls per

sediment treatment. Stock slurry (60 ml) was poured into each bowl and the volume brought to 240 ml by adding 180 ml FSW. Bowls were capped with a lid, and a plastic pipette tip connected to an airline was inserted through a hole in the cap so that a continuous but gentle flow of bubbles came out of the tip suspended ~1 cm below the water surface; the outflow of the air pump used was passed through 2 wash bottles (one empty, the second with distilled water) before it reached the tips to ensure air of high quality. Bowls were then left undisturbed until Day 0 when the water in the bowls was clear and sediments had settled and formed a layer ~1 cm thick.

Initiation, procedure and termination of bioassay. There were duplicate clam sets (A and B) of each sediment, but 4 replicated bowls with sediment (A1, A2, B1 and B2) per treatment; this was designed to allow observation of juvenile burying activity into settled sediment by switching clams between bowls labelled with the same letter every sixth day as follows: on Day 0, 1 set of clams was introduced in each of 2 bowls per treatment (A1 and B1), and the number of juveniles failing to be totally buried was recorded at lapses of 2.5 min for the first 10 min, then every 5 min up to 40 min, and then at 10 min intervals to complete a 1 h observation; clams were then left undisturbed until Day 6. On this day, all individuals within a bowl (labelled A1 or B1) were sieved out of the sediment with a tea strainer, carefully observed under a microscope and dead clams (i.e. showing gaping valves and not reacting to gentle probing) removed for later identification and measurement; finally, live A1 and B1 juveniles were introduced in bowls with settled sediment labelled A2 and B2, respectively, and burying time monitored as before for 1 h. On Day 12, clams were dug out from sediment in bowls A2 and B2 and, after checking and removing dead ones, similarly shifted to bowls with settled sediment A1 and B1, respectively. Alternation of bowls containing juveniles and burying assay into settled sediment was repeated on Days 18, 24, 30 and 36 (see Table 1 for a diagram of procedure). After a 1 h burying trial on Day 36, clam sets were dug out again, sized to the nearest 166 µm, blotted dry for 2.5 h, weighed and frozen.

Partial exchange or total renewal of water was accomplished in each bowl every 3 or 6 d as follows (see also Table 1): on days when a bowl was going to receive clams (including bowls 1 on Day 0), one-third of the total volume (i.e. 80 ml) was siphoned out beforehand and fresh FSW (80 ml) introduced carefully to produce minimal disturbance on settled sediment. On days when clams were going to be sieved out of a certain bowl and also in bowls 2 on Day 0, as much overlying water as possible was decanted beforehand to a graduated cylinder trying not to discard any sediment or clams; then, the same amount of FSW as collected in the cylinder was added to the sediment containing juveniles, plus distilled water to reach a total of 180 ml. The resulting slurry plus bivalves was sieved through the tea strainer to a transient bowl; the slurry (juveniles were retained in sieve) was then poured back to the original bowl and left undisturbed till the next sixth day when it received clams again. On Day 3 of the experiment and every sixth day thereafter, 80 ml of overlying water was switched with equal volume of FSW as above only in bowls containing clams; pH and salinity were measured in the 160 ml resulting from pooling per treatment the volumes siphoned out of bowls. When 80 ml FSW was exchanged in bowls with or to receive clams, ~2 700 000 cells of the flagellate

Table 1. Diagram of procedure applied to sediment bioassay bowls. Asterisks indicate days when pH and salinity were measured in the 160 ml (80 ml from bowl A*i* + 80 ml from bowl B*i*, where *i* = 1 or 2) water switched per treatment. For further explanation see text

180 ml renewal	Bowls A1 and B1 80 ml switch and algae	Clams in		Day of bioassay		Clams in	Bowls A2 and B2 80 ml switch and algae	180 ml renewal
	+	+		0				+
	+	+		3*				
+				6	shift clams →	+	+	
				9*		+	+	
	+	+	← shift clams	12				+
	+	+		15*				
+				18	shift clams →	+	+	
				21*		+	+	
	+	+	← shift clams	24				+
	+	+		27*				
+				30	shift clams →	+	+	
				33*		+	+	
	+	+	← shift clams	36				+

Isochrysis galbana (Parke) were also added as supplementary food to render a minimum final concentration of ~15 cells μl^{-1} in each bowl.

RESULTS

Sediment chemistry

The concentrations of heavy metals as extracted with nitric acid from sediment aliquots are given in Table 2. Although some metals reach maxima in samples from Torridge (Co, Mn), Guernica (Ag, Ni) and Cracknore (Cu, Pb), levels of Cd, Cr, Fe and Zn are highest in those from Bilbao; higher concentrations of some metals in every sample are ascribable to their regional geological background and to site-specific contamination. The estimated dry weight of the sediment samples used in the bioassay, their organic content and their levels of organotin are also shown in Table 2. Dry weight of samples ranged from 31 to 43 g, and the organic content from 3.5 to 5%; butyltins were undetectable in sediments of both negative control sites (Torridge and Guernica), low in Bilbao and high in Cracknore deposits (i.e. TBT + DBT = 0.447 μg Sn g^{-1} dry wt).

Water chemistry and quality

Concentrations of Cd, Cu and Zn in supply and bioassay overlying waters renewed on Day 18 and pooled per treatment are given in Table 3; maximum levels were detected in water from Bilbao bowls for every metal, notably Zn (447 $\mu g\ l^{-1}$). TBT in overlying waters (Table 3) ranged from below detection limits in control samples to 36 ng Sn l^{-1} in that of Cracknore; Cd, Cu, Zn and TBT K_p (sediment-water partition coefficient) values are also given for each treatment. Values of pH and salinity in overlying waters as monitored throughout the bioassay (Table 3) remained consistent in every case at ~8 and ~24‰, respectively.

Acute toxicity of Bilbao sediments

When the juveniles of *Scrobicularia plana* were first deposited on Bilbao sediments and observed for 1 h some clear avoidance behaviour was displayed. There were some individuals which probed the sediment with the foot but did not attempt to dig into it; rather, they started crawling on the sediment surface, and some of them reached and climbed up walls of the vessel to drift away on the water surface by means of fine translucent byssal threads. However, after extensive probing, most of the juveniles had buried into the sediment by 10 min. This burial was not permanent since a number of clams were observed to dig themselves out shortly afterwards; once unearthed, they crawled all over the sediment for long periods, stopping intermittently to probe the substratum. When bowls were checked on Day 3, only a few juveniles were observed to be unburied. The inhalant siphon of some buried individuals was fully extended, probably filtering the overlying waters; no siphon was seen to be cropping the sediment around the burrow entrance and, after close observation, no mark was found indicating that cropping (and therefore ingestion) of sediment had occurred at all. The sediment surface was smooth and untouched except for a number of tracks left by the clams when crawling throughout. No juvenile was found to be dead on Day 6, and when they were switched to bowls A2 and B2 reactions to sediments were less intensive than on Day 0. Many of the clams which managed to bury entered the substratum obliquely, and some did not stay beneath for long;

Table 2. Concentration ($\mu g\ g^{-1}$ dry wt, Fe%) of metals in bioassay sediments (the frozen fraction < 100 µm) as extracted with nitric acid; and concentration (μg Sn g^{-1} dry wt) of organotins, estimated dry wt of sediment samples used and their organic content. Total = TBT + DBT. nd: not detectable

Sediment	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Torridge	0.6	0.1	9	40	25	2.6	650	22	33	136
Guernica	3.6	0.5	7.4	91	41	2.5	279	26	30	146
Cracknore	0.7	1.1	7	50	189	2.6	173	16	186	494
Bilbao	1.7	3.9	6.7	106	118	2.7	299	22	126	646

Sediment	TBT	DBT	Total	%TBT	Dry wt	% organics
Torridge	nd	nd			43.0	4.39
Guernica	nd	nd			31.1	5.05
Cracknore	0.269	0.177	0.447	60	35.0	3.5
Bilbao	0.016	0.010	0.026	62	41.1	3.69

Table 3. Concentration of heavy metals (µg l^{-1}) and TBT (ng Sn l^{-1}) in supply and bioassay water (volumes renewed on Day 18 pooled per treatment). nd: not detectable; K_p (sediment-water partition coefficient) in thousands except for Cd. Also given are mean ± SD of pH and salinity in overlying waters as monitored in days throughout the test (see Table 1)

Water	Cd	K_p	Cu	K_p	Zn	K_p	TBT	K_p	pH	Salinity (‰)
Supply	1.7	–	7.8	–	nd	–	nd	–		
Torridge	9.6	8	6.1	4.1	11	12	nd	–	8.2 ± 0.1	24.5 ± 1.0
Guernica	19	26	14	2.9	16	9.1	nd	–	8.0 ± 0.1	24.2 ± 1.2
Cracknore	6.0	182	16	12	70	7.1	36	7.5	7.9 ± 0.2	24.5 ± 1.0
Bilbao	22	176	30	4.0	447	1.4	14	1.1	8.2 ± 0.1	24.0 ± 0.6

however, periods of crawling were brief and for shorter distances than on Day 0. On Day 9, ~50% of clams were found unburied, and when checked on Day 12 heavy mortalities (mean 82.5 ± 5%) had occurred in both Bilbao vessels. Mortality increased slightly after that day, and only 6 clams survived in 1 of the 2 replicated Bilbao bowls until the end of the bioassay; no trace of cropping and ingestion of sediment was found in containers for the duration of the test.

Chronic toxicity of Cracknore sediments

Torridge, Guernica and Cracknore sediments were readily accepted as burying substrata by juvenile *Scrobicularia plana*: most clams had dug in by 2.5 min with apparent ease. Burial was permanent and only very few individuals were observed to be unearthed throughout the test. Many clams displayed siphon activity shortly after burying, sampling and cropping the sediment around the burrow entrance; dark particles of sediments could be seen being ingested through the white-translucent siphons. As a result of this feeding activity, all the sediment surface in bowls containing the 6 juvenile sets considered had been processed in a few days, presenting an uneven and finely granulated appearance; it also showed abundant distinct formations of sediment ejected through the exhalant siphon (i.e. pseudofaeces) and fecal pellets. All sediment bowls with clams showed this same aspect for the duration of the test. Growth of juvenile sets after 36 d exposure to bioassay sediments was calculated as detailed in the companion paper for the parallel low-TBT toxicity test: the blotted dry weight of clam sets at the conclusion of the bioassay (Table 4) was expressed as a percentage of the mean weight of initial sets (Table 4) after correcting for the individual weight (Table 1 in companion paper) of the very rare mortalities which occurred (Table 4). When transformed data were analysed by ANOVA and Student-Newman-Keuls test (SNK, $\alpha = 0.05$; Table 5), clams in the Cracknore sediments were shown to have gained significantly less weight (mean 36%) than those in either the Torridge or Guernica treatments (mean gain 196 and 140%, respectively). The observed presence of new rings in the shells of most individuals (see Table 4 for total shell length gained by clam sets) also indicates clear growth which in some control juveniles reached 1.5 mm in length. Assuming that all clams in a given set grew at a constant and common rate throughout the experiment, the individual shell growth rate could be calculated to have been ~12 µm d^{-1} in control treatments while only ~4 µm d^{-1} in Cracknore sediments (Table 4).

Original data on the burying activity of each set of clams during the 1 h assay every sixth day were transformed using the formula described in the companion paper; the burying times calculated for each individual observation are plotted in Fig. 1. While mean burying time of individuals held in both control sediments was always less than 4 min, burying time of Cracknore-treated clams exceeded that time from Day 24 onwards. ANOVA and the post hoc SNK test displayed no significant difference between mean burying time

Table 4. *Scrobicularia plana*. Number of survivors in each duplicated sediment treatment and growth parameters (mean ± SD) of clam sets at conclusion of bioassay: total blotted dry weight, total shell length gained and estimated individual shell growth rate

Treatments	No. of survivors Dupl. A	Dupl. B	Total weight (mg)	Total shell gain (mm)	Shell growth rate (µm juvenile^{-1} d^{-1})
Initial	40	40	15.25 ± 0.05	–	–
Torridge	40	38	44.70 ± 1.20	17.58 ± 1.25	12.51 ± 0.57
Guernica	40	40	36.55 ± 4.15	17.75 ± 1.58	12.33 ± 1.10
Cracknore	40	39	20.60 ± 0.90	5.42 ± 0.42	3.81 ± 0.34

Table 5. *Scrobicularia plana*. ANOVA of percentage weight gain and burying time of juvenile sets after exposure to sediments during bioassay. Variances of all 3 data sets had been shown to be homogeneous by Cochran's test: $C < C_{95}$ (3,1). ns: not significant, $^{*}p < 0.05$, $^{**}p < 0.01$

Source	df	MS	*F*	p
Weight gain Day 36				
Sediment	2	13146	24.3	0.014*
Error	3	542		
SNK: Torridge = Guernica > Cracknore				
Burying time Day 0				
Sediment	2	1.20	3.02	0.19 ns
Error	3	0.40		
Burying time Day 36				
Sediment	2	3.49	34	0.008**
Error	3	0.10		
SNK: Cracknore > Torridge = Guernica				

of clams in all 3 treatments on Day 0, but it showed that juveniles exposed to Cracknore sediments buried significantly slower on Day 36 than those kept in either negative control sediment (Table 5).

DISCUSSION

Frozen heavy metal polluted sediments from Bilbao were acutely toxic to juveniles from the Torridge *Scrobicularia plana* population, killing more than 80% of individuals in 12 d. Sediments first induced some remarkable instances of avoidance behaviour (emergence, crawling, drifting), suggesting that clams would have moved into a sediment of a lesser toxicity had it been available (see McGreer 1979); later reduction in the refusal response may have resulted from either diminished condition and fatigue of individuals and/or from amelioration of toxic conditions in bowls through repeated renewal of the supernatant water. Frozen TBT-polluted sediments from Cracknore did not elicit any avoidance response or any mortality in *S. plana* juveniles, but they were chronically stressful in that Cracknore-exposed clams grew significantly less than juveniles in control treatments; in addition, after 36 d exposure, individuals buried more slowly into Cracknore sediments, than into control substrata. These results may be subject to criticism because the bioassay suffers 2 of the 4 limitations most sediment toxicity tests have: (1) disruption of sediment geochemistry and the kinetic activity of bedded contaminants through sampling, storage and handling, and (2) toxicological uncertainties (Lamberson et al. 1992).

As for the first point, collection of intertidal superficial sediments at low tide is possibly the least disturbing sampling method that can be applied, and handling as described above mimics to some extent resuspension of the most unstable and oxic sediment layers. However, storage procedure is likely to have affected the toxicity of some contaminants (see for instance Schuytema et al. 1989), but it was selected because freezing is the best possible way to store sediments for several months as required by bioassay characteristics; in addition, no storage technique totally respects integrity of field samples, and frozen sediments have been shown to be reasonably stable for their heavy metal (Thomson et al. 1980) and organotin (Quevauviller & Donard 1990) content. It is therefore recognised that observations would need to be confirmed by repeated trials using freshly collected sediments.

As for the second caveat, in investigating the cause of toxicity of coastal sediments we first encounter the problem of determining the actual exposure levels of, perhaps, thousands of chemicals and, secondly, quantifying the route of exposure to select those phases of concern for a given biological species. Since sediments from the banks of the heavily industrialised Bilbao estuary have been characterised as considerably polluted by metallic and organic compounds (Ruiz 1993 and own data), contamination of Bilbao sediments used in bioassay with a myriad of potentially toxic substances is strongly suspected. On the other hand, there is neither evidence nor suspicion of Cracknore sediments being seriously polluted by chemical contamination other than TBT. Due to analytical constraints, only the concentrations of 10 heavy metals and butyltins were determined in sediment samples (Table 2), and of some metals and TBT in overlying waters of bioassay bowls (Table 3). Interstitial water was not considered because non-migrating deposit-feeding bivalves do not interact with pore water as much as other groups (non-tubicolous amphipods, worms). *Scrobicularia plana* is primarily a deposit-

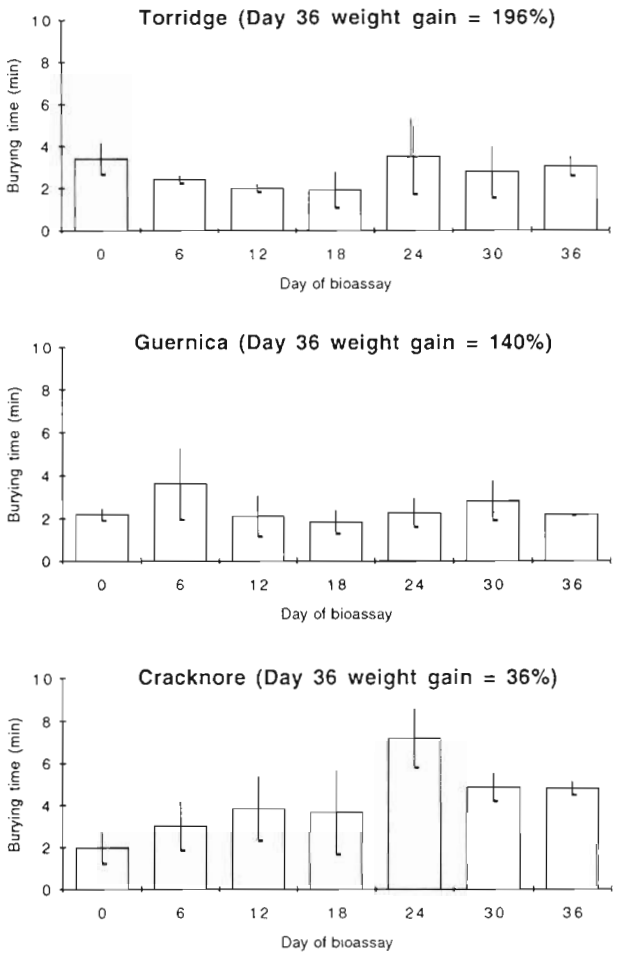

Fig. 1 *Scrobicularia plana*. Burying time (mean ± SD) of juveniles after exposure to negative control (Torridge and Guernica) and TBT-contaminated (Cracknore) sediments during bioassay

feeder in which tissue concentrations of heavy metals and TBT are largely controlled by the levels of these compounds in surface sediments and their partitioning between different sediment constituents such as humics, organic carbon and hydrous oxides of both Fe and Mn (Luoma & Bryan 1982, Langston & Burt 1991). Further, the feeding mode of *S. plana* accounts for the rarity of horizontal migrations (Hughes 1969), and this allows the walls of its relatively permanent burrows to be well ventilated and therefore oxidized; this may act as a partial barrier to remobilized metals (cf. Bryan 1985) which may not have sufficient time to equilibrate with pore water within burrows. In this respect, mass balance results indicated that uptake of hexachlorobenzene (HCB) by the gut from ingested solids was the single most important phase of exposure in the deposit-feeding clam *Macoma nasuta* (Conrad), accounting for 63 to 89% of HCB tissue residues (Boese et al. 1990); these authors concluded that the importance of ingested solids to pollutant tissue residues increases for compounds with high K_{ow} (octanol-water partition coefficient). Since TBT is rather hydrophobic (K_{ow} = 5500 in sea water of 25‰; Laughlin et al. 1986), it is likely that active ingestion of TBT-polluted Cracknore sediments enhanced organotin body burdens in juveniles and, consequently, constituted the main factor resulting in the toxic effects suffered by clams; in addition, the 36 ng Sn l^{-1} of TBT in overlying waters (i.e. within the range reducing *S. plana* juvenile growth; see companion paper) surely contributed to observed results.

Since juveniles in Bilbao treatments did not ingest any substantial amount of sediment, dissolved toxicants must have resulted in observed lethal effects. However, whether concentrations detected in overlying waters (Table 3) totally explain heavy clam mortalities is dubious. The 12 d LC_{50} (concentration killing 50% of the population considered) for juvenile (2 to 3 mm) *Scrobicularia plana* held in water was reported to be 325 µg l^{-1} for Cu and 8000 µg l^{-1} for Zn (Bryan & Gibbs 1983); toxicity of Cu and Zn to the related clam *Abra tenuis* (Montagu) was observed to be additive. When similar-sized *S. plana* spat from Mylor Creek (typical concentrations in sediment of 1117 µg Cu g^{-1} and 980 µg Zn g^{-1}) were exposed for 18 d to Mylor Creek and Restronguet Creek sediments (~490 µg Cu g^{-1} and ~494 µg Zn g^{-1}), substantial mortalities (80%) only occurred in Restronguet Creek treatments; levels in the common overlying water were 40 µg Cu l^{-1} and 300 µg Zn l^{-1} (Bryan & Gibbs 1983). Results of Mylor Creek, Restronguet Creek and Bilbao sediment tests constitute yet another example of how chemical analyses provide indications of the relative contamination among sites, but not a measure of their potential for deleterious effects (Long 1992). On the other hand, Akberali et al. (1981) showed that 500 µg l^{-1} was the threshold level of dissolved Zn inducing siphonal withdrawal and valve closure in adult *S. plana* ~4 cm long, but exposure did not result in significant mortalities during 14 d; similarly, concentrations of Cu in the range 20 to 80 µg l^{-1} have proved sublethally stressful in 30 d to juvenile clams (see companion paper). It can then be concluded that levels of dissolved Zn and Cu in bioassay bowls stressed the juveniles, but synergism with other metals, organometals (including TBT present at detectable levels) and other toxicants should be considered to account for the acute toxicity of Bilbao sediments. Interestingly, small *S. plana* spat were collected from a clam population in the mudflat of Bilbao concurrently with sediment samples for bioassay (Ruiz 1993); this fact constitutes the preliminary indication that the native population of *S. plana* in Bilbao is tolerant of local toxic conditions. Increased tolerance to Cu was reported for

the *S. plana* population of Restronguet Creek (Bryan & Gibbs 1983), where the indigenous *Nereis diversicolor* has been recently shown to have inherited tolerance to Cu and Zn (Hateley et al. 1989) as suggested by Bryan & Hummerstone (1971). Further research on this peculiar *S. plana* population of Bilbao is clearly warranted, particularly considering the toxicological uncertainties raised by the use of frozen, stored sediments in the current study.

With respect to the other 2 possible shortcomings of sediment bioassays listed by Lamberson et al. (1992), i.e. (1) sensitivity to natural sediment features and laboratory conditions and (2) ecological relevance of test, they are not thought to apply to the present technique. Water quality was maintained within safe limits (Table 3), and all samples of fine superficial deposits were collected at mudflats containing recruiting populations of *Scrobicularia plana*, except at Cracknore; behaviour of juveniles in the Cracknore treatment clearly shows that deleterious effects cannot be due to natural unsuitability of sediments. In addition, given the *S. plana* pseudofaeces production and high organic content (Hughes 1969), it is thought that sediment organic matter (Table 2) supplemented by algal foodstuff was not a limiting factor for juvenile growth in any bowl. As for the ecological relevance of the selected end-points, it has already been extensively discussed (see companion paper), and their relation to bivalve population dynamics is explicit; further validation was conferred *a priori* by field data and, thus, bioassay results confirm the suspicion of sediment ecotoxicity to *S. plana* at TBT concentrations ~0.3 µg Sn g^{-1} (Langston et al. 1990).

Why *Scrobicularia plana*?

An immediate question raised in view of the likely ecotoxicity of TBT is why other invertebrate species usually cohabiting TBT-affected estuarine habitats are not suffering a decline similar to that shown by *Scrobicularia plana*. A response is equally immediate: given the limited financial commitment, it is difficult to monitor a representative selection of the whole variety of species found along European coasts, and the general lack of documented reports does not necessarily mean absence of impact. The overall conclusion of Bryan & Gibbs (1991), that untold consequences of releasing TBT are likely to have occurred through subtle toxicity to the reproduction and early life stages of marine organisms, must be accepted. For the sake of brevity, studies of molluscan species inhabiting mostly soft substrata provide important clues as to the extent of damage.

Thus, Minchin et al. (1987) found a strong correlation between declining populations of scallops and flameshells and increased use of organotin net-dips on salmonid farms along the Irish coast, but their suspicion of detrimental effects of TBT on the reproduction and larval development of the bivalves could not be proved. Similarly, circumstantial evidence linking poor recruitment of *Littorina littorea* (L.) in estuaries of the English East Coast with substantial levels of dissolved TBT was shown (Matthiessen et al. 1991), but only very recently impaired periwinkle reproductive and larval hatching success have been found to be induced by 100 to 330 ng TBT l^{-1} in laboratory experiments (P. Matthiessen pers. comm.). The fact that there are some characteristics of the life cycle of this gastropod which are shared by *Scrobicularia plana* (i.e. longevity and planktonic veliger development) leads to the suggestion that, if *L. littorea* populations have not been harmed as much as those of the clam, it is probably because of their multiple periods of reproduction throughout the year. Since clams in Southern Europe recruit 2 or even 3 times a year (Bachelet 1981, Essink et al. 1991), this may also account for the absence of reports on affected *S. plana* populations in meridional areas well known to have been polluted by TBT (e.g. Arcachon Bay); successive larval and juvenile cohorts distributed from April to October and average autumn-winter conditions less adverse than in northern waters probably ameliorate effects of TBT and aid in maintenance of populations.

On the other hand, if no consistent decline of populations such as that reported for *Scrobicularia plana* by Essink et al. (1991) has been noticed in similar studies on other common sediment-dwelling bivalves [see Desprez et al. (1991) and Ducrotoy et al. (1991) for data about *Macoma balthica* (L.) and *Cerastoderma edule* (L.), respectively], an inverse correlation between environmental levels of TBT and bivalve recruitment has been observed for years in UK embayments (Langston et al. 1987, 1990). Thus, a conspicuous impoverishment of bivalve spat was found at sites in Southampton Water from the mid 1980s, and only some of the less polluted sites have shown a few signs of re-established recruitment in 1991–92 (W. J. Langston unpubl.); of a range of species surveyed [*S. plana*, *M. balthica*, *Mya arenaria* (L.), *Abra tenuis*], only *A. tenuis* seems to have bred successfully in the presence of TBT. Since this probably monotelic clam lays its eggs (July-August in UK waters) in a mass within the sediment and, following direct development, miniature adults are hatched (Gibbs 1984), it is likely that its peculiar life cycle (lasting only 1 to 2 yr) has led to enhanced survival. The remainder of these bivalve species have planktonic larvae which are exposed to considerable risks (both natural and anthropogenic) during their development and, particularly, in near-bottom waters at the time of settlement. Nevertheless,

for species reproducing only once a year in North Atlantic European waters, larval settling before water TBT concentrations reach seasonal maxima in mid-late summer (see for instance Langston et al. 1987) will usually allow spat to grow to 3 to 5 mm by the end of August (e.g. for *M. balthica* and *M. arenaria* in the Wadden Sea settlement occurs in spring; Günther 1991 and 1992, respectively). Burying capabilities afforded by 3 to 5 mm length guarantees a minimum depth refuge for overwintering. Since *S. plana* populations at northern latitudes concentrate spawning in August, early stages have a shorter time to grow before conditions become less favourable and, thus, it is quite possible that significant reductions induced by TBT on the larval (Ruiz 1993) and juvenile (see also Ruiz et al. 1994) growth rate are a factor of critical importance in the failure of recruits to prosper.

In addition, a multitude of factors, both physiological and ecological, may interact to result in the observed rarity of successful *Scrobicularia plana* recruitment. For instance, it is generally accepted that burial depth in bivalves is largely determined by length of siphons (Zwarts & Wanink 1989) and shape of shell (Trueman 1983). It could be speculated that, in tidal flats where several bivalve species coexist sharing a common predator pressure, TBT (both dissolved and sediment-bound; see also Ruiz et al. 1994) will deprive *S. plana* spat of their natural strategies to avoid predation (i.e. ability to grow fast by secreting a thin shell and attainment of deep reburial rapidly). This would divert crab attention from equally shallow-buried but thicker-shelled *Cerastoderma edule* of a similar size (since they will be probably rejected in favour of a prey with less-resistant shell and, therefore, shorter handling time; see Boulding 1984) and also from similarly shelled spat of species such as *Abra tenuis*, *Macoma balthica*, *Mya arenaria* (which, for reasons described in the previous paragraph, will have reached a safer size and depth refuge). Predator foraging would therefore concentrate on the increasingly vulnerable *S. plana* recruits. This indirect effect of TBT is likely to be of high environmental relevance because decapod crustaceans appear relatively resistant to TBT (see review by Bryan & Gibbs 1991) due to their fairly efficient ability to degrade it (Rice et al. 1989) and, as a result, crab activity in the field would not be substantially diminished by organotin pollution.

The *Scrobicularia plana* spat sediment toxicity bioassay: suitable for routine use in Europe?

Even though we do not intend to review current sediment-testing techniques and their reasoning, we wish to emphasize the potential of the present bioassay since there is an almost total absence of marine whole sediment bioassays using European autochthonous species (see Chapman et al. 1992). *Scrobicularia plana* is a coastal bivalve well distributed in both Atlantic and Mediterranean Europe, it is tolerant of a considerable salinity range and it accumulates pollutants mainly from solid phase sediments (through active deposit-feeding) but also from overlying waters; the usefulness of adult specimens to monitor organometallic and heavy metal pollution has been extensively proved (Langston & Burt 1991 and Bryan & Langston 1992, respectively). Its spat are relatively easy to collect in the field, to handle, to feed and to maintain in the laboratory with no major effort for both growth and toxicological studies (see Bachelet 1981, Bryan & Gibbs 1983). Thus, it has been feasible to run the present experiment to test survival and growth in a way similar to that standardised for juvenile polychaetes (Pastorok & Becker 1991). In addition, other sublethal indices reflecting fitness of spat of this and/or other intertidal or subtidal bivalve species, such as reburying activity (assessed as herein or otherwise, e.g. as with amphipods; see Swartz et al. 1985), are of high environmental relevance for early infaunal stages inhabiting the less stable layers of sediments (see also companion paper). Finally, bioaccumulation resulting from exposure to sediments may also be measurable.

Among the drawbacks this bioassay may present, 3 are considered foremost: (1) its inability to provide a way to elucidate possible mechanisms of toxicity; thus, the present procedure is one more plainly descriptive bioassay; (2) its restricted potential to become a third generation test (i.e. tests investigating toxicant effects on reproductive output); this disadvantage also applies to most current sediment tests and can apparently only be overcome by using short-lived species usually of a lesser importance for the local ecosystem; and (3) the limited availability of sufficient specimens to perform routine bioassays. Lacking a commercial source of *Scrobicularia plana* spat, individuals must be collected in the field; this restrains the working season, particularly in northern waters where the clam only reproduces once a year (Essink et al. 1991) and small spat can only be captured during a few months. The problem of mistaking juveniles of *S. plana* for those of other bivalve species which cohabit estuaries and are of similar external morphology (notably *Abra tenuis;* see Gibbs 1984) is considered to be minor for careful toxicologists with a basic training in bivalve taxonomy. Finally, although the use of different populations with differential sensitivity and pre-test history may render any intercalibration exercise impracticable, the current trend is towards finding the most appropriate bioassays for specific areas of concern by using indigenous species (Chapman & Long 1983). Bearing in mind

the likely ecotoxicity of TBT to *S. plana* populations in estuaries throughout the northeastern Atlantic and the persistence of butyltin in their deposits, it is concluded that the *S. plana* spat sediment bioassay certainly possesses the potential to become a very useful tool for assessment and regulatory purposes within Europe.

CONCLUSIONS

Frozen Bilbao sediments proved fatal to *Scrobicularia plana* juveniles unable to avoid them; it seems possible that the native clam population at Bilbao has developed a tolerance of local toxic conditions originated from heavy metal and, allegedly, other pollution. Sublethal effects of frozen Cracknore samples strongly suggest that field sediments polluted by TBT may act as a trap to *S. plana* recruits: sediments would allow prompt settlement of drifting juveniles (and, presumably, competent pediveliger larvae) which, by means of active deposit-feeding, may experience some growth; nevertheless, this would only be ~20 to 25% (as weight gain) of the potential growth experienced in TBT-unaffected sediments. In the best case, incorporation to the reproductive stock of the population — which in UK waters occurs by the second summer after settlement, when clams are ~2 cm long (Hughes 1971) — will be delayed about 9 yr. In the most probable scenario, juvenile bivalves forced by their size to stay in shallow sediment layers will suffer the highest mortality rate because of predation, loss and exposure to extreme temperatures (Zwarts & Wanink 1989). If washed out by wave action or current scour, debilitated spat would not rebury quickly, thus prolonging exposure and increasing risk of death (see also companion paper). This hypothesis is further supported by the observation of inert but live *S. plana* spat lying on the sediment surface during low tide in TBT-affected areas within Poole Harbour (N. D. Pope pers. comm.). Effects of sediment TBT will be worsened by concurrent exposure to increasing levels of dissolved TBT during the non-winter yachting season; joint action of other chemicals having similar gradients of contamination cannot be discarded.

Results of the bioassay constitute sound evidence of deleterious effects of TBT-polluted field sediments on local infauna, confirming concern about the environmental relevance of TBT accumulated in deposits (Langston et al. 1990, Waite et al. 1991, Dowson et al. 1992). Our observations may help to explain the widespread decline of *Scrobicularia plana* populations (Essink et al. 1991), addressing particularly the reported non-winter disappearance of clam spat. Superficial sediments containing levels of TBT of similar magnitude to those used here and shown to produce sublethal stress to juvenile *S. plana* have recently been reported for coastal areas throughout Atlantic Europe, Sado and Tejo estuaries in Portugal, Oléron Island and Arcachon Bay in France, Rhine and Scheldt estuaries in The Netherlands (Quevauviller & Donard 1990), East Coast estuaries in the UK (Waite et al. 1991, Dowson et al. 1992), and, more specifically, in sites within Poole Harbour and Southampton Water where *S. plana* populations have been decimated (Langston et al. 1987, 1990, Langston & Burt 1991, own data). It is therefore concluded that TBT in superficial sediments is likely to have greatly aided in preventing the successful settlement of *S. plana* spat in a number of European areas; in addition, the continued presence of moderate levels of deposit-bound TBT may render mudflats unsuitable for the development of juveniles of this and possibly other sediment-dwelling bivalves.

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