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Survey of Imposex in Dogwhelks *(Nucella lapillus)* from North Sea Coasts

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Following a North Sea Task Force (NSTF) initiative in 1991, and funding from the Department of Environment (DOE), The Scottish Office Agriculture, Environment and Fisheries Department Marine Laboratory (SOAEFD), Aberdeen, was awarded a contract to assess the impact of tributyltin compounds in dogwhelks in all North Sea coastal waters. These waters were surveyed in 1991 and 1992 to evaluate the distribution and effects of tributylin (TBT) compounds on the common dogwhelk, *Nucella lapillus*. Wild and, where necessary, transplanted dogwhelks were analysed for imposex.

Historical records indicate that *Nucella lapillus* was once present, on suitable substrates, around the whole of the North Sea coastline. Wild dogwhelks could be collected in all countries except Belgium and Germany, where the species is present only on Helgoländ (in numbers too small to be sampled).

All wild populations of dogwhelks showed imposex. Near sites of intense boating activity, all populations examined contained some sterile females. At all sites where transplants were recovered, the dogwhelks were affected by TBT. Prolonged survival of dogwhelks at any of these sites would not be expected, except perhaps at Tørsminde in Denmark.

All sites studied were categorized with reference to their potential for the maintenance of self-sustaining *Nucella* populations. All sites in the southern part of the North Sea (i.e. the coastlines of Belgium, The Netherlands, Germany and southern Denmark) were placed in Category C (reduced egg capsule production) or D (adult females expected to be sterile).

In Norway, sites near large harbours had only sterile females in the population (Category D), whilst those further from harbours probably had reduced egg capsule production (Category C). In Sweden, all wild sites were affected but probably had no reduction in egg capsule production (Category B).

In France and the UK, sites near large harbours or areas of small-boat activity probably had reduced egg capsule production (Category C). Sites further from sources of TBT contamination probably do not have reduced egg capsule production (Category B). One site on the north east coast of Scotland shows effects consistent with an area distant from a source of TBT pollution (Category A).

Keywords: TBT; butyltin; dogwhelk; *Nucella lapillus*; imposex

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INTRODUCTION

Tributyltin (TBT) compounds have been used as biocides in antifouling paints on small boats, larger ships and other marine structures, including cooling pipes and fish farming equipment. As these compounds can produce adverse effects on non-target organisms in the marine environment there has been considerable environmental concern about the release of TBT into the environment. In the UK, the main focus of concern has been TBT release from small pleasure craft, particularly in areas where water movement is restricted such as sheltered estuaries, sea lochs and marinas.[1] In

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Table 1 Distribution and sampling of the dogwhelk (*Nucella*)

		Numbers of samples obtained (60 individuals per sample)							
		Wild					Transplants		
Country	Presence of wild dogwhelks	Adults	Sub-adults	Juveniles	Total tin[a]	Tributyltin[a]	Adults	Total tin	Tributyltin
Norway	Along most of coast where conditions are suitable	15	9	5	R	A	—	—	—
Sweden	Only found on the outer islands of the archipeligos	2	—	1	AJ	—	—	—	—
Denmark	Present on most suitable substrates from Grenan Point in the north to as far south as Thyborøn	6	1UtA	5	R	A	3	3	1
Germany	Wild dogwhelks only present on Helgoländ in numbers too small to sample	—	—	—	—	—	16	11	8
Netherlands	The only populations found were on Texel in the Wadden Sea, and at locations in Zeeland	—	—	—	—	—	10	—	—
Belgium	Unsuitable coastline	—	—	—	—	—	4	2	2
France	Found along most of the coast from the Belgian border to Brittany	16	1UtA	15	R	A	—	—	—
UK	Populations were found at sites distant from harbours and marinas, along the south coast, and on suitable substrates away from intense shipping on the east coast	19	2UtA	16	AJ	A	3	2	—

[a] R, a range of age/size classes sampled; A, adults; UtA untoothed adults; J, juveniles (10–15 mm).

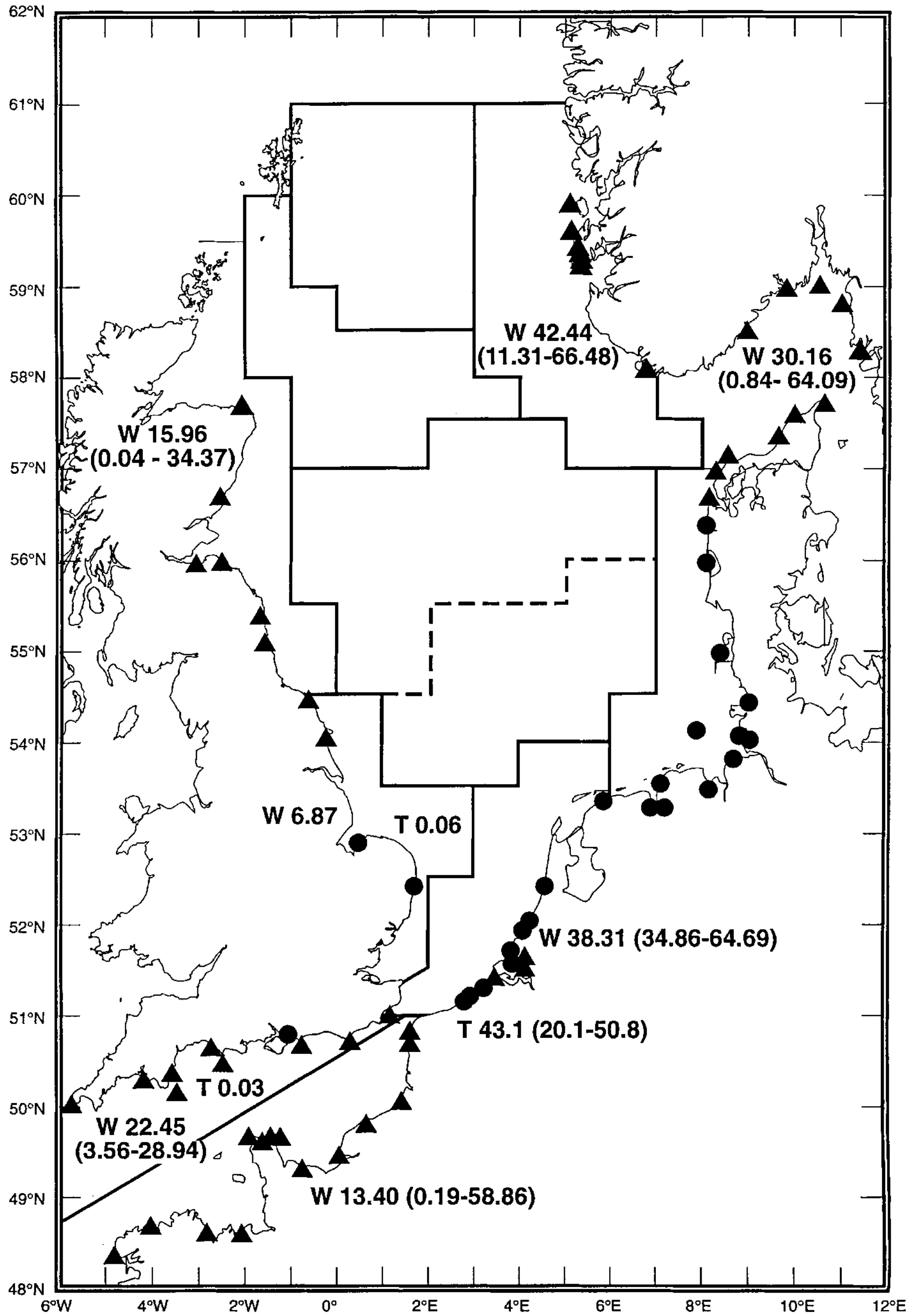

Figure 1 Distribution of sampling sites for wild dogwhelks (▲), and sites at which transplanted dogwhelks were located (●), by North Sea Task Force Sub-Region and Median RPSI values (min–max) in wild (W, ▲) and transplanted (T, ●) adult dogwhelks.

France, TBT is thought to have been instrumental in the decline of the French oyster industry.[2] The hazardous nature of TBT compounds was increasingly recognized in the 1980s and resulted in restrictions on their use. TBT was included as a voluntary parameter in both the Joint Monitoring Programme of the Oslo and Paris Commissions, and in the North Sea Task Force Monitoring Master

Plan.[3] In 1991, the UK Department of the Environment provided core funding for a coordinated monitoring exercise throughout the North Sea coastal areas, in collaboration with appropriate authorities in all other North Sea countries, to assess the distribution and impact of TBT compounds. Despite legislation restricting the use of TBT in France, a presence or increased incidence of a biological effect (imposex) of TBT usage was recorded at sites along the French Coast.[4] Minchin *et al.*[5] also reported increased imposex measurements in animals from shipping areas between 1987 and 1993.

The basis of the survey was the assessment of imposex in dogwhelk (*Nucella lapillus*) populations. Female dogwhelks develop a penis and vas deferens;[6,7] the extent of development of these male sexual characteristics (imposex) is dependent upon exposure to TBT.[8] A number of gastropods show related responses,[9–13] but the dogwhelk appears to be the most sensitive, developing the imposex characteristics in three months when exposed to TBT concentrations of only 2 ng l^{-1},[14] or less I. M. Davies, M. J. C. Harding, S. K. Bailey, S. M. Shanks and R. Länge, (1997). Sublethal effects of tributyltin oxide on the dogwhelk *Nucella lapillus* (L.), *Mar. Ecol. Prog. Ser.*, **158**:191–204. The specificity of the response to TBT, and the high sensitivity of *Nucella*, together with its wide geographical distribution, the biology of the species, and its robustness in handling, all combine to make the dogwhelk an appropriate bioindicator of TBT pollution along the North Sea coast. Since the phenomenon of imposex does not regress, any improvement in the imposex condition of dogwhelks resulting from the legislative controls restricting the use of TBT will first be seen in the juveniles. Less affected cohorts should then become available to recruit to the adult population. Generally, the RPSI (see the Methods section) in the juveniles is lower than that in the adults[15] in dogwhelk populations where exposure to TBT has ceased, or is decreasing. In populations that continue to be exposed to TBT, the RPSI in juveniles is higher.[16] Therefore, where possible, juveniles were sampled, together with adults, from the natural populations.

Information was obtained from laboratories in collaborating North Sea States about the distribution of wild populations of *Nucella* (Table 1), and indicated that it would not be possible to sample natural populations of dogwhelks from all countries. In some areas, suitable hard intertidal substrate was absent. A strategy was therefore adopted by which the existing natural populations of *Nucella* were sampled, where possible. For sampling sites without natural *Nucella* populations, dogwhelks would be transplanted, from an area distant from TBT contamination, for a period of three months. In this way, a broad survey of sites of interest could be carried out, providing a unified programme over the whole North Sea coastal area.

In the present work we take it that the effects discussed are caused by TBT and total tin as a surrogate for TBT, is indicative of the cause of the phenomena.

METHODS

Sampling

Sixty wild toothed adult dogwhelks were sampled from each of 63 sites on the coast of the North Sea, and transplants were placed at a further 44 sites (Table 1; Fig. 1) in 1991 and 1992. The location and a brief description of each site are given by Harding *et al.*,[17] who have also provided more detailed descriptions.[18] In general, where the opportunity existed, sites immediately adjacent to large commercial harbours were not selected for sampling as it is well recognized that such areas are subject to significant contamination from TBT. However, some transplants were undertaken in these areas, for example to meet the requirements of individual collaborating countries, or in areas where other suitable transplant sites were not available.

Juveniles (10–15 mm) were also collected from wild populations, where possible. A complete size range was collected from a few sites to provide a further indication of the state of TBT contamination. Only the penis size was recorded for most juvenile dogwhelks, due to the difficulty in assessing the early stages of vas deferens development.

Transplanted samples

Transplants consisted of netlon bags containing dogwhelks [with mussels (*Mytilus edulis*) as a food source] from Loch Ewe, a remote part of north-west Scotland, where the dogwhelks showed a low degree of imposex (incidence 17%, RPSI 0.01%, VDSI 0.58; see below for definitions). Mussels were obtained from Loch Torridon (NW Scotland), and contained <0.02 mg kg^{-1} TBT-Sn. After discussion with local scientists, the bags were suspended from suitable places, which included piers, buoys and pontoons, and were permanently submerged throughout the tidal cycle. The procedure was tested in Loch Ewe, Scotland, where bags were suspended for three months from a buoy in the sea, and in an indoor tank. The survival of both dogwhelks and mussels was good, with no mortalities among dogwhelks in the bag suspended in the loch and 81% survival in the bag suspended in the indoor tank.

The percentage of transplanted animals surviving the three-month exposure was recorded at each site, and the concentration of tin was measured in the feed mussels recovered from three countries (Germany, Belgium and The Netherlands).

Determination of imposex

Adult dogwhelks were analysed for imposex using the methods described by Gibbs *et al.*[14] The incidence of imposex (the percentage of females in a sample showing signs of both penis and vas deferens development), the relative penis size index (RPSI; the mean bulk (♀l^3) of the female penis (♀l = penis length) expressed as a percentage of the mean bulk (♂l^3) of the male penis length (♂l) in a sample), and the vas deferens sequence index (VDSI classification of developmental stage) were all recorded.

Chemical analysis

Dogwhelk and mussel tissue (from the transplants) was frozen at −20 °C before analysis for total tin and/or TBT content. Methods for total tin and TBT were adapted from McKie,[19] as modified by Bailey,[20] using a graphite furnace atomic absorption spectrophotometer after solvent extraction clean-up of the extracts, and transfer of the analyte to nitric acid. The limit of detection was 0.02 mg kg^{-1} (two standard deviations of repeated analysis of samples containing 200 ng of tin). The results are expressed in wet tissue weight. The methods for both analyses used approximately 3 g (wet weight) homogenized tissue samples (∼10 whole dogwhelks).

RESULTS

Wild populations

The results for the wild populations of adult dogwhelks are summarized for each subdivision of the North Sea (as adopted by the North Sea Task Force) in Fig. 1 (RPSI) and Fig. 2 (VDSI), and given in detail for the individual sites in Appendix 1. (The data for the wild population at Thyborøn in Denmark were included with the rest of the wild populations on that coastline.)

Populations of wild dogwhelks were sampled in all countries except Germany and Belgium. Imposex was found in natural dogwhelk populations from all the sites in Norway, Sweden, Denmark, the Netherlands, France and the UK. The incidence of imposex (VDS stage 2 or above) in these populations was 100% at all sites in Norway, Sweden, Denmark (except site Nørre Vorupør, 97%), Germany, France (except the sites Pointe de Barfleur, 60%; Cap de la Hague, 95%; and Pte de Perharidy, 91%) and the UK (except Tarbat Ness, 50%; Skirza, 80%; Skateraw, 97%; and Loch Ewe, 17%).

The RPSI values in adult dogwhelks from wild populations around the coast of the North Sea showed a range of values from those associated with no obvious point source distant from TBT contamination, below 1%,[21] to values of 60–65%. In general, low levels of imposex were found furthest away from sources of TBT, and higher values closer to sources such as harbours.

The lowest levels of imposex were found in Denmark at Nørre Vorupør (0.8%, 2.7 RPSI and VDSI respectively), France at Pointe de Perharidy (0.4%, 3.2), and the UK at Tarbat Ness (0.04%, 1.8) and Skirza (0.17%, 2.3). All these areas are distant from obvious sources of TBT.

Slightly higher RPSI and VDSI values were found in dogwhelks from sites where there was small-boat activity or large vessels in passage. Ramshl Island (N1, 24.4%, 4.3), Yevesøy Island (N2, 34.1%, 4.5) and Vadøy (N3, 41.4%, 5.1) in Norway; Bredholmen (S1, 26.6%, 4.0) in Sweden; le Conquet (F16, 45.3%, 4.2), le Havre (F6, 25.1%, 4.3) and Cherbourg (F10, 27.6%, 4.8) in France and Brixham (UK4, 27.4%, 4.3), Folkestone (UK9, 28.9%, 4.4) and Fraserburgh (UK17, 30.0%, 4.1) in the UK are all in areas with maritime activity (either from aquaculture marinas or harbours, e.g. fishing ports).

Kråga Island (N12, 11.3%, 4.2) and the entrance to Oslo fjord (N15, 21.33%, 4.1) in Norway and Ursholmen (S2, 10.2%, 4.0) in Sweden are sites where the main input of TBT would probably have been from the passage of large ships.

The worst-affected animals were found in populations from harbour areas, where sheltered waters, close passage of ships and static leaching

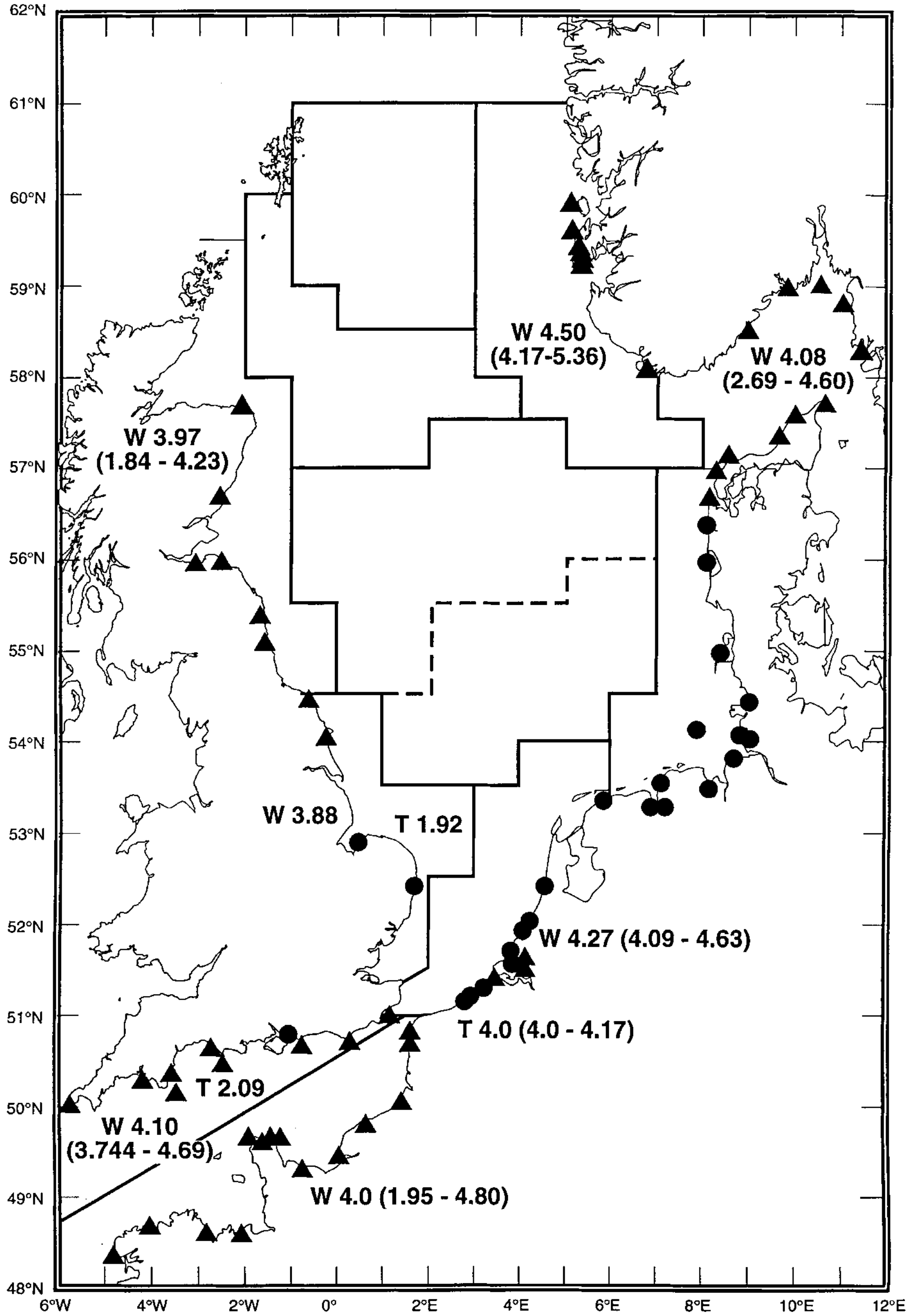

Figure 2 Median VDSI values (min–max) in wild (W, ▲) and transplanted (T, ●) adult dogwhelks

from the ships in port would be conducive to maintaining relatively high concentrations of TBT in the water. Harbours in Norway [Haugesund (N4–N10, 36.3–66.5%, 4.8–5.4) and Kopervik (N11, 63.2%, 5.3)], Denmark [Grenen Point (D1, 40.2%, 4.3) and Hirtshals D2, 64.1%, 4.4], The Netherlands, France [Boulogne (F3, 58.9%, 4.7)] and the UK at the industrialized dock at Montrose

(UK16, 34.4%, 4.0) all showed high imposex values.

All adult females collected in Norway, Sweden and The Netherlands had developed a complete vas deferens (vas deferens stage 4),[14] as had those from populations adjacent to harbours in Denmark, France and the UK. Sterile females (reproductive tracts blocked with vas deferens tissue, vas deferens stage 5 or 6) were found in all populations sampled from Norway; half the females from harbour sites in Denmark and The Netherlands were affected, and in all sites in France and the UK which had VDS greater than four. The worst-affected females were found from the anchorage at Vadøy (N3, VDSI 5.1) and from the harbour areas at Haugesund (N4, VDSI 5.4) and Kopervik (N11, VDSI 5.3) where none of the females collected was capable of reproduction.

The juvenile/subadult samples (Appendix 2) showed similar patterns to those found in the adult survey, with the highest RPSI values found at sites near intense boating activity. Some sites Yevesøy Island (Norway), Ursholmen (Sweden), Løkken, Hantsholm and Nørre Vorupør (Denmark), Dinard and the four easterly sites in France (F1–4), Portland Bill, Blyth Ferry and Fraserburgh (UK) all showed juvenile/subadult imposex levels lower than (if only slightly) or similar to those of the adults, indicating decreasing exposure to TBT.

In general, however, the results of the juvenile/subadult survey showed higher imposex values in the younger animals than in the adults. This was the case at most sites sampled for juveniles in Norway, France, the UK and Denmark (Grenen Point and Hirtshals). These populations were still being exposed to TBT concentrations in the water at levels sufficient to result in high values of imposex.

Transplants

Details of the sampling stations, and the results, are shown in Appendix 3 and summarized for each subdivision of the North Sea in Fig. 1 (RPSI) and Fig. 2 (VDSI). When recovered, most of the transplant bags had growth of fouling organisms on them, generally barnacles and sea squirts. The survival of dogwhelks in the transplant bags was very variable, ranging from 0 to 78%. Low survival was probably the result of poor water quality or low-salinity water, and in some cases may have indicated why wild populations were absent.

Imposex developed in the transplanted dogwhelks in all five countries where transplants were used. Following the three months' exposure, the incidence of imposex was 100% at all but two sites in Germany (Schlüttsiel, 97%) and Helgoland (inner, 89%), and at all sites in The Netherlands and Belgium. The incidence of imposex in the three sites in Denmark varied from 25 to 100% and was 63 and 68% at the two sites in the UK.

As with the dogwhelks from the wild populations, the imposex levels in transplanted animals were highest near harbour areas. The transplanted dogwhelks at the port of Esbjerg in Denmark (RPSI 30.1%, VDSI 4.0); the harbours of Husum, Büsum and Norddeich in Germany (15.7–44.4%, 3.9–4.0); Scheveningen, Scharendijke and Colijnsplaat in The Netherlands (20.1–50.8%, 4.0–4.2), and Zeebrugge (43.2%, 4.2) and Blankenberge (43.1%, 4.0) in Belgium, all showed imposex values considerably higher than the pre-transplant animals from Loch Ewe (0.01%, 0.6).

Some animals were rendered sterile (VDS stage 5) during the three-month exposure period. Sterile females were found at Büsum harbour (Germany), Scharendijke (The Netherlands) and Zeebrugge (Belgium). All females from the transplant sites in Germany, The Netherlands and Belgium showed vas deferens development after the three-month exposure. If the exposure period had been longer, it is likely that larger proportions of the females would have become sterile.

There were only three sites at which transplants were recovered and the imposex values were low: Tørsminde in Denmark (0.01%, 0.68) and two sites in the UK (Brancaster Staithe, 0.06%, 1.9; and Hayling Island, 0.39%, 2.1),

Concentrations of tin and TBT in dogwhelk tissue

The concentrations of total and tributyltin for wild adults, the size-range classes and the transplant animals respectively are given in Appendices 1–3, and summarized for each subdivision of the North Sea in Fig. 3.

In general, the highest total tin values in wild adult dogwhelks were found at sites of intense shipping activity, e.g. Haugesund (N5, 0.12 mg kg^{-1}) and Kopervik (0.11 mg kg^{-1}) harbours in Norway, the ports of Hirtshals (D2, 0.08 mg kg^{-1}) in Denmark, and Boulogne (0.10 mg kg^{-1}) and le Havre (0.09 mg kg^{-1}) in France. Elevated values were also reported from the various size classes.

Of the transplanted animals, the highest values were again found in dogwhelks from harbour areas. The total tin concentrations in transplanted dogwhelks from harbour areas in Denmark

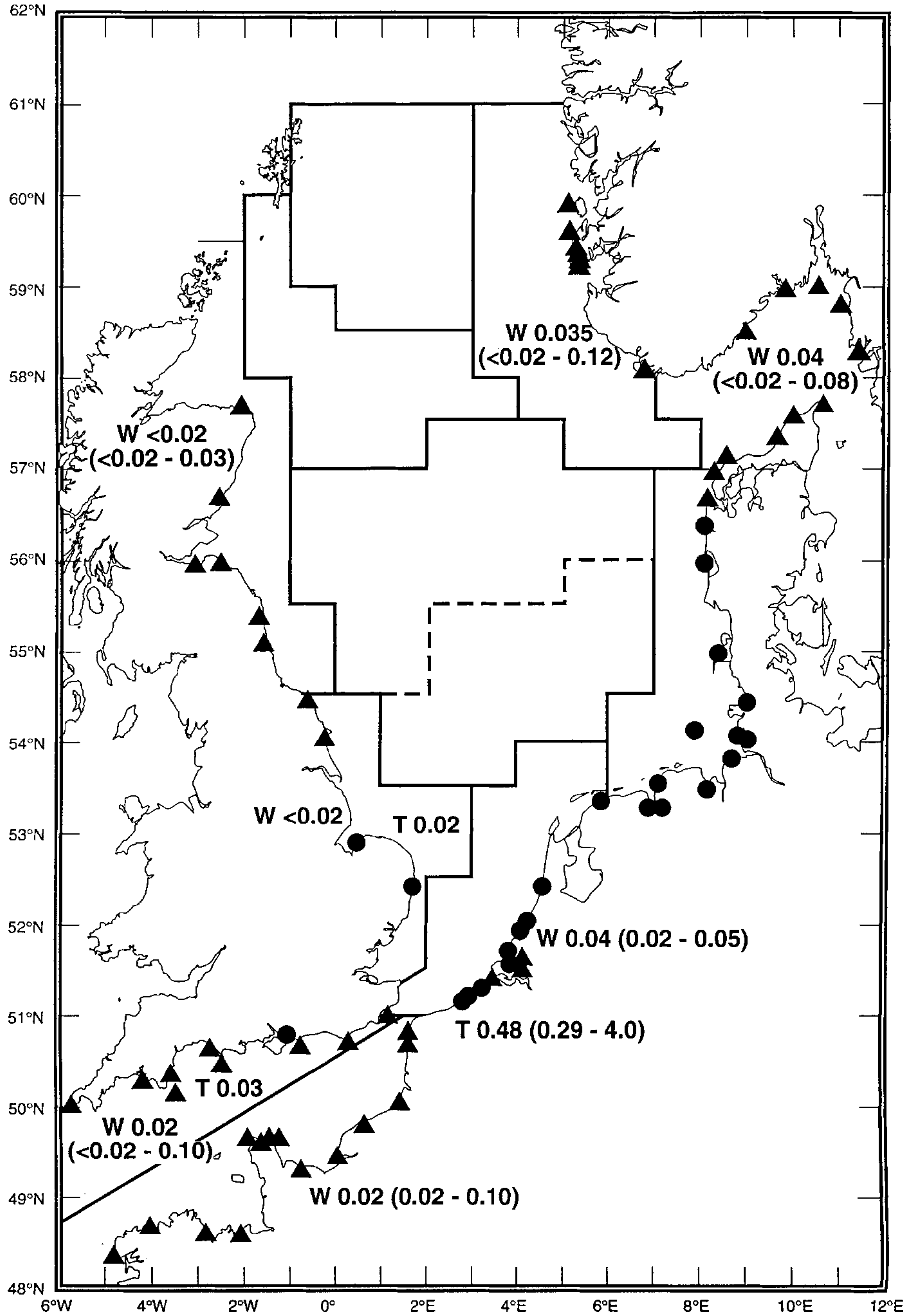

Figure 3 The median concentration (min–max) of total tin (mg/kg wet) in wild (W, ▲) and transplant (T, ●) adult dogwhelks.

(Esbjerg, 0.2 mg kg^{-1}), Germany (inner Büsum, 0.51 mg kg^{-1}; Norddeich harbour, 0.48 mg kg^{-1}), The Netherlands (Scheveningen 0.58 mg kg^{-1}; Scharendijke 0.52 mg kg^{-1}), Belgium (Zeebrugge, 0.51 mg kg^{-1}; Blankenberge, 0.45 mg kg^{-1}), were approximately two to five times the highest value found in the wild animals (0.12 mg kg^{-1} at Haugesund harbour wall.

Total tin concentrations in the dogwhelks from sites with small-boat activity were lower than in the

Table 2 Concentrations of tin in the feed mussels in the transplant bags[a]

Country	Site	Code no.	Total tin concn (mg kg^{-1} wet wt)
Germany	Schlüttsiel	G3	0.09
	Husum, inner	G4	0.32
	Husum, outer	G5	0.20
	Büsum, inner	G6	0.69[b]
	Meldorfer Hafen	G8	0.02
	Helgoländ, inner	G9	0.16
	Cuxhaven	G11	0.06
	Wilhelmshaven, inner	G12	0.14[c]
	Wilhelmshaven, outer	G13	0.26
	Norddeich harbour	G14	0.45
Belgium	Zeebrugge	B1	0.36
	Blankenberge	B2	0.03[c]
Netherlands	Delfzijl	NL7	0.22
	Scheveningen	NL11	0.25
	Hoek van Holland	NL12	0.07
	Scharendijke	NL13	0.39
	Colijnsplaat	NL14	0.30[b]
	Vlissingen	NL15	0.39

[a] Wt of homogenized tissue: 3 g wet wt.
[b] Mean of two values.
[c] Mean of three values.

dogwhelks from larger harbours. For example, Yevesøy Island in Norway (0.03 mg kg^{-1}), Bredholmen (0.04 mg kg^{-1}) in Sweden, and le Conquet in France (0.07 mg kg^{-1}) had small-boat activity, which was reflected in the small elevations in total tin concentrations above background values in the Loch Ewe animals (<0.02 mg kg^{-1}). Other sites at which dogwhelks showed detectable total tin concentrations were on commercial shipping routes, e.g. the entrance to Oslo fjord in Norway (0.03 mg kg^{-1}) and Hayling Island in the UK (0.03 mg kg^{-1}).

The tin concentrations in the feed mussels (Table 2) generally showed similar patterns to the tin concentrations in the transplanted dogwhelks, with the higher concentrations being found in animals from areas with high boating activity. The dogwhelks feeding on these animals were therefore exposed to tin in their feed as well in the surrounding water.

The dogwhelks from the harbour sites, both wild and transplants, showed high RPSI and VDSI values together with the elevated tissue total tin concentrations. However, some sites had total tin concentrations below or at the detection limit and also elevated imposex values: for example, Tromø Island in Norway, Texel in The Netherlands, Dinard in France and Selsy Bill and Montrose in the UK. This may suggest decreasing exposure of these dogwhelk populations to TBT.

DISCUSSION AND CONCLUSIONS

The uniformity of the response of *N. lapillus* to TBT contamination within the geographical area of this study has been demonstrated experimentally.[22] Dogwhelks from four sites between Shetland and Cornwall, covering over 10° of latitude, showed consistent responses to exposure to TBT in seawater.

The present survey has utilized the VDS classification scheme of Gibbs *et al.*[14] A more complex classification scheme was presented by Oehlmann *et al.*[23] and included several developmental routes of imposex, particularly between Stages 0 and 4. This scheme was consistent with the simpler scheme of Gibbs *et al.*,[14] but also accommodated rare stages observed in populations in parts of northern France. The uniformity of the geographical response, the classification systems and the rarity of reported anomalies (e.g. Pte du Cabellou, France;[22] Dumpton Syndrom,[24]), support the comparability of this data set, both internally and for other studies.

Due to the volume of data produced by this

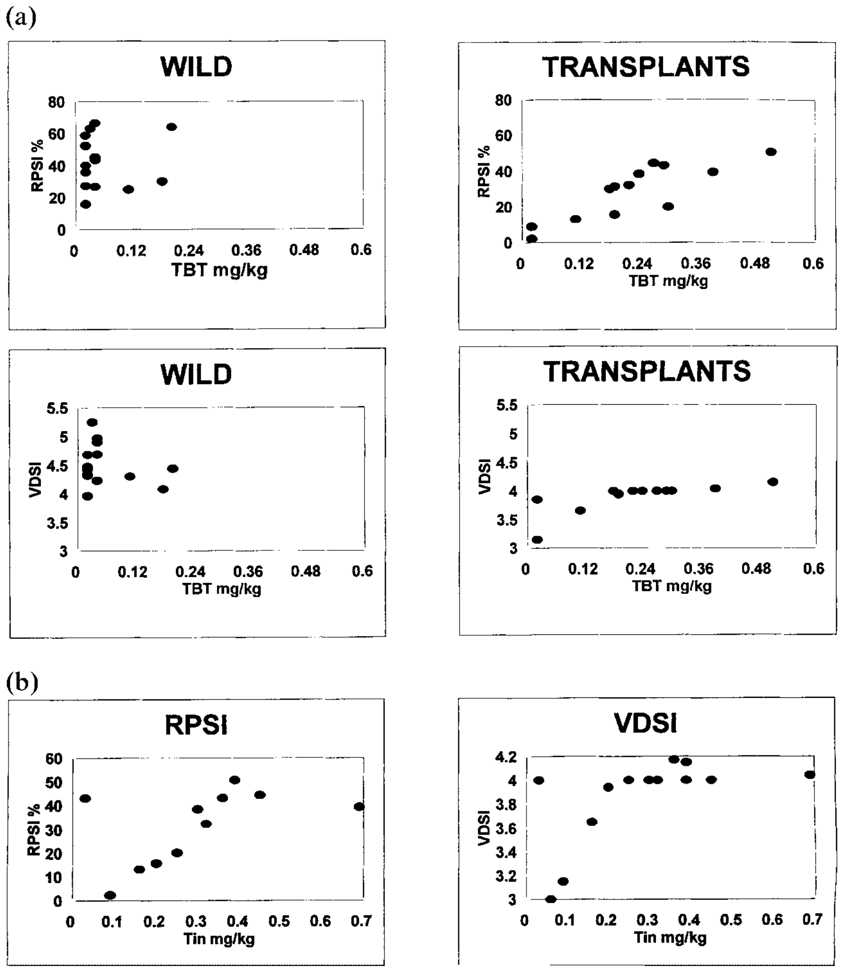

Figure 4 (a) Relationship between RPSI and TBT concentration in both wild and transplanted adult dogwhelks. (b) RPSI and VDSI, in dogwhelks, and tin concentration (mg kg^{-1} wet weight) in feed mussels in the transplant bags.

survey, the following discussion is limited to general points of interest. More detailed presentations of the results for individual countries[18] are available from the authors.

All wild populations of dogwhelks were affected by TBT. At only four sites, one in Denmark, one in France and two in north-east Scotland, the adult dogwhelks had RPSI values below the 1% value normally associated with areas distant from TBT contamination.[21] However, adults from these sites all had VDSI values above that in the Loch Ewe population from which the animals for the transplants were taken; and the Danish dogwhelks from Nørre Vorupør also had detectable traces of tin in their tissues. It is not expected that production of juvenile dogwhelks would be affected at these sites.

At all sites in The Netherlands and Norway, and sites near large harbours and areas of intense boating activity in all other countries, there were sterile females in the populations. The production of egg capsules may be reduced at these sites and it is possible that populations may eventually be depleted if insufficient juveniles are produced.

Previous observations [16] indicated that at sites where the juveniles had higher RPSI values than the adults, exposure to TBT was continuing, and reductions in exposure (e.g. in response to national and international control measures) were not yet taking place. This situation was found at sites in Denmark, near large harbours and shipping channels, and at some sites in France and the UK.

At all sites where transplants were recovered, the

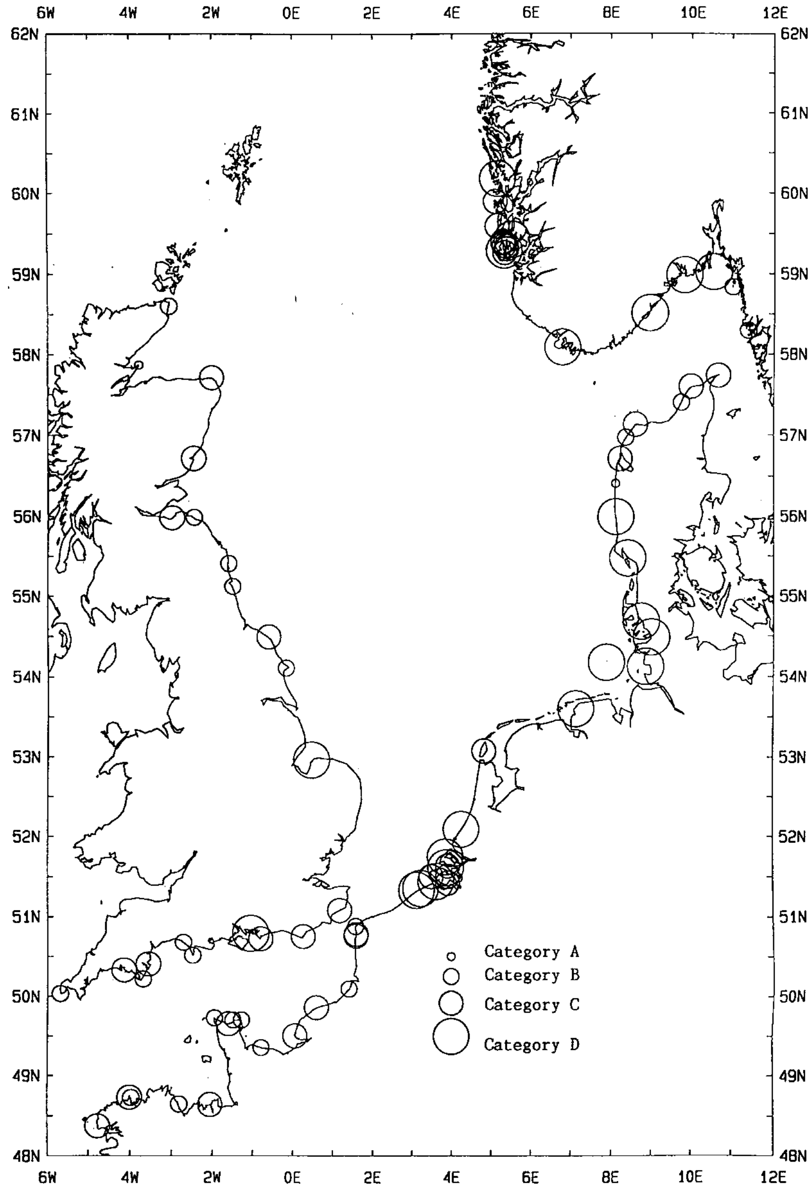

Figure 5 Categorisation of sites along the North Sea coast.

dogwhelks had been affected by TBT. Prolonged survival of dogwhelks would not be expected at any of these sites, except perhaps at Tørsminde in Denmark, where the dogwhelks showed little change in imposex over the transplantation period. At some sites in Germany, Belgium and the Netherlands there were sterile females in the transplanted populations after only three months exposure.

The RPSI and VDSI values in the transplanted animals were positively correlated with the concentrations of TBT in both their own tissues (Fig. 4a) and in the feed mussels (Fig. 4b). Some wild populations of dogwhelks showed high RPSI values but low tissue TBT concentrations, possibly suggesting that the concentration of TBT in the water had decreased at these sites. Imposex in dogwhelks is largely irreversible (I. M. Davies, M. J. C. Harding, S. K. Bailey, S. M. Shanks and R. Länge, (1997). Sublethal effects of tributyltin oxide on the dogwhelk *Nucella lapillus* (L.), *Mar. Ecol. Prog. Ser.*, **158**:191–204) but metabolism and degradation processes may have led to reduction in TBT levels in the tissues.

Overall assessment

In order to make an overall presentation of the effects of TBT by combining information from both wild and transplanted populations of dogwhelks, a categorization scheme has been devised relating to the impact of TBT on the potential of the study sites for the maintenance of self-sustaining populations. Four categories have been recognized, largely based on the VDSI values in wild populations.

Category A: Dogwhelk populations showing little, if any, effect of exposure to TBT beyond that associated with areas distant from sources of TBT (VDSI <2).

Category B: Dogwhelk populations showing some effects of exposure to TBT, but not to the extent that any significant effect on production of egg capsules would be expected ($2 \leq$ VDSI <4).

Category C: Dogwhelk populations showing more marked effects of exposure to TBT, to the extent that reductions in the production of egg capsules would be expected ($4 \leq$ VDSI <5).

Category D: Dogwhelk populations showing severe effects of exposure to TBT, to the extent that production of egg capsules would be prevented (VDSI ≥ 5).

In order to accommodate the transplant sites in this categorization scheme, it is necessary to compare the results from the field with data from tank experiments. I. M. Davies, S. K. Bailey and M. J. C. Harding (1998) Tributylin inputs to the North Sea from shipping activities and potential risk of biological effects. *ICES Journal of Marine Science*, **55**:34–43, reported that dogwhelks from Loch Ewe exposed to TBT concentrations in sea water of 2, 8, 32 and 128 ng $^{-1}$ for three months developed VDSI values of 1.0, 1.7, 3.3 and 3.9 respectively. At most transplant sites, dogwhelks showed VDSI values of 3.3 or more, suggesting concentrations of TBT in water at the transplant sites of 30 ng $^{-1}$ or more. Comparison with Ref. 14 indicates that these sites should be placed in Category D.

The results of this categorization of sites are presented in Fig. 5. All sites in the Southern Bight of the North Sea were placed in Category C or D. Wild dogwhelk populations at any of the transplant sites in Germany, The Netherlands or Belgium would be expected to be unable to reproduce. The wild dogwhelks sampled in The Netherlands all showed imposex to the extent that egg capsule production would be expected to be reduced.

In France, all sites showed evidence of contamination by TBT. Egg capsule production would probably be reduced at sites near large harbours or areas of small-boat activity (Category C) but not at sites more distant from sources of TBT contamination (Category B).

In the UK, all transplants, and wild samples near large harbours or areas of small-boat activity, showed signs of TBT contamination, with probable reduction in egg capsule production (Category C). Sites away from these areas, although affected, probably did not have reduced egg capsule production (Category B). One site on the north-east coast of Scotland was placed in Category A.

In Denmark, transplanted dogwhelks at the large harbours were all affected and wild populations at these sites would be expected to be sterile (except at Thorsminde). All the wild dogwhelks showed effects of TBT contamination; those near the large harbours may have had reduced egg capsule production (Category C). All the sites in Norway showed effects of TBT contamination. The sites near the large harbours had only sterile females in the population (Category D), whilst those further away probably had reduced egg capsule production (Category C). In Sweden, populations at wild sites were affected, but probably had no reduction in egg capsule production (Category B).

Modelling of the distribution of inputs of TBT to the North Sea from large vessels, and assessment of the resulting risk of biological effects, indicated that the areas at relatively greatest risk were ICES areas 4 and 5, in the south-eastern North Sea (I. M. Davies, S. K. Bailey and M. J. C. Harding (1998) Tributylin inputs to the North Sea from shipping activities and potential risk of biological effects. *ICES Journal of Marine Science*, **55**:34–43). This model also suggested much lower levels of risk in areas 1, 2, 3i, and 7i, in the north and north-central North Sea. In general terms, the results of the modelling broadly reflected the distribution of effect (imposex) observed in the dogwhelk survey. Areas of the Belgian, Dutch and German coasts showed very limited opportunity for the survival of wild populations of dogwhelks (if suitable substrates were present), while the intensity of effect was rather lower in areas further north, and on the French coast. The model did not allow any detailed assessment of the degree of impact at individual sites.

A range of European national and international statutory controls on the use of tributyltin compounds in antifouling paints were introduced at various times, mainly from the mid to late 1980s (e.g. in the UK, The Control of Pollution (Antifouling Paints and Treatments) Regulations 1987). There has therefore been considerable interest in the rate at which the effects of TBT, e.g. on dogwhelk populations, have declined.

Variations in RPSI measurements can be attained by using different methodology, e.g. narcotization of the animals can cause an increase in the penis length, especially the male,[12] and sampling at different times throughout the year when the male penis length varies naturally[23] (See also I. M. Davies, M. J. C. Harding, S. K. Bailey, S. M. Shanks and R. Länge, (1997). Sublethal effects of tributyltin oxide on the dogwhelk *Nucella lapillus* (L.), *Mar. Ecol. Prog. Ser.*, **158**:191–204) can also affect the RPSI value. The existence of morphological types of *N. lapillus* with a vas deferens of differing lengths and the absence of a penis,[25] and the methodological differences, contribute to uncertainty in comparison of RPSI values from different studies. VDSI values are considered to be the most useful parameter to measure the intensity of imposex[26] in allowing assessment to be made of the reproductive capability of the current population and the potential reproductive capacity of juveniles. The RPSI measurement can be used to indicate relative exposure to TBT,[5] but differences in RPSI measurements between studies may be misleading.

Fioroni *et al.*[4] reported the presence or increase in pseudohermaphroditism in *N. lapillus*, from sites along the French coast of the English Channel and sites near Roscoff in France. VDSI values have been reported for *N. lapillus* collected at sites along the French coastline between 1988 and 1992 by Oehlmann *et al.*,[26] who observed that despite the restrictions on the use of TBT, the levels of imposex in *N. lapillus* remained relatively constant during the period 1989–1992, with the highest frequency of Stage 4 individuals. The same VDSI value of four for the Roskoff site was reported by Oehlmann *et al.*[26] and in the present study.

Spence *et al.*[27] reported RPSI values and the sterile percentage (VDS 5 and 6) for populations of *N. lapillus* along the UK coastline of the English Channel, with some sites along the north-east coastline of England. These data were collected between 1986 and 1989, and showed the severity of imposex and TBT contamination along the English Channel, reflecting the high level of boating activity. A number of the sites had only adult males present and some sites were entirely devoid of dogwhelks. The severity of the situation along the English coastline reported by Spence *et al.*[27] probably overrides the variability in the methodologies and sampling times between the studies, and comparison with the present data indicates that some populations may have shown a level of recovery. Detailed assessment of recovery at individual sites south and north-east coasts of England would require detailed sampling at the same locations, as by Spence *et al.*[27]

Recovery, expressed as reductions in VDSI and/or RPSI, has been reported for oil terminals [e.g. by Harding *et al.*,[28] and by Evans *et al.*[29] (RPSI only)], mariculture areas[5,16] and areas influenced by boating and commercial shipping (e.g. Refs 29, 30). Evans *et al.*[29] reported large reductions in RPSI in the Clyde Sea area from 27–75% in 1987 (Bailey and Davies, 1989) to less than 1–15% in 1994 (although measurement techniques differed). Changes in Sullom Voe were less marked (34–57% in 1987[31] to 8–25% in 1994). Evans *et al.*[29] resampled a small number of sites in Norway and reported reductions in RPSI, but they did not report VDSI values. They noted that, whilst severe imposex could still be found in places, its occurrence was highly localized round sources of TBT, such as harbours etc. Elsewhere, populations appeared healthy, even at locations where earlier surveys had reported VDSI values that would markedly reduce the ability of the females to produce young. These observations, although limited, may therefore indicate that populations can recover from imposex, provided a proportion (perhaps rather small) of the females continue to breed successfully. In such populations, the resources of space and food would be available to a relatively small number of juveniles, and these may consequently show enhanced growth rate (cf. Ref. 32) and comparatively low mortality, thereby aiding the recovery of the populations.

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Appendix 1: Sampling sites and results of the wild adult dogwhelk populations around the North Sea coastline

Country	Site	Number		RPSI[a] (%)	VDSI	Tin (mg kg^{-1})	
		Females	Males			Total	TBT
Norway	Ramshl Island N1	25	38	24.4	4.28	0.02	—
	Yevesøy Island N2	38	24	34.1	4.50	0.03	—
	Vadøy N3	34	27	41.4	5.06	0.04	—
	Haugesund harbour mouth N4	36	24	54.9	5.36	0.06	—
	Haugesund harbour wall N5	26	31	43.5	4.97	0.12	0.04
	Haugesund harbour bridge N6	33	27	66.5	4.90	0.09[b]	0.04
	Haugesund harbour south N7	34	27	52.5	4.47	0.06	<0.02
	Haugesund harbour south/east N8	34	26	48.4	4.44	0.02[b]	—
	Haugesund harbour south of Fosenøy N9	32	28	36.3	4.78	0.03	—
	Haugesund harbour south N10	27	33	39.2	4.48	0.03	—
	Kopervik harbour N11	8	9	63.2	5.25	0.11	0.03
	Kråga Island N12	35	25	11.3	4.17	0.02[b]	—
	Tromø Island N13	31	25	33.1	4.48	<0.02	—
	Fuglö Island N14	30	30	36.1	4.60	0.04	—
	Entrance to Oslo fjord N15	31	24	21.3	4.06	0.03[b]	—
				24.3	4.04		—
Sweden	Bredholmen S1	34	27	26.6	4.0	0.04[b]	—
	Ursholmen	32	15	10.2	4.0	0.03	—
Denmark	Grenen Point D1	32	28	40.2	4.32	0.06	<0.02
	Hirstshals D2	27	34	64.1	4.44	0.08*	0.20*
	Løkken D3	33	29	5.1	3.68	0.02	—
	Hanstholm D4	26	35	30.2	4.08	0.05	0.18
	Nørre Vorupør D5	38	22	0.8	2.69	0.02	—
	Thyborøn D6	2	2	59.7	4.50	0.04[d]	—
Netherlands	De Val NL1	28	36	35.9	4.42	0.05	<0.02
	Zuidbout NL2	30	31	47.8	4.14	0.04[b]	—
	Heerenkeet NL3	8	16	64.7	4.63	0.05	—
	Texel NL4	11	16	34.9	4.27	0.02	—
	Zoutelande NL5	34	27	38.3	4.09	0.04	—
France	Cap Gris Nez F1	38	23	14.5	3.97	0.02	—
	Wimereux F2	34	26	22.5	4.03	<0.02	—
	Boulogne F3	25	36	58.9	4.68	0.10	<0.02*
	Le Bois-de-Cise F4	31	28	9.6	4.00	0.04	—
	Veulettes-sur-Mer F5	27	34	10.2	4.04	<0.02	—
	Le Havre F6	30	31	25.1 (30.9)	4.30	0.09[c]	0.11
	Ste Honorine-des-Pertes F7	23	38	2.7	3.83	<0.02	—
	Pointe de Barfleur F8	23	38	0.19 (0.22)	1.95	<0.02[c]	—
	Cap Lévy F9	34	29	3.1	3.53	<0.02	—
	Cherbourg F10	20	40	27.6	4.80	0.03	—
	Cap de la Hague F11	19	41	4.2 (5.0)	3.63	0.02	—
	Dinard F12	34	27	21.2	4.12	0.02	—
	St Quay-Portrieux F13	27	34	12.3	3.89	<0.02	—
	Roscoff F14	7	54	23.4	4.00	0.03	—
	Pte de Perharidy F15	11	50	0.4 (0.5)	3.18	<0.02	—
	Le Conquet F16	32	29	45.3 (49.8)	4.23	0.07	0.04
United Kingdom	Land's End UK1	31	30	3.6	3.74	<0.02	—
	Bovisand UK2	28	21	26.9 (30.0)	4.69	0.05	0.04
	Start Point UK3	29	33	18.9	4.0	<0.02	—
	Brixham UK4	21	39	27.4	4.33	0.02	<0.02
	West Bay UK5	34	28	11.2	3.97	<0.02	—
	Portland Bill UK6	32	29	12.5	4.00	0.02	—

Appendix 1: Continued

Country	Site	Number				Tin (mg kg^{-1})	
		Females	Males	RPSI[a] (%)	VDSI	Total	TBT
	Selsey Bill UK7	18	20	27.3	4.14	<0.02	—
	Eastbourne UK8	33	29	22.5	4.10	<0.02	—
	Folkestone UK9	26	34	28.9	4.36	<0.02	—
	Sewerby UK10	26	36	6.9	3.88	<0.02	—
	Whitby UK11	23	38	24.2	4.04	<0.02	—
	Blyth Ferry UK12	28	33	16.0	3.96	<0.02	<0.02
	Boulmer UK13	29	33	6.01	3.97	0.02	—
	Skateraw UK14	29	33	2.8	3.66	<0.02	—
	Port Seaton UK15	26	34	23.8	4.23	0.03	—
	Montrose UK16	28	33	34.4	4.04	<0.02	—
	Fraserburgh UK17	30	32	30.0	4.10	0.03	—
	Tarbat Ness UK18	32	29	0.04	1.84	<0.02	—
	Skirza UK19	20	40	0.17	2.30	<0.02	—
	Loch Ewe UK20	39	21	0.01	0.58	<0.02	—

[a] Where two values are given for RPSI, the value in parentheses was calculated excluding females with a complete vas deferens, but no penis (pseudohormaphroditism; Ref. 25).
[b] Mean of two values.
[c] Mean of three values.
[d] Mean of four values.

Appendix 2: Sampling of size/age classes in the countries bordering the North Sea

Country	Site	Juveniles (<15 mm)			Sub-adults (15–21 mm)			Tin total (mg kg^{-1})	
		No.	RPSI	VDSI	No.	RPSI	VDSI	Juveniles	Untoothed
Norway	RamshI Isad N1	—	—	—	29	29.3	4.18	<0.02	<0.02
	Yevesøy Island N2	12	25.9	3.40	38	19.1	3.85	<0.02	—
	Vadøy N3	9	63.1	4.00	34	52.3	4.00	0.03	—
	Haugesund harbour mouth N4	40	60.2	4.47	51	63.1	4.39	0.06	0.04
	Haugesund harbour mouth N5	—	—	—	39	77.6	4.43	—	0.10[a]
	Haugesund harbour bridge N6	—	—	—	—	—	—	—	—
	Haugesund harbour south N7	—	—	—	23	73.9	4.33	—	0.06
	Haugesund harbour south/east N8	—	—	—	—	—	—	—	—
	Haugesund harbour south of Fosenøy N9	—	—	—	—	—	—	—	—
	Haugesund harbour south N10	—	—	—	—	—	—	—	—
	Kopervik harbour N11	—	—	—	—	—	—	—	—
	Kråga Island N12	14	17.7	4.00	40	11.4	4.04	<0.02	0.02
	Tromø Island N13	—	—	—	16	39.5	4.25	—	0.03
	Fuglö Island N14	—	—	—	—	—	—	—	—
	Entrance to Oslo fjord N15	15	32.3	4.00	41	28.4	4.00	—	0.03[a]
Sweden	Bredholmen S1	—	—	—	—	—	—	—	—
	Ursholmen S2	69	4.2	4.00	—	—	—	0.02	—
Denmark	Grenen Point D1	52	84.7	—	—	—	—	0.05	—
	Hirstshals D2	75	86.4	—	—	—	—	0.06	—
	Løkken D3	41	4.1	3.40	—	—	—	<0.02	—
	Hanstholm D4	51	27.7	4.00	—	—	—	0.02	—
	Nørre Vorupør D5	40	0.0	—	40[b]	2.8	2.38	0.03	<0.02
France	Cap Gris Nez F1	122	7.0	—	—	—	—	—	<0.02[a]
	Wimereux F2	41	20.4	—	55[b]	16.9	4.00	—	<0.02
	Boulogne F3	98	51.3	—	—	—	—	—	0.07

Appendix 2: continued

		Juveniles (<15 mm)			Sub-adults (15–21 mm)			Tin total (mg kg^{-1})	
Country	Site	No.	RPSI	VDSI	No.	RPSI	VDSI	Juveniles	Untoothed
	Le Bois-de-Cise F4	61	1.5	—	—	—	—	0.12[c]	—
	Veulettes-sur-Mer F5	59	50.6	—	—	—	—	0.03	—
	Le Havre F6	61	33.8 (57.3)	—	—	—	—	0.09	—
	Ste Honorine-des-Pertes F7	60	2.7 (4.6)	—	—	—	—	<0.02	—
	Pointe de Barfleur F8	92	9.0	—	—	—	—	—	<0.02[d]
	Cap Levy F9	62	31.0	—	—	—	—	<0.02	—
	Cherbourg F10	92	80.5	—	—	—	—	—	0.04
	Cap de la Hague F11	—	—	—	—	—	—	<0.02	—
	Dinard F12	62	19.7	—	—	—	—	0.02	—
	St Quay-Portrieux F13	60	8.1	—	—	—	—	<0.02	—
	Roscoff F14	61	70.4	—	—	—	—	0.03	—
	Pte de Perharidy F15	57	52.9	—	—	—	—	<0.02	—
	Le Conquet F16	61	70.0	—	—	—	—	0.04	—
United Kingdom	Land's End UK1	61	26.3	—	—	—	—	<0.02	—
	Bovisand UK2	—	—	—	—	—	—	—	—
	Start Point UK3	60	35.5	—	—	—	—	<0.02	—
	Brixham UK4	60	35.9	—	50	25.2	4.0	0.02	—
	West Bay UK5	—	—	—	—	—	—	—	—
	Portland Bill UK6	10	10.4	—	—	—	—	<0.02	—
	Selsey Bill UK7	—	—	—	—	—	—	—	—
	Eastbourne UK8	61	45.8	—	—	—	—	<0.02	—
	Folkestone UK9	60	56.4	—	—	—	—	<0.02	—
	Sewerby UK10	14	10.8	—	—	—	—	<0.02	—
	Whitby UK11	39	44.4	—	—	—	—	<0.02	—
	Blyth Ferry UK12	81	13.2	—	20	4.5	3.78	0.04	—
	Boulmer UK13	62	15.5	—	—	—	—	<0.02	—
	Skateraw UK14	64	20.1	—	—	—	—	<0.02	—
	Port Seaton UK15	63	33.3	—	—	—	—	<0.02	—
	Montrose UK16	63	48.5	—	—	—	—	0.03	—
	Fraserburgh UK17	60	24.8	—	—	—	—	0.02	—
	Tarbat Ness UK18	60	2.0	—	—	—	—	<0.02	—
	Skirza UK19	60	0.2	—	—	—	—	<0.02	—
	Loch Ewe UK20	—	—	—	—	—	—	—	—

[a] Untoothed adults and juveniles combined.
[b] Untoothed adults.
[c] Mean of two samples.
[d] Mean of three samples.

Appendix 3: Summary of transplant sites and results.

Country	Site	Survival (%)	Incidence of imposex	RPSI[a] (%)	VDSI	Tin (mg/kg^{-1})[b] Total	TBT	Comments
Denmark	Tørsminde D7	78	24.25	0.01	0.68	<0.02	—	Dogwhelks had laid egg capsules inside the bag
	Hvide Sande D8	23	70	1.4	2.0	0.02	—	Low survival may have been due to low-salinity water from Ringkobing fjord
	Esbjerg D9	42	100	30.1	4.0	0.2	0.18	
Germany	Inner List G1	0	—	—	—	—	—	Bag missing
	Outer List G2	0	—	—	—	—	—	Bag missing
	Schlüttsiel G3	75	97	2.3	3.15	0.07	0.02	
	Husum inner G4	43	100	32.3	4.0	0.28	0.22	
	Husum outer G5	48	100	15.7	3.94	0.20	0.19	
	Büsum inner G6	58	100	39.3	4.04	0.51[c]	0.39	
	Büsum outer G7	77	100	9.0	3.85	0.07	<0.02	
	Meldorfer Hafen G8	7	—	—	—	0.11	—	Excessive growth of sea squirts on bag preventing water circulation
	Helgoland inner G9	69	89	13.1	3.65	0.23[d]	0.11	
	Helgoland outer G10	0	—	—	—	—	—	Bag missing
	Cuxhaven G11	4	100	—	3.0	0.13	—	
	Wilhelmshaven inner G12	1	—	—	—	0.19	—	Poor water quality probably affected survival
	Wilhelmshaven outer G13	0	—	—	—	—	—	
	Norddeich harbour G14	50	100	44.4	4.0	0.48	0.27	
	Norddeich marina G15	50	100	31.4	3.95	0.26	0.19	
	Emden G16	0	—	—	—	—	—	Poor water quality probably affected survival
Netherlands	Delfzijl NL6	0	—	—	—	—	—	Low-salinity (5–15‰) water probably affected survival
	Delfzijl NL7	0	—	—	—	—	—	
	Holwerd NL8	—	—	—	—	—	—	
	Den Helder NL9	0	—	—	—	—	—	Bags vandalized
	IJmuiden NL10	0	—	—	—	—	—	
	Scheveningen NL11	58	100	20.1 (24.1)	4.0	0.58	0.30	
	Hoek van Holland NL12	0	—	—	—	—	—	Low-salinity (5–20‰) water probably affected survival
	Scharendijke NL13	39	100	50.8	4.15	0.52	0.51	
	Colijnsplaat NL14	73	100	38.5	4.0	0.29[c]	0.24	
	Vlissingen NL15	4	100	—	4.0	0.31	—	Poor water quality probably affected survival
Belgium	Zeebrugge B1	64	100	43.2	4.17	0.51[c]	0.15	
	Blankenberge B2	45	100	43.2	4.0	0.45	0.29[c]	
	Oostende B3	0	—	—	—	—	—	Bag probably torn on underwater projections and dogwhelks lost
UK	Nieuwpoort B4	0	—	—	—	—	—	Transplants not recovered
	Lowestoft UK21	—	—	—	—	—	—	Transplants not recovered
	Brancaster Staithe UK22		68	0.06	1.92	<0.02	—	
	Hayling Island UK23		63	0.39	2.09	0.03	—	

[a] Where two values are given for RPSI, the value in parentheses was calculated excluding females with a complete vas deferens but no penis Ref. 25.
[b] Wet weight.
[c] Mean of two values.
[d] Mean of three values.