MONITORING OF TRIBUTYL TIN CONTAMINATION IN SIX MARINE INLETS USING BIOLOGICAL INDICATORS.

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ABSTRACT

Dogwhelk and periwinkle specimens were collected from six bays/estuaries in 2000, where Tributyl Tin (TBT) contamination was suspected. In four estuaries, shells of dead Pacific oysters were collected from 7 sites in the vicinity of culture installations. Observations on imposex in dogwhelks, intersex in periwinkles and shell thickness in the Pacific oysters were used to assess the degree of TBT contamination.

The results showed low levels of contamination, which are unlikely to have detrimental effects to mollusc culture or fisheries in Mulroy Bay, Valentia Harbour or Tralee Bay. Thickening of oyster shells was detected in Carlingford Lough, Waterford Harbour, Cork Harbour and Fountainstown. The small degree of thickening was considered unlikely to affect marketability.

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INTRODUCTION

Tributyl tin (TBT) is an effective anti-fouling agent that has been used in marine paints for shipping and for fish cages. It is sufficiently toxic to harm many marine organisms at very low concentrations and to impair reproductive performance in a number of molluscan species. During the 1980's several Pacific oyster culture operations in Europe were abandoned due to high levels of TBT, that resulted in low meat yields and highly distorted shells (Alzieu *et al*.,1982).

A cause and effect relationship was established between TBT contamination and reductions in meat weight and increased shell thickness in Pacific oysters (Alzieu *et al.,* 1982). The consequence of shell thickening is that in extreme cases the oysters are unmarketable. Oysters even moderately contaminated may suffer from reduced soft tissue growth. In Ireland, grossly distorted oysters were reported from Cork Harbour and Baltimore, Co. Cork in 1985 (Minchin *et al*., 1987). In view of the year-class failures of 1992-1995 of scallops in Mulroy Bay, the local community became concerned that any releases of TBT could have adverse consequences for local aquaculture and scallop ranching. At this time salmon cages were treated with a TBT flexible net-dip coating in Mulroy Bay (Minchin, 1995).

An Irish Bye-law was passed in April 1987 (Anon., 1987), prohibiting the use of TBT on all vessels under 25 m except under special circumstances. This ban applied to freshwater and marine areas and included structures, such as fish cages. Ireland was the first country to ban the use of all organotins on vessels less than 25 m.

In order to evaluate the effectiveness of the 1987 legislation it was decided as part of the OSPAR program that certain regions in Ireland should be studied every six years. The different areas for examination were where leachates of TBT were predominantly from salmon farming, small boat activity (i.e. vessels <25m) or shipping activity (where vessels were predominantly over 25m and up to 100,000 DWT). By 1993, there had been an apparent reduction in the level of contamination in regions of salmon farming and small boat activity. However, in areas where there was shipping, levels were generally found to have increased (Minchin, *et al.* 1995). This led to further investigations of some fishing and shipping ports that had moderate to high levels of contamination. Ports with dry-dock or boat lifting facilities appeared to have higher levels of contamination. (Minchin *et al.* 1996 & 1997).

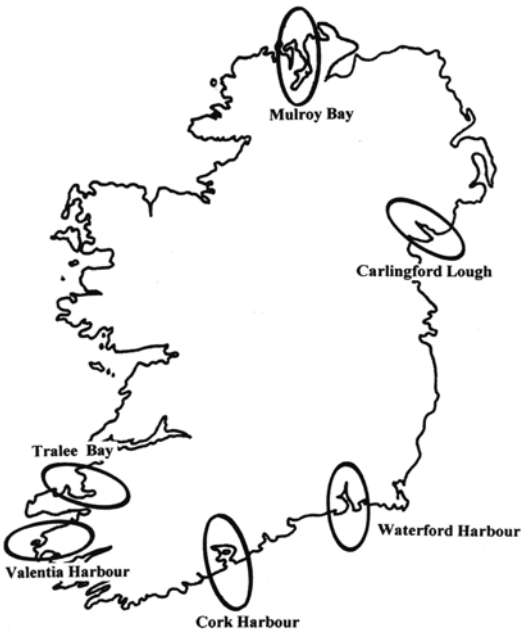
Unexpectedly high levels of TBT contamination were found in Baltimore Harbour in 1999, as indicated from the high values of dogwhelk imposex. This is a condition whereby TBT results in the development of a penis and other male features in female marine snails. In addition, Pacific oysters near Baltimore were found to be so grossly distorted, that they were unmarketable. A more detailed investigation ensued and indicated that the source the TBT contamination was due to the application of TBT based paints (Minchin & Duggan, 1999).

This investigation, which was carried out in 2000, is part of the monitoring process set by the OSPAR Program in 1987 to visit certain marine regions every six years to monitor TBT contamination.

MATERIALS AND METHODS

The standard methods as recommended by OSPARCOM were employed using biological indicators for assessing relative levels of contamination (Minchin & Davies, 2000). Three indicator species were used: *Nucella lapillus* (dogwhelk), *Littorina littorea* (periwinkle) and *Crassostrea gigas* (Pacific oyster). Six areas were chosen to carry out the study (Figures 1 & 2).

Site Description



a) Mulroy Bay: The recent decline of veliger larvae of the king scallop (*Pecten maximus*), in Mulroy Bay has been claimed to be due to the leaching of TBT. There is one main site in the bay where inputs could occur. Sampling took place at five stations close to a boatyard and also at a control site outside Mulroy Bay, on the west side of the Rosguill Peninsula, Co Donegal (Figure 2a).

b) Carlingford Lough: There are important Pacific oyster culture activities in this region. Farming takes place as close as 500 m from Greenore Port, Co Louth. Other possible TBT sources include Carlingford Pier and Warrenpoint (Figure 2b).

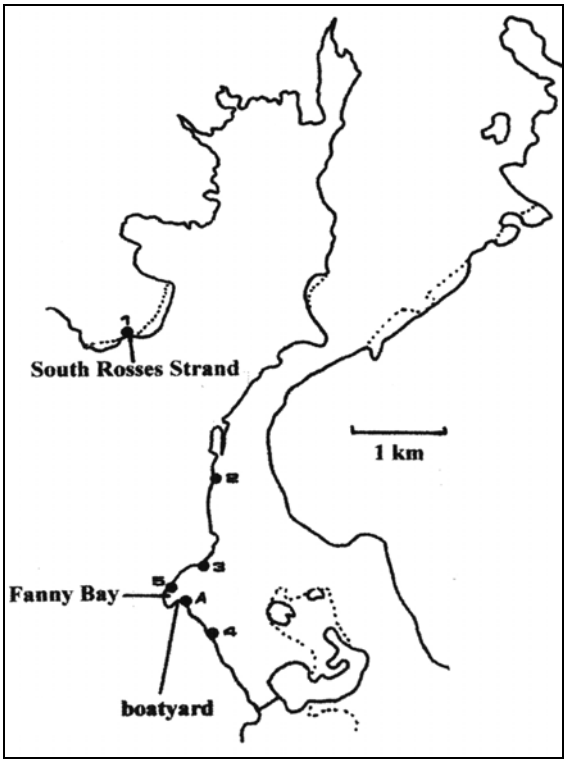
c) Waterford Harbour: In the early 1990's, Pacific oysters were found with gel within the shell lamellae. This is a condition only known to be induced by the presence of TBT. Possible sources of contamination include Dunmore East, New Ross, Waterford and vessels mooring in the channel (Figure 2c).

Figure 1. Sampling sites used in study.

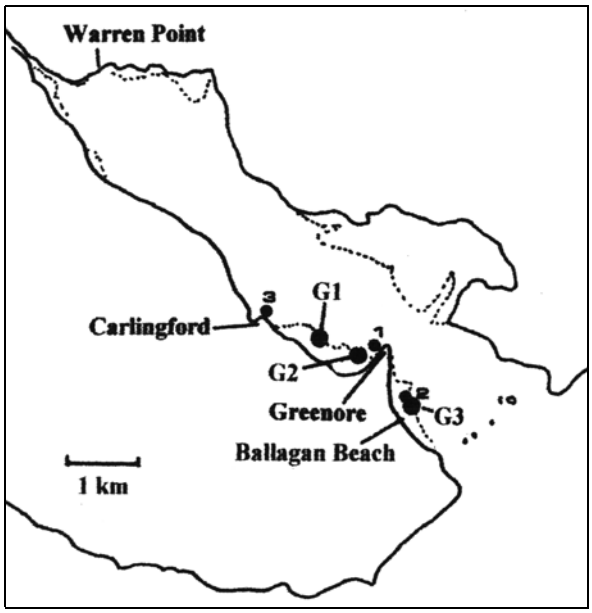
d) Cork Harbour: This region has been under study for some years (Minchin *et al*., 1987, 1995, 1996). Two sites were selected: 'Cork Harbour', with stations on the north and east channels of Great Island and at Fountainstown, 3 km to the southwest of the Harbour mouth. (Figure 2d).

e) Valentia Harbour: In this channel there is an important scallop fishery and some efforts have been made to culture scallops in trays before their release in the fishery area. Pacific oysters are also cultivated in this region. A boatyard was built in 1993, under the strict condition that no TBT paints would be applied to vessels (Figure 2e).

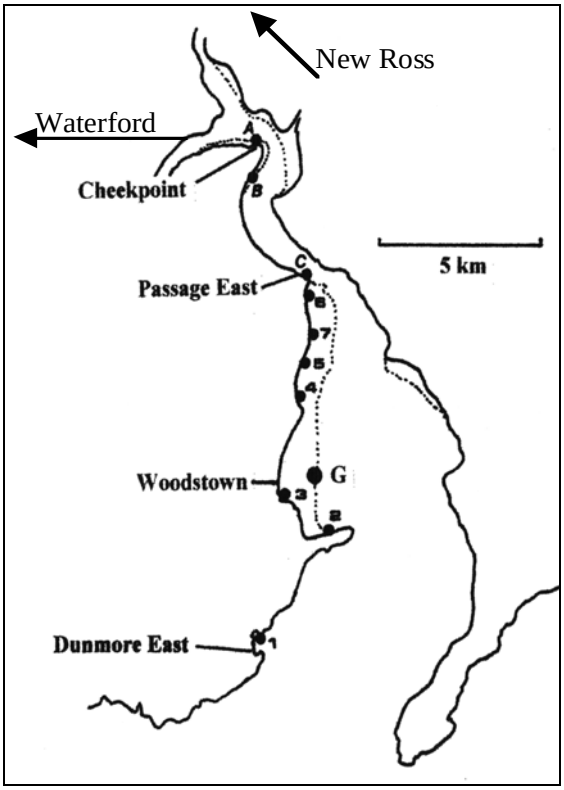
f) Tralee Bay: This bay has the largest fishery for wild native oyster *Ostrea edulis* in Europe. Most vessels engaged in the seasonal fishery are based in Fenit. There are also other fishing activities and a small angling industry. There is some shipping activity but those vessels entering the bay are normally small with the exception of ocean-going barges for the export of large cranes (Figure 2f).



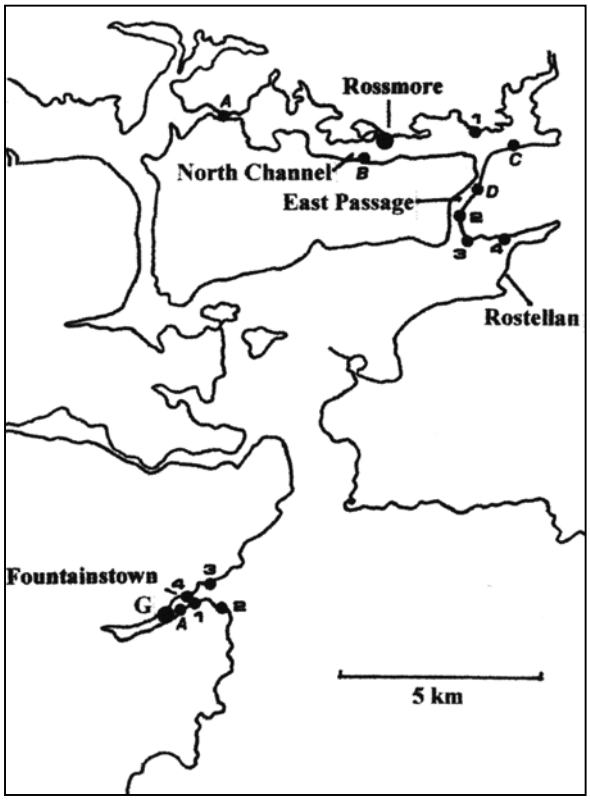
a) Mulroy Bay



b) Carlingford Lough

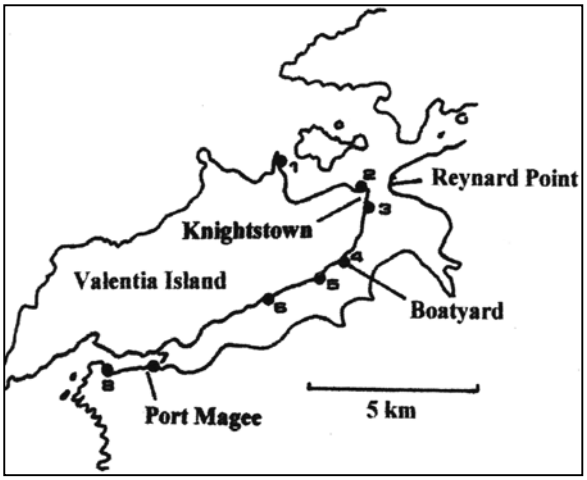


c) Waterford Harbour

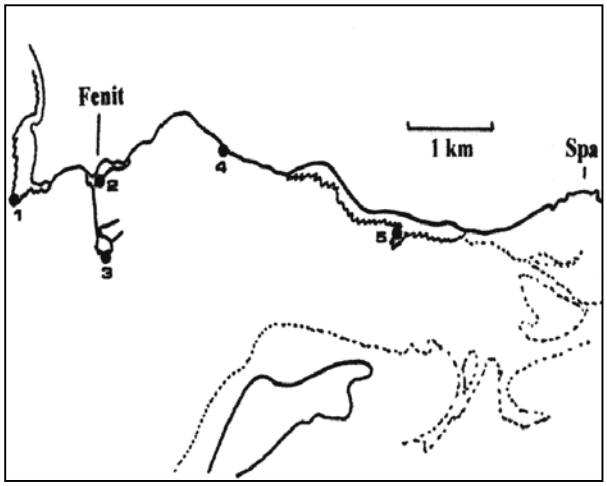


d) Cork Harbour

Figure 2. Sampling stations used in study.



e) Valentia Harbour



f) Tralee Bay

Figure 2. Sampling stations used in study.

MATERIALS AND METHODS (contd.)

Bioindicator species.
The following indicator species were used in this study:

Nucella lapillus, the dogwhelk
TBT simulates an androgenic hormone in this and many other neogastropod snails, causing the formation of male organs superimposed upon the normal female reproductive tract. In severe conditions the vas deferens seals the vagina causing obstruction to the release of egg capsules. This condition not only renders the female snail sterile but can also result in mortality. Six stages of male organ superimposition are observed, ranging from 1, the least effect to 6 – the worst case likely to be observed. Specimens were collected in the mid-low intertidal zone. Where samples (±40) could not be completed using unparasitised adults, juveniles were included. Parasitsitism can result in hormonal imbalance in snails (Minchin & Davis, 2000).

Littorina littorea, the periwinkle:
Intersex is a condition where a female gradually develops male characteristics. The intersex index (ISI) defined by Bauer *et al.*(1995) assigns values from 0 to 4. Periwinkles do not become extinct in heavily contaminated areas because individuals recruit from the plankton and not from recently hatched crawling individuals from an egg case, as with dogwhelks. This provides an alternative indicator of TBT contamination, useful in areas where dogwhelk population numbers are low or are absent.

Samples of ±50 individuals were taken from the mid-shore where dogwhelks could not be found. When necessary the sample was completed with specimens collected from the upper shore. Specimens were anaesthetised in a 7% solution of magnesium chloride and distilled water for two hours or more before examination. Forty individuals were examined per site.

Crassostrea gigas, the Pacific oyster
Waldock *et al.*, (1995) used the ratio of shell length to shell thickness (length/thickness) as an index of contamination. In this study the practice of measuring maximum length of the flat shell and thickness at the position of the adductor muscle, was adopted.

There is a great deal of variation within a population and consequently a large sample size is required to obtain a meaningful value. Indicators of contamination from TBT include the formation of an adductor pit in the upper (flat) shell and the occurrence of a gel between shell lamellae of the thickened flat valve. Measurements were made from shells associated with trestles and from within oyster-bags at cultivation sites.

RESULTS

Values of the contamination indices for dogwhelk and periwinkle are given in Tables 1 and 2 and for oyster shell thickness index in Table 3.

a) Mulroy Bay: (Figure 2a) A boatyard is situated near the entrance to Mulroy Bay in the sheltered Fanny Bay. High values for VDSI and RPSI, 4.6 and 28.2 respectively, were observed at Station 5, within Fanny Bay near the boatyard. These values declined with distance from this point and Station 1, the control site outside Mulroy Bay, showed no sign of contamination. The boatyard was evidently well maintained and no containers of TBT antifouling were noted on or near the premises.

b) Carlingford Lough: (Figure 2b) A possible source of contamination, apart from the port of Greenore, is the port of Warrenpoint well within the Lough which has a regular turnover of vessels involved in container traffic. Sampling took place in the vicinity of oyster culture stations close to Carlingford and Greenore. VDSI values, approximately 4.1, were similar at the three stations, but RPSI was much higher at Carlingford Pier. Oyster shell thickness indices were low, and showed no significant variation between the three stations. The presence of gel within shell lamellae was observed.

c) Waterford Harbour: (Figure 2c) Sampling at two of the three potential sources of TBT (Waterford Port and New Ross), was impossible due to the absence of the indicator species. The nearest rocky shore was at Cheekpoint, 8 km downstream from Waterford Port.

VDSI values were close to 4.1 at all seven stations. The highest RPSI, 31.6, was observed at Station 7, south of Passage East. Dogwhelks were absent from the shore between Cheekpoint and Passage East, but ISI values for periwinkle were low, less than 1.0. From Passage East to Dunmore East sterile dogwhelks were found, indicating that there was a degree of contamination. Shell indices of oyster at Woodstown Strand ranged from 9 to 33, suggesting moderately low contamination.

d) Cork Harbour: (Figure 2d) Within Cork Harbour imposex values were greater than at the nearby estuary of Fountainstown. ISI values in this region were all comparatively low and did not indicate high levels of contamination. There were indications of shell thickening in oysters in the North Channel. The lowest value, 0.2, was found in the East Passage. Gel was found in the lamellae of some shells.

e) Valentia Harbour: (Figure 2e) Fishing vessels land their catches at Reynard Point. These vessels and larger craft are known to shelter here. Two low level point sources were identified as, the boatyard on Valentia Island and Port Magee, a fishing port. All other sites had very low values of less than 1.0. Dogwhelks sampled from the slip of the boatyard had low imposex values.

f) Tralee Bay. (Figure 2f) The region with highest levels of imposex was at Fenit Port and associated pier, where VDSI values reached 3.7. Elsewhere in the Bay values were less than 2.9. RPSI values were also highest in the port region and low in the other sampled areas.

Comparison between 1999 and 2000 studies.

The oyster shell index values in Cork Harbour (from this study), Baltimore Harbour, which was severely contaminated in 1999 (Minchin & Duggan, 1999) and the 1999 control area of Dungarvan Bay where no evidence of TBT contamination has been observed are seen in Figure 3. The values seen in Cork Harbour which are similar to the data recorded in the other three oyster sites during this study, lie between the two extremes.

				Number of females in imposex grades							*% sterile*
	Station	*VDSI*	*RPSI*	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*females*
Mulroy Bay	1	0.0	0.0	30	0	0	0	0	0	0	0
	2	1.63	0.3	5	3	6	4	1	0	0	0
	3	3.24	4.2	0	0	3	7	7	0	0	0
	4	4.24	20.7	0	0	0	0	14	2	1	18
	5	4.64	28.2	0	0	0	0	4	7	0	64
Carlingford Lough	1	4.09	14.4	0	0	0	0	20	2	0	9
	2	4.04	12.6	0	0	0	1	20	2	0	9
	3	4.06	36.9	0	0	0	0	17	1	0	6
Waterford Harbour	1	4.13	22.8	0	0	0	0	13	2	0	13
	2	4.07	16.4	0	0	0	0	14	1	0	7
	3	4.11	25.1	0	0	0	0	17	2	0	11
	4	4.10	22.2	0	0	0	0	19	2	0	10
	5	4.08	12.8	0	0	0	0	24	2	0	8
	6	4.09	23.2	0	0	0	0	21	5	0	22
	7	4.13	31.6	0	0	0	0	14	0	1	0
Cork Harbour	1	4.08	15.9	0	0	0	0	12	1	0	8
	2	4.11	22.7	0	0	0	0	16	2	0	11
	3	4.11	25.0	0	0	0	0	17	2	0	11
	4	4.12	19.0	0	0	0	0	15	2	0	12
Fountainstown	1	3.86	5.0	0	0	0	3	18	0	0	0
	2	4.06	15.1	0	0	0	1	13	2	0	13
	3	4.00	14.6	0	0	0	0	17	0	0	0
	4	3.92	7.0	0	0	0	1	11	0	0	0
Valentia Harbour	1	0.68	0.0	8	7	2	0	0	0	0	0
	2	0.95	0.1	7	8	4	1	0	0	0	0
	3	0.50	0.0	14	9	0	1	0	0	0	0
	4	2.53	4.9	6	1	2	6	0	0	0	0
	5	0.71	0.0	8	6	3	0	0	0	0	0
	6	0.38	0.0	11	4	1	0	0	0	0	0
	7	3.29	5.0	0	3	0	2	8	1	0	7
	8	0.44	0.0	11	6	1	0	0	0	0	0
Tralee Bay	1	2.81	1.8	0	1	6	10	4	0	0	0
	2	3.72	6.2	0	1	0	4	20	0	0	0
	3	3.86	10.3	0	0	0	4	16	1	0	5
	4	2.77	2.0	0	4	2	11	5	0	0	0
	5	2.38	1.4	1	3	4	5	3	0	0	0

Table 1. Dogwhelk imposex: means from 40 specimens from each station.

			Numbers of females in intersex grades			*%*
	Station	*ISI*	*0*	*1*	*2*	*sterile*
Mulroy Bay	A	0.4	12	4	1	0
Waterford Harbour	A	0.4	9	6	0	0
	B	0.6	5	9	0	0
	C	0.5	11	10	0	0
Cork Harbour	A	0.3	14	7	0	0
	B	0.3	14	7	0	0
	C	0.4	11	7	0	0
	D	0.2	10	3	0	0
Fountainstown	A	0.4	13	7	0	0

Table 2. Periwinkle intersex index: means from 40 specimens per station. No specimens in ISI grades 3 and 4 were observed.

	Station	*Sample size*	*Minimum*	*Shell index Mean*	*Maximum*
Carlingford Lough	G1	38	9.9	**14.9**	26.1
	G2	67	7	**15.8**	33.3
	G3	80	8.3	**16.0**	31.2
Waterford Harbour	G	67	9.2	**18.8**	33.1
Cork Harbour	G	125	6.8	**12.5**	24.2
Fountainstown	G	78	9.2	**17.8**	37.7

Table 3. Pacific oyster shell thickness index from dead shells.

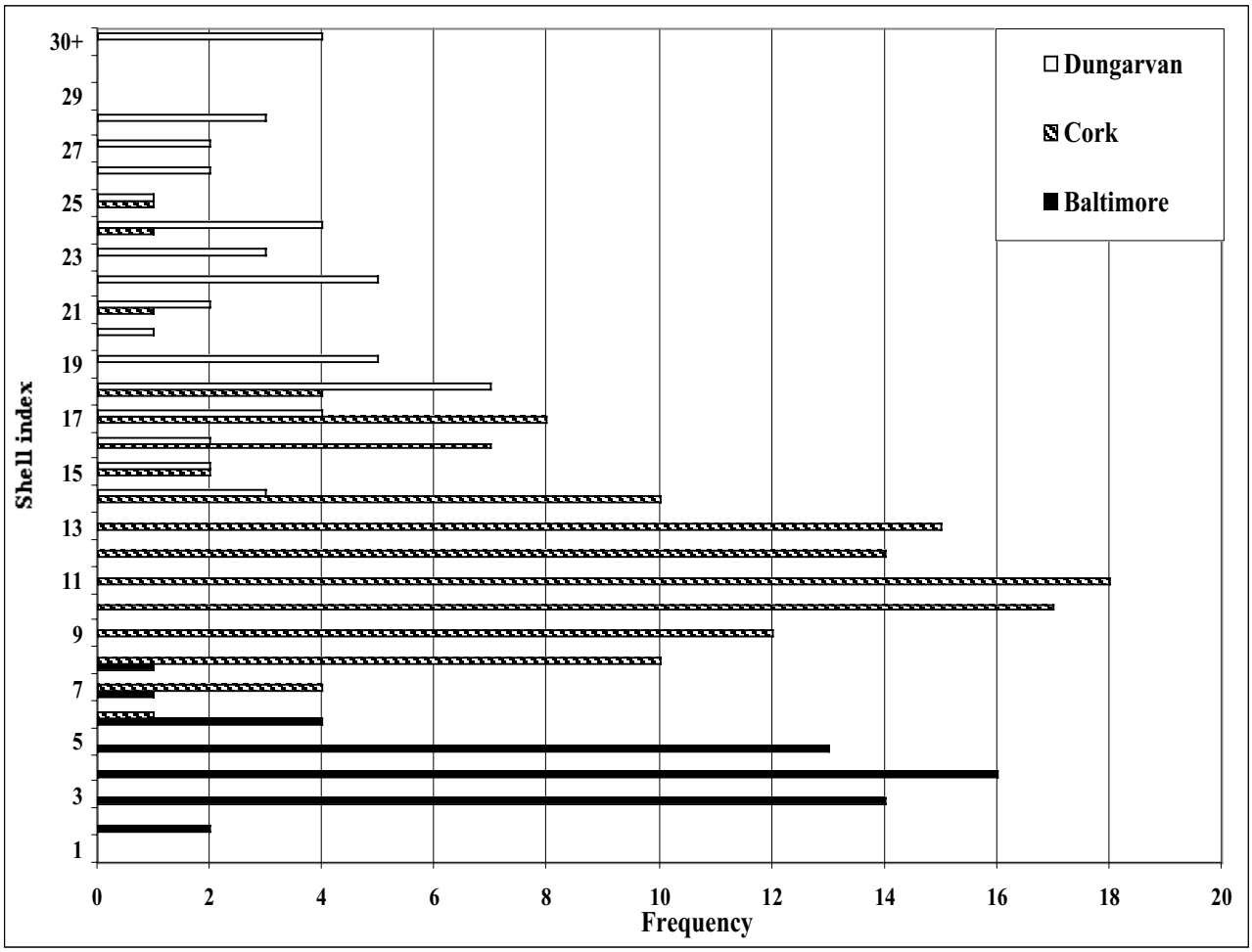


Figure 3. Shell index frequencies for oysters from Baltimore Harbour (September 1999), with high levels of contamination, the North Channel of Cork Harbour (this study) and from Dungarvan Bay (September 1999), an area with very low levels of TBT contamination.

DISCUSSION

This investigation, is part of the monitoring process set by the OSPAR Program in 1987 to visit certain marine regions every six years to monitor TBT contamination.

a) Mulroy Bay: The strong currents at the sampling stations, close to the entrance of Mulroy Bay are likely to disperse widely any TBT released. All indications strongly suggest that in Fanny Bay there had been some relatively recent inputs of TBT. Possible sources include the removal of old paint scraped from the hulls of boats. This degree of contamination is very unlikely to have any effect on mollusc culture in the Broadwater and North Water of Mulroy Bay.

b) Carlingford Lough. The high RPSI value at Carlingford Harbour suggested that it is a possible source of contamination, though the port of Warrenpoint may also have contributed to this contamination. It is remarkable that a population of dogwhelks survives at the breakwater at Greenore Point, despite the shipping berths being within 100m. The strong currents in this region may rapidly disperse any possible contamination. The low levels of oyster shell thickening and dogwhelk imposex nearby support this view.

c) Waterford Harbour: The relatively constant levels of contamination in Waterford Harbour may be due to the current patterns or to the leaching of TBT from many points. These include ships awaiting entry into the estuarine ports while anchored between Cheek Point and Passage. Dunmore East has a lifting facility and many of the vessels serviced in this port are over 25m so TBT antifouling paints may legitimately be used. Contamination within Waterford Harbour, although detectable, is not considered likely to have a significant effect on mollusc culture.

d) Cork Harbour: Moderate levels of contamination were detected in the upper estuary, with marginally lower values at Fountainstown, outside Cork Harbour.

e) Valentia Harbour: Levels of contamination are low. It is possible that there is good circulation of the water that results in a high dilution of TBT if present.

f) Tralee Bay: Indications of contamination are low, with a possible small rise in the region of Fenit Pier. The slightly elevated levels may be derived from occasional shipping traffic. Leaching of TBT from the crane transport barges may cause the symptoms observed. The main part of the native oyster bed would not be expected to be subject to harmful effects arising from TBT contamination at this level.

CONCLUSION

The four partly enclosed bays or estuaries examined in this study, demonstrated similar moderate levels of contamination. Lower levels of contamination were seen at Valentia and Fenit, the two sites where a greater degree of circulation of ocean water occurs. Here the highest indication values of TBT contamination observed are so low, that even the small port and boatyard areas examined were unlikely to cause any significant impact from TBT on aquaculture or mollusc fisheries.

The planned phasing out of TBT, as recommended by the International Maritime Organisation, is likely to lead to a decline of TBT contamination and the three shipping ports may be expected to experience improvements of water quality.

The study has shown that wild molluscs and cultivated oysters in all six sites examined show measurable effects of contamination by TBT. In all cases the effects are small and, from the point of view of commercial production, negligible. It is concluded that the Bye-law of 1987, severely restricting the application of TBT, has been effective in reducing TBT contamination and that it is unlikely that a significant increase in contamination will take place in the future.

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