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Monitoring and Assessment of Butyltins in Atlantic Coastal Waters

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Tributyltin and its degradation products were measured in seawater samples in 1986–87 at different locations of the French Atlantic coast, including marinas and oyster culture areas. Concentrations were in the range of <2–1 500 ng TBT l^{-1}, <1–194 ng DBT l^{-1}, and <1–200 ng MBT l^{-1}. In Arcachon Bay, the results showed that there was a good agreement between TBT levels in seawater and the frequency of oyster shells anomalies monitored at the same time.

Butyltins are the most toxic compounds used as antifouling agents in marine paints. Their high degree of toxicity against mollusc reproduction is well documented (His & Robert, 1983–85; Thain, 1983; Gibbs & Bryan, 1986). Furthermore, sublethal effects on shell calcification mechanisms of the Pacific oysters *Crassostrea gigas* have been reported in France (Alzieu *et al.*, 1982), UK (Waldock & Thain, 1983) and San Diego Bay (Stephenson *et al.*, 1986).

France was the first country to regulate the use of organotin antifouling paints. This action was supported by the fact that disturbances in Arcachon Bay oyster production (reproduction failure, decrease in adult growth, shells anomalies) were linked with the seawater organotin contamination. Since January 1982 the use of antifouling paints containing organotins has been forbidden for boats of less than 25 m long. The survey conducted between August 1982 and November 1985 in Arcachon Bay has shown the decrease in tin and organotin levels in seawater and oyster flesh (Alzieu *et al.*, 1986). This decrease since August 1984 is particularly obvious and seems to be associated with the improvement of the shell quality. This survey was expanded in 1986 to various sites of the Atlantic coast: Anse de Camaret (Brest), marinas at La Trinité and La Rochelle. The measurements were focused on the determination of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) levels in sea water and were complemented by a biological survey using oysters shell malformation as a contamination indicator.

Materials and Methods

Sampling strategy

The surveys were carried out in the following zones (Fig. 1): Anse de Camaret, inside Brest roadstead; mooring areas, ship docks and fishing port are the main sources of organotins at the vicinity of one experimental salmon farm; La Trinité marina, located on the rivière d'Auray estuary, capacity 970 boats; La Rochelle, Port des Minimes, capacity of the marina 2500 boats; Oléron Island, in the area of the marina of Boyardville (300 boats); Arcachon bay sampling was undertaken at the marinas of La Vigne (268 boats) and Arcachon (1700 boats), as well as the non-polluted reference station of Lahillon.

Except for the Anse de Camaret, sampling was carried out three times a year, corresponding to wintering, ship's cleaning, painting and mooring periods. Anse de Camaret was sampled only in June 1987 but at fourteen stations.

In addition, the development of shell thickening in oysters held at three locations in Arcachon Bay was recorded in animals sampled between March and December.

Water sampling techniques

Water samples (250 ml) were collected at 1 m from the sea bed, 2 h after high tide on neap tides. Glass sampling and storage bottles were washed by soaking for 24 h in a diluted hydrochloric acid solution (10%) and rinsing in deionized water. All water samples were immediately acidified by 5 ml of acetic acid, blocked by a teflon screw cap and stored at room temperature.

Analytical techniques

All samples were analysed during the week following the sampling. The automatized hydride generation/atomic absorption spectrometry technique (Michel, 1987), was used for determination of TBT, DBT, and MBT levels. The concentration given for each sample is the mean value obtained from five replicates. This method provides detection limits of around 1 ng l^{-1} for DBT and MBT, and 2 ng l^{-1} for TBT.

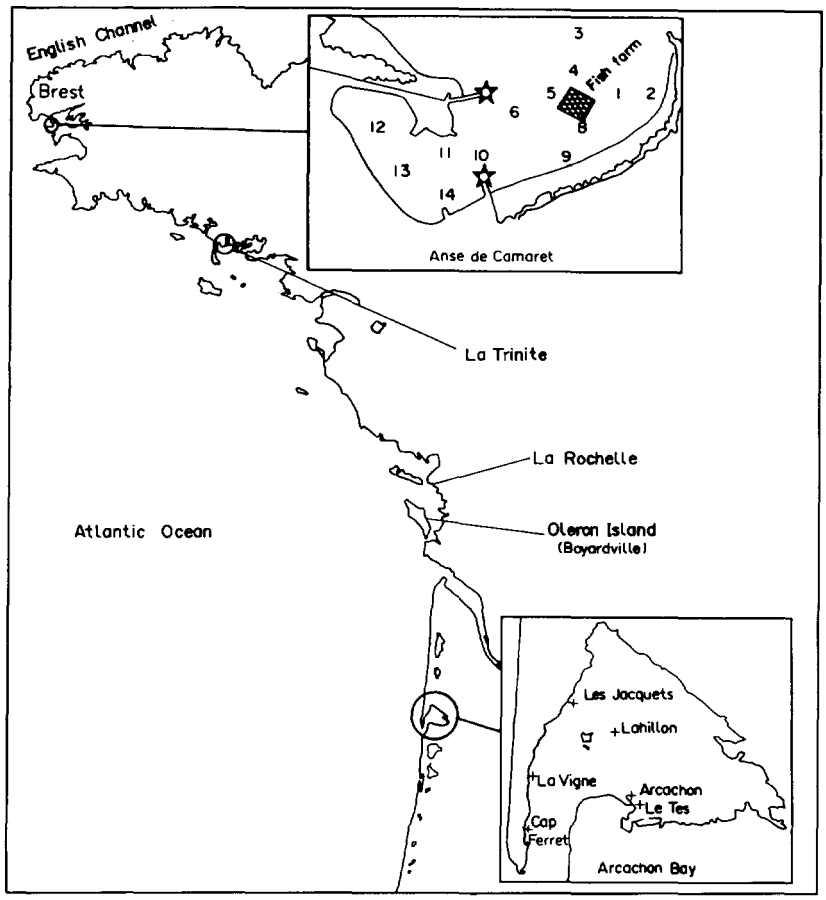

Fig. 1 Sampling sites.

Oyster shell thickness index

Each month, from March to December, oyster growth was measured on samples taken from three areas of Arcachon Bay: Cap-Ferret, in the south-west, an oceanic station, receiving the inputs from La Vigne marina; Les Jacquets in the north, a continental station, and Le Tes, in the east of the bay, the nearest from Arcachon marina. At the same time, thirty oysters were examined for the presence of absence of chambers, using the shell thickness index described by Alzieu *et al.* (1986).

A: the percentage of oysters with at least one chamber, generally in the upper valve;

B: the percentage of oysters with chambers in both valves simultaneously, i.e. acute abnormality.

Results

Water contamination

Concentration of contaminants in water samples, expressed as ng l^{-1} of TBT, DBT, and MBT ions, are shown in Table 1 for the Anse de Camaret and Table 2 for the other sampling zones. All values were in the range of <2–1500 for TBT, <1–194 for DBT, and <1–200 for MBT. In general, for the same sample the concentrations decreased in the following order: TBT>DBT>MBT. However, some samples having low TBT contamination (less than 10 ng l^{-1}) showed higher DBT concentration. This particular distribution was clearly established at Lahillion in March 1986, July 1986 and 1987. In contrast in October, when TBT value reached 51 ng l^{-1}, DBT and MBT were lower. These observations may be the result of lower degradation rate for DBT in particular environmental conditions.

Inside the marinas, TBT was in the range of 8–57 ng l^{-1} at Arcachon, 9–69 at La Trinité, 2–89 at La Vigne, 13–119 at La Rochelle, and 39–1500 at Boyardville. Boyardville appeared to be particularly highly polluted compared with the other marinas, especially when its low boat capacity is taken into account.

With regard to oyster culture areas, TBT levels were not detectable at Boyardville bank and were <5 ng l^{-1} at Lahillon, except in October 1986 when unusually high value (51 ng l^{-1}) was recorded.

During the sampling period a strong peak of contamination was observed in October at Oleron Island stations. At the same time, a less extensive peak was observed in Arcachon Bay at La Vigne and Lahillon stations and in La Rochelle marina. This temporary increase of the contamination may be due to cleaning and repainting of boats prior to the winter season.

In the Anse de Camaret, the level of seawater contamination was below the detection limit, i.e. <4 ng l^{-1} for TBT and <10 ng l^{-1} for DBT and MBT.

Shell thickness index

A and B values reported in Table 3 show that during the 1986–87 survey, the ratio of chambered oysters was greater during the summer, corresponding with the end of the oyster shell growth period. For index A the maximum range was 60–70 at Cap Ferret and Le Tes; 80–90 at Les Jacquets. In comparative terms, chambering in its acute form (index B) was worst in

TABLE 1
Organotin levels in seawater samples (ng l^{-1}) at Anse de Camaret.

Sampling stations	1	2	3	4	5	6	7	8	9	10	11	12	13	14
TBT	<2	<2	<2	<2	<2	<2	<2	3	2	4	<2	<2	<2	<2
DBT	<2	<2	<2	2	<2	2	<2	5	3	9	2	<2	<2	<2
MBT	8	2	6	14	4	10	6	7	8	8	4	3	4	3

TABLE 2
Organotin levels in seawater samples (ng l^{-1}) at La Trinité, La Rochelle, Oléron Island, and Arcachon Bay.

Sampling	1986			1987		
	March	July	October	March	July	October
La Trinité marina						
TBT	10*	14	44	69	9	37
DBT	5	4	8	27	10	5
MBT	7	10	n.a.	10	10	11
La Rochelle marina						
TBT	78	20	119	36	37	13
DBT	5	12	18	28	35	3
MBT	3	10	11	2	19	6
Oléron Island						
Boyardville marina						
TBT	39	235	1500	150	284	133
DBT	8	50	40	44	194	19
MBT	4	21	200	2	46	7
Boyardville pier						
TBT	2	5	57	2	1	<2
DBT	11	3	4	1	1	<1
MBT	5	4	4	1	1	<1
Boyardville bank						
TBT	<2	<2	n.a.	<1	<1	<2
DBT	17	<2		<1	<1	<1
MBT	7	<2		<1	<1	<1
Arcachon Bay						
Arcachon marina						
TBT	9	8	57	50	12	10
DBT	15	<1	13	2	9	3
MBT	4	6	14	<1	4	6
La Vigne marina						
TBT	2	2	89	2	30	<2
DBT	1	7	19	<1	14	<1
MBT	3	<2	8	<1	6	<1
Lahillon						
TBT	<2	<2	51	2	5	2
DBT	5	3	6	<1	6	1
MBT	2	3	8	<1	5	1

September and October. The most affected samples were found at Les Jacquets. In contrast no value of index B was noticed at Le Tes in 1986.

Two general conclusions can be drawn from the data in Table 2: malformations appeared earlier in 1987 (April) than in 1988 (June), at each of the three sampled stations; this difference was also observed in TBT seawater contamination values reported in Table 2; the slow decrease in chambering during autumn 1986 was not observed in 1987.

On the basis of these two observations, 1987 was characterized by a longer period of chambering than 1986.

Discussion

Data reported in Tables 1 and 2 represent a first level evaluation of TBT and its breakdown products in various waters of the French Atlantic coast. These 2 yr of monitoring data show that the seawater contamination in marinas is usually lower than 100 ng l^{-1} for TBT; except in Boyardville where abnormally high concentrations, up 1500 ng l^{-1}, were recorded. This high contamination in Boyardville marina seawater could not be entirely explained by inputs from illegal uses of organotin antifouling paints. This conclusion is supported by the fact that the theoretical paint leach rate corresponding with 1500 ng l^{-1} for TBT in seawater marina is around 1,5 µg cm^{-2} day^{-1}. This calculation, made with the very unlikely assumption that all boats were painted with organotin antifouling paint, give a leaching rate close to the limit of the effectiveness of the paints. In contrast, in the well flushed areas, like Anse de Camaret, Boyardville bank, and stations not

TABLE 3
1986–87 Shell malformation indices A and B at three stations of Arcachon Bay.

		Cap-Ferret			Les Jacquets			Tes		
	Month	Heal.	A	B	Heal.	A	B	Heal.	A	B
1986	March	100	0	0	100	0	0	100	0	0
	April	100	0	0	100	0	0	100	0	0
	May	100	0	0	100	0	0	100	0	0
	June	70	30	0	60	40	0	57	43	0
	July	67	33	3	17	83	0	33	67	0
	August	47	53	3	10	90	12	34	66	0
	September	27	73	3	7	93	17	36	64	0
	October	47	53	10	37	63	17	44	56	0
	November	53	47	0	37	63	7	50	50	0
	December	80	20	0	0	0	0	50	50	0
1987	March	100	0	0	100	0	0	100	0	0
	April	93	7	0	83	17	0	76	24	0
	May	77	23	0	70	30	0	63	37	0
	June	–	–	–	–	–	–	–	–	–
	July	40	60	0	13	87	4	17	83	6
	August	60	40	0	10	90	0	33	67	6
	September	30	70	12	20	80	40	40	60	0
	October	47	53	3	13	87	7	13	87	17
	November	54	46	0	13	87	20	17	83	13

Heal = non affected.

directly influenced by input sources (Lahillon), TBT levels were usually lower than 5 ng l^{-1}.

These results are in accordance with those found by Waldock *et al.* (1987) in UK estuaries in 1986. These authors have reported TBT values in the range of less than 1 ng l^{-1} in estuaries to 1500 ng l^{-1} in marinas having poor water exchange. Half the results obtained, in April–December, were equal to or above the Environmental Quality Target concentration fixed by the UK authorities (20 ng l^{-1}).

Similar values were reported for water samples from Chesapeake Bay, where organotin antifouling paints are not subject to restriction. In marina waters, Hall *et al.* (1987) found mean concentrations of TBT ranged from 51 to 408 ng l^{-1}, with peak values around 1000 ng l^{-1}. In the same bay, Hugget *et al.* (1986) reported that TBT levels in recreational waters and marinas ranged from <1–100 ng l^{-1}.

With regard to the impact of seawater TBT contamination on bivalves, the deleterious effects on calcification mechanisms of *Crassostrea gigas* is now well documented. We have recently found, through long-term experiments, that TBT concentrations as low as 2 ng l^{-1} were able to produce gel formation and shell chambering in adults (Alzieu *et al.* unpublished data). Compared to this threshold value, the levels found in seawater sampled in Arcachon Bay could explain why malformed oysters are still persistent at Lahillon, since concentrations there were in the range of <2–5 ng l^{-1} with a peak of 51 ng l^{-1}.

The high sensitivity of the malformation indices to the variation of TBT seawater contamination is apparent from Fig. 2 where values observed before (1980–82) and after the organotin ban in antifouling paints are reported (1980–85 data were taken from Alzieu *et al.*, 1986). For index A development, Fig. 2 shows three different periods corresponding with: high values (>90) in 1980–82; progressive decline in 1983–85 to 40; increase in malformations in 1986–87 up to 70.

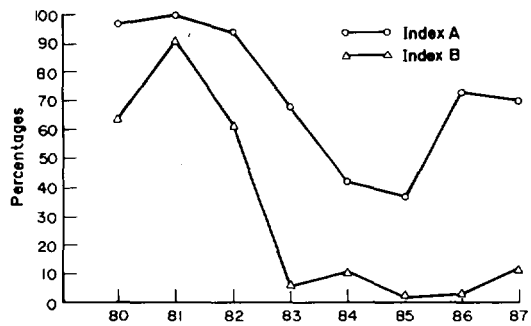

Fig. 2 Shell malformation indices at Cap Ferret Station in 1980–87.

In contrast, the development of index B was very different: after the rapid decline observed in 1982, values remained around 10 in 1983–87.

These observations and results lead us to conclude that:

- abnormal inputs of TBT still exist, specially in Boyardville marina, in spite of the regulation of antifouling paints;
- although contamination levels are low or below the minimum detectable in oyster culture areas, a significant number of cultured oysters are still affected by malformations induced by organotins;
- biological surveys using indices A and B, as described above, is a very efficient indicator of water contamination.

These findings are consistent with our previous assessment based on total tin/organotin measurements in oysters tissues (Alzieu *et al.*, 1986), where we concluded that "the prohibition of the use of organotin in antifouling paints was an effective action for both protection and conservation of the oyster stock". Furthermore, analytical techniques detecting the TBT ng l^{-1} level performed in the 1986–87 survey, proves that there is a real need for improvement in the enforcement of existing regulations.

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Characterization of PCBs by Principal Component Analysis (PCA of PCB)

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Tabulated results of PCB measurements in terms of individual chlorobiphenyls are converted into planar graphs for visual examination. Relationships among the chlorobiphenyls are also detected in the process.

Results of PCB determinations are now almost exclusively reported in terms of individual chlorobiphenyls. This leads to long tables that are difficult to comprehend and some of the more subtle features of the data may escape attention. The data are multidimensional, their dimension given by the number of chlorobiphenyls reported. For a geometric display the number of dimensions must be decreased to 2–3. This may be accomplished by principal component analysis (PCA, see for example Sasaki & Takahashi, 1987). PCA was used on a number of occasions to study the patterns of PCB mixtures (see Schwartz *et al.*, 1987, and Stalling *et al.*, 1987 for recent examples). The technique has not been applied as frequently as warranted by its potential, possibly because of its perceived complexity. In this note, PCA is demonstrated on the data of Duinker *et al.* (1988) on PCBs in organs and tissues of porpoises and whales, to further illustrate its application and simplicity.

To measure PCB in environmenal samples, Duinker *et al.* selected seventeen chlorobiphenyls that can be measured unambiguously and accurately. Concentrations of the individual chlorobiphenyls were then expressed as percentages of their sum. The data set is a table (matrix) with 33 rows and 17 columns. Duinker *et al.* presented the scaled data in bar graphs, displaying several samples side-by-side.

For PCA, each sample (row) is a point in a seventeen dimensional space. Similarity of samples is inversely proportional to their distance in this space. To give all the chlorobiphenyls (columns, dimensions) an equal weight, the data are centred by subtracting mean row and scaled by dividing by the respective (column) standard deviations.

For a visual pattern recognition the data are projected by PCA from the original 17-dimensional space, on a plane (2 dimensions) selected in a way that preserves most of the information in the data. This is usually measured by the retained variance. In the present example, 76% of the variance is retained and the projection is presented in Fig. 1. In the literature, such a plot is called the principal components plot, theta plot or the scores plot. The axes are linear combinations of the original variables (chlorobiphenyls). In the projection, similar samples are close together.

As can be seen from Fig. 1, samples of whales are separated by a wide margin from the samples of porpoises. This is immediately obvious from the display. The same conclusion can be reached from the bar graphs of Duinker *et al.* (1988), but only after some