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Scale of classification based on biochemical markers in mussels: application to pollution monitoring in European coasts

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A battery of biochemical parameters is used to evaluate the response of mussels to a contaminated coastal environment. In the European BIOMAR programme, a multimarker approach was developed, establishing a scale for the classification of the water quality in European coastal sites. AChE activity is highly sensitive to organophosphorus and carbamate insecticides but also to heavy metals. Catalase activity and lipid oxidation (evaluated as MDA) are markers of oxidative stress, GST activity is related to the conjugation of organic compounds and BPH activity is a marker of planar compounds (e.g. PAHs). These parameters were measured either in gills (AChE, GST) or digestive gland (BPH, GST, CAT, MDA). Contamination levels were estimated by measurement of PAHs and heavy metals in animals. For each biomarker, a discriminatory factor was calculated (maximum variation range/confidence interval) and a response index was allocated. For each site, a global response index was calculated as the sum of the response index of each of the five biomarkers. As a result of our calculation method, the quality of the coastal environment at each site can be classified with a five level scale. Mussels were collected during five cruises in 1995–1996 on the Baltic and Mediterranean coasts. The results show that water quality ranged from class 1 (clean areas in some sites of the French Riviera, Spanish Costa Brava and the Baltic coast) to class 5 (high pollution in main harbours, e.g. Kiel and Toulon). Some areas fall into class 4, e.g. Carteau, Cortiou, Barcelona, Warnemunde, Swinemunde, Ebro delta. The global Biomarker Index was positively correlated with the level of PAHs in mussels in Baltic transects. A number of other contaminants or stressors may be present in the marine environment and the Biomarker Index appeared to be relevant to classify coastal environmental pollution.

Keywords: molecular biomarkers, *Mytilus edulis*, *Mytilus galloprovincialis*, benzo(*a*)pyrene hydroxylase, glutathione S-transferase, catalase, malonedialdehyde, acetylcholinesterase, Baltic Sea, Mediterranean Sea.

Introduction

Over the past decade, molecular and cellular biomarkers have been extensively studied in pollution monitoring of aquatic environments (McCarthy and Shuggart 1990, Huggett *et al*. 1992). Biomarkers were selected among early molecular events occurring in the toxicological mechanisms of main contaminants (PAHs, PCBs, heavy metals, pesticides, etc.) and are potentially useful tools for detecting either exposure to, or effects of, chemicals. Benzo(*a*)pyrene hydroxylase (BPH) activity was used in a number of field studies in mussels and some correlations were found with PAH pollution (Suteau *et al*. 1988, Garrigues *et al*. 1990, Narbonne *et al*. 1991, Michel *et al*. 1994). Lipid peroxidation (malonedialdehyde MDA formation), glutathione S-transferase (GST) and catalase (CAT) activities were found to be modulated by metal or organic contaminants under both field and laboratory

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conditions (Pellerin-Massicotte 1994, Prakash and Rao 1995, Regoli and Principato 1995). Measurements of cholinesterase activities have been used as biomarkers of exposure for some pesticides and other pollutants in wildlife (Boquené *et al*. 1990, Najimi *et al*. 1997). These data may be useful to scientists in evaluating the specificity of responses to natural or anthropogenic changes, but it is very difficult for the environmental manager to interpret increasing or decreasing changes in biomarker values. Except for some examples in limited areas and time (Bayne *et al*. 1988, McCarthy and Shugart 1990), the translation of biochemical data into environmental information is limited, due to the difficulty in interpreting the temporal and spatial extent of biomarker variation. The practical approach carried out by the official agencies in charge of environmental surveys is to establish indices of environmental quality taking into account chemical or biological criteria, in order to classify the sites being monitored on a scale from 'clean' to 'highly polluted' (usually four levels for microbiological criteria, or five levels for chemical criteria). Marine mussels are commonly used as sentinel organisms for the detection of environmental pollution in coastal waters due to their capacity to accumulate several organic and inorganic contaminants (Goldberg *et al*. 1975, Livingstone 1991). The aim of this paper is to evaluate biomarkers in bivalves in relation to their potential utility as indices of marine environmental quality by using the results obtained during the BIOMAR programme (1994–1998). This programme, supported by the EU Commission, was designed to validate the multimarker approach in pollution monitoring of European coastal environments.

Materials and methods

Sample collection and preparation

Mussels—*Mytilus edulis* and *galloprovincialis*—were collected from stations on the European coast either by grab or by skin divers (in water depth up to 40 m). The sites from the German coast of the Baltic sea consisted of three transects from main harbours (Kiel, Warnemünde, Swinemünde) to the open sea. Three sampling cruises took place in the same area (March 1995, October 1995, November 1996). Coastal sites from the Mediterranean sea (Italy, France and Spain) were expected to present a wide variety of contamination patterns, from preserved areas in Corsica to highly polluted harbours on the continental coast (Toulon, Marseille, Barcelona). The BIOMAR I cruise on the French and Sardinian coasts took place in October 1995 and the BIOMAR II cruise on the French and the Spanish coasts took place in August 1996. Gills and digestive glands were dissected and immediately stored in liquid nitrogen prior to analysis. Those for chemical analysis were wrapped in aluminium boxes and stored at −20°C prior to analysis.

Biochemical measurements

Biochemical determinations were carried out as previously described (Labrot *et al*. 1996). In gills, AChE and GST activities were measured in the post-mitochondrial fraction, (S9) by using acetylthiocholine (Ellman *et al*. 1961) and 1-chloro-2-4-dinitrobenzene (Habig *et al*. 1974) respectively. In the digestive gland, BPH activity was measured in the microsomal fraction (Michel *et al*. 1994), GST and CAT activities (Clairborne 1985) were determined in the cytosolic fraction. The concentration of endogenous MDA was also measured in the cytosol after reaction with thiobarbituric acid (Buege and Aust 1978). All these data were expressed in relation to protein concentration determined according to the method of Lowry *et al*. (1951).

Chemical analysis

The freeze-dried mussel tissues were extracted as reported by Baumard *et al*. (1997). The aromatic fraction was analysed by gas chromatography coupled to mass spectrometry and 14 PAHs among those recommended as priority pollutants by the EPA were measured. Metal concentrations (Hg, Cd, Zn, Cu, Pb) were determined by Atomic Absorption Spectrophotometry as described by Abdullah and Steffenak (1988). The metal content in animals is expressed as the sum of these five metals.

Table 1. Index given for each biomarker response according to their rank in a scale related to the discriminatory factor.

	Discriminatory factors				
	1	2	3	4	5
Index of response	4	8			
	3	6	10		
	2	4	7	12	
	1	2	4	8	14

Statistical procedure and indices calculation

Biomarker data were analysed by carrying out a one-way ANOVA and Tukey test using the statistical software (release 5.1, Statsoft Inc. Ed., 1998). For each parameter at each site the mean (average) was calculated. An average confidence interval (CI) for each parameter is determined at the desired significance level (usually 0.05) for each experiment (cruise). The response factor (RF) is the ratio between the higher and the lower mean, the response range (RR) is the arithmetic difference between the higher and the lower mean. A discriminatory factor is calculated as DF = RR+CI/CI. This factor served to determine the number of significant differences among the sites being compared. By using this scale, each biomarker response must be ranked '1' or '2' when there are two levels and from '1' to '5' when there are five places between lower and higher mean. In order to standardize the biomarker response a response index (RI) is attributed for each result according to their rank position as indicated in table 1. For each site a global biomarker index (BI) is calculated as the sum of five individual biomarkers measured. Using the multimarker approach, the selected biomarkers include drug-metabolizing enzymes, indicators of oxidative stress and cholinesterase activity.

Results

Biomarkers and discriminatory factors

Biomarker measurements (average) obtained for each site studied in the Baltic and Mediterranean Seas are presented in table 2. The results indicated high variability in biomarker data due to both intersite (supposed to be due in part to difference in pollution level) and to intrasite (seasonal variability) sources. This variability is analysed in table 3 and expressed in terms of discriminatory levels. The maximum response factor was observed for CAT/MDA ratio and the minimum RF for GST in digestive gland. Discriminatory levels were higher for biomarkers measured in gills (especially for GST from 3 to 5 DL) than in digestive gland (DL from 2 to 4).

Biomarker index

Table 4 shows the index response attributed to each biomarker. The results of biomarker index calculation show that the highest value was found in Kiel harbour (K0) and the lowest index was obtained on the Spanish Mediterranean coast (Cala Monjoy). High BI values (higher than 40) were also found in main harbours (Warnemunde, Swinemunde, Toulon, Barcelona) or in sites exposed to industrial or domestic water release (Cortiou, Carteau, Ebro delta). Samples collected inside Toulon and Barcelona harbours were significantly higher than those collected outside. These last values are similar to those obtained in sites located near by (French Riviera, Costa Brava). Low BI values (18–26) were obtained in Corsica, French Riviera and Spanish Costa Brava. In the Baltic Sea, the lower BI values

Table 2. Results of biomarker measurements for each site studied during BIOMAR cruises in 1995–1996.

		AChE G	GST G	GST DG	CAT DG	MDA DG	CAT/MDA DG	BPH DG
Baltic Sea	Kiel 0	7	201	160	68	nd	nd	0.36
March 1995	Kiel 1	8.9	175	189	70	nd	nd	0.39
	Kiel 2	13.2	202	263	102	nd	nd	0.43
	Warnemunde 1	9	203	201	74	nd	nd	0.32
	Warnemunde 2	6.5	123	234	84	nd	nd	0.33
	Warnemunde 3	9.7	124	223	88	nd	nd	0.34
	Swinemunde 1	6.1	147	205	64	nd	nd	0.31
	Swinemunde 2	7.8	140	210	72	nd	nd	0.17
	Swinemunde 4	7.7	137	179	84	nd	nd	0.15
	Swinemunde 5	7.7	78	244	100	nd	nd	0.13
Baltic Sea	Kiel 0	11.5	476	147	48	2.7	28	nd
October 1995	Kiel 2	11.9	430	176	60	1.5	50	nd
	Warnemunde 2	11.4	337	209	26	3.7	7	nd
	Warnemunde 3	12.4	344	208	30	3.8	8	nd
	Warnemunde 4	11.1	309	201	32	2.5	15	nd
	Swinemunde 2	13.8	330	138	36	2.1	21	nd
	Swinemunde 3	12.6	283	143	47	2.6	18	nd
	Swinemunde 4	11.9	169	123	49	2.5	21	nd
Baltic Sea	Kiel 1	7	311	160	18	0.9	18	0.6
November 1996	Kiel 2	7	362	188	26	0.8	31	0.7
	Warnemunde 1	8	243	128	24	1.1	20	0.9
	Warnemunde 2	9	275	150	24	0.8	26	0.7
	Warnemunde 3	11	279	154	28	1.2	22	0.4
	Swinemunde 2	11	546	165	30	0.7	39	0.5
	Swinemunde 3	11	757	178	29	0.6	42	0.6
	Swinemunde 4	9	255	134	31	0.9	34	0.7
Mediterranean Sea	Cortiou	11	273	124	21	4.3	4.9	1.2
October 1995	Planier	19	331	114	18	3.5	5.1	0.8
	Toloun out	19	343	101	19	2.3	8.1	0.9
	Toulon in	21	432	117	17	1.7	10.1	1.3
	Sanguinaires	28	476	94	16	4.9	3.3	1.4
	Ajaccio	13	292	103	15	2.8	5.5	1.4
	Punta Rossa	24	371	113	21	4.7	4.5	1.4
	Toro	18	332	116	17	4.6	3.8	1.1
	Porto Vecchio	27	294	87	17	4.4	3.9	1.2
	Bonifacio	28	435	103	20	4.5	4.4	1.3
	Porto Torres	16	207	97	17	4.1	4.2	1.1
	Fourmigue	15	300	86	12	2.6	4.8	1
Mediterranean Sea	Planier	18	317	108	57	2.6	27	1.7
August 1996	Carteau	17	176	87	21	2.1	15	1.9
	Cap d'Agde	17	275	97	44	3	16	0.9
	Leucate	15	219	112	39	2.3	20	1.3
	Port Vendres out	16	249	114	39	2.8	14	1
	Port Vendres in	15	233	126	47	1.8	29	1
	Cala Monjoy	18	244	122	36	2.6	15	0.9
	Calella	22	282	118	37	2.4	16	1.3
	Barcelona in	10	218	85	22	1.5	18	1.8
	Barcelona out	16	196	91	18	3.1	7	1
	Tarragona	20	168	106	27	3.2	9	1.7
	Ebro	7	157	82	10	1.9	6	1.7

Glutathione S-transferase (GST); nmol min^{-1} mg^{-1} prot, acetylcholinesterase (AChE); nmol min^{-1} mg^{-1} prot, malonedialdehide (MDA); nmol min^{-1} mg^{-1} prot, benzopyrene hydroxylase (BPH); pmol min^{-1} mg^{-1} prot, catalase (CAT); µmol min^{-1} mg^{-1} prot, nd; not determined, G; gills, DG; digestive gland.

Table 3. Discriminatory levels obtained for the biomarkers studied in 1995–1996 for the BIOMAR programme.

		Response factor (RF)	Response range (RR)	Confidence interval (CI)	Discriminatory factor (DF)	Discriminatory levels (DL)
Baltic Sea March 1995						
AChE	Gills	2.14	7.07	2.75	3.57	4
GST	Gills	2.57	124	40.87	4.03	4
GST	Digestive gland	1.64	103	53.2	2.86	3
CAT	Digestive gland	1.55	36	26	2.38	3
BPH	Digestive gland	5.35	0.456	0.386	2.18	2
Baltic Sea October 1995						
AChE	Gills	1.23	2.61	3.16	1.82	2
GST	Gills	2.81	307	60.7	6	5
GST	Digestive gland	1.68	84	30.5	3.8	4
CAT	Digestive gland	2.32	34.6	16.4	3.11	3
CAT/MDA	Digestive gland	7.16	43.5	29	2.5	2
Baltic Sea November 1996						
AChE	Gills	2.06	6.13	2.16	2.84	3
GST	Gills	3.77	605	100.5	6.05	5
GST	Digestive gland	1.68	83.8	29.5	2.83	3
CAT/MDA	Digestive gland	5.57	60.6	25.1	2.41	2
BPH	Digestive gland	4.45	0.87	0.64	1.36	2
Mediterranean Sea October 1995						
AChE	Gills	2.55	17.4	7.39	3.35	3
GST	Gills	2.08	225	63.5	4.53	3
GST	Digestive gland	1.42	38.1	25.96	2.46	2
CAT	Digestive gland	1.71	9.42	7.64	2.23	2
CAT/MDA	Digestive gland	3.27	8.25	4.53	2.82	3
BPH	Digestive gland	1.61	0.54	0.866	1.62	2
Mediterranean Sea August 1996						
AChE	Gills	3.94	18.16	5.4	3.36	4
GST	Gills	2.42	206	57.4	3.61	4
GST	Digestive gland	1.79	58.9	24.86	2.37	3
CAT	Digestive gland	16.13	66.8	21.12	3.16	4
CAT/MDA	Digestive gland	12.04	42.4	22.6	1.8	3
BPH	Digestive gland	3.60	1.77	0.8	2.21	3

RF = Higher mean/lower mean, RR = Higher mean/lower mean, CI; confidence interval at $P = 0.05$, DF = RR+CI/CI, DL; entire number of discriminatory levels from DF calculation.

were observed in Swinemunde sites for the October cruise. Table 5 shows the relationship between biomarker index and chemical levels (PAHs and metals) measured in mussels. For samples collected in the Baltic Sea during the three cruises, a significant correlation ($P < 0.05$) was found with PAH contamination. No correlation was found between BI and measured contaminants for the Mediterranean sites.

Discussion

The aim of this paper is not to discuss in detail the results obtained with the different parameters measured during BIOMAR cruises but to validate the use of a biomarker index for the quality assessment of the coastal environment (main chemical and biochemical results were presented and discussed in two doctoral theses (Baumard 1997, Mora 1998). AChE activities were lower in sites from the

Table 4. Index of biomarker responses for each site studied during BIOMAR cruises in 1995–1996.

		AChE G	GST G	GST DG	CAT DG	CAT/MDA DG	BPH DG	Biomarker index (global)
Baltic Sea	Kiel 0	12	12	12	12	nd	8	56
March 1995	Kiel 1	4	7	6	12	nd	8	37
	Kiel 2	2	12	3	3	nd	8	28
	Warnemunde 1	4	12	6	12	nd	8	42
	Warnemunde 2	12	3	6	6	nd	4	32
	Warnemunde 3	4	3	6	6	nd	8	26
	Swinemunde 1	12	3	6	12	nd	8	42
	Swinemunde 2	7	3	6	12	nd	4	33
	Swinemunde 4	7	3	6	6	nd	4	27
	Swinemunde 5	7	2	3	3	nd	4	19
Baltic Sea	Kiel 1	8	14	4	6	8	nd	40
October 1995	Kiel 2	8	8	7	12	8	nd	43
	Warnemunde 2	8	4	12	3	4	nd	31
	Warnemunde 3	8	4	12	3	4	nd	31
	Warnemunde 4	8	4	12	3	4	nd	31
	Swinemunde 2	4	4	4	3	4	nd	19
	Swinemunde 3	8	2	4	6	4	nd	24
	Swinemunde 4	8	1	2	6	4	nd	21
Baltic Sea	Kiel 1	12	8	6	*12	3	8	37
November 1996	Kiel 2	12	8	12	*6	6	8	46
	Warnemunde 1	6	14	3	*6	3	8	34
	Warnemunde 2	6	14	6	*6	3	8	37
	Warnemunde 3	3	14	6	*6	3	4	28
	Swinemunde 2	3	2	6	*12	12	4	27
	Swinemunde 3	3	1	12	*12	12	8	36
	Swinemunde 4	6	14	3	*12	6	8	37
Mediterranean Sea	Cortiou	12	12	12	*8	3	8	47
October 1995	Planier	6	12	12	*8	3	4	37
	Toulon out	6	6	6	*8	6	4	28
	Toulon in	6	12	12	*4	12	8	50
	Sanguinaires	3	6	6	*4	3	8	26
	Ajaccio	12	6	6	*4	3	8	35
	Punta Rossa	6	12	12	*8	3	8	41
	Toro	6	12	12	*4	3	4	37
	Porto Vecchio	3	3	3	*4	3	8	20
	Bonifacio	3	6	6	*8	3	8	26
	Porto Torres	6	6	6	*4	3	8	29
	Fourmigue	6	3	3	*4	3	4	19
Mediterranean Sea	Planier	3	2	6	*12	12	12	35
August 1996	Carteau	3	12	12	*3	6	12	45
	Cap d'Agde	3	3	6	*6	6	4	24
	Leucate	3	6	6	*6	6	6	27
	Port Vendres out	2	6	6	*6	6	4	24
	Port Vendres in	3	6	3	*6	12	4	28
	Cala Monjoy	2	3	3	*6	6	4	18
	Calella	2	3	3	*6	6	6	20
	Barcelona in	6	6	12	*3	6	12	42
	Barcelona out	3	6	12	*3	3	4	27
	Tarragona	2	12	6	*3	3	12	35
	Ebro	12	12	12	*2	3	12	51

nd: not determined,
* Not used for total index calculation.
G: gills, DG: digestive gland.

Table 5. Correlation coefficient of linear correlation between chemical analysis and global biomarker response index.

		Biomarker index (BI)	Metals (μg g^{-1} dry wt)	PAHs (ng g^{-1} dry wt)	*r*
Baltic Sea	Kiel 0	56	nd	2808	
March 1995	Kiel 1	37	171	4305	
	Kiel 2	28	48	1188	BI/Metals
	Warnemunde 1	42	160	600	–0.127
	Warnemunde 2	32	208	268	
	Warnemunde 3	28	160	271	BI/PAHs
	Swinemunde 1	42	48	nd	0.540
	Swinemunde 2	33	220	302	
	Swinemunde 4	27	nd	370	
	Swinemunde 5	19	157	404	
Baltic Sea	Kiel 1	40	216	866	
October 1995	Kiel 2	43	261	537	
	Warnemunde 2	31	415	104	BI/Metals
	Warnemunde 3	31	429	119	–0.453
	Warnemunde 4	31	nd	135	
	Swinemunde 2	19	340	125	BI/PAHs
	Swinemunde 3	24	337	164	0.719
	Swinemunde 4	21	320	200	
Baltic Sea	Kiel 1	37	nd	361	
November 1996	Kiel 2	46	nd	342	BI/Metals
	Warnemunde 1	34	nd	191	nd
	Warnemunde 2	37	nd	92	
	Warnemunde 3	28	nd	112	BI/PAHs
	Swinemunde 2	27	nd	100	0.680
	Swinemunde 3	36	nd	118	
	Swinemunde 4	37	nd	221	
Mediterranean Sea	Cortiou	47	111	131	
October 1995	Planier	37	135	305	
	Toulon out	29	128	288	
	Toulon in	50	118	373	BI/Metals
	Sanguinaires	27	122	26	0.279
	Ajaccio	36	130	208	
	Punta Rossa	41	109	37	BI/PAHs
	Toro	37	80	31	0.346
	Porto Vecchio	21	76	26	
	Bonifacio	27	128	121	
	Porto Torres	30	79	390	
	Fourmigue	20	95	64	
Mediterranean Sea	Planier	35	168	79	
August 1996	Carteau	45	127	48	
	Cap d'Agde	22	114	39	
	Leucate	28	144	50	BI/Metals
	Port Vendres out	24	187	40	0.335
	Port Vendres in	28	138	336	
	Cala Monjoy	18	117	37	BI/PAHs
	Calella	21	138	82	0.147
	Barcelona in	43	167	335	
	Barcelona out	27	127	60	
	Tarragona	35	277	51	
	Ebro	51	177	25	

nd: not determined; PAHs: 14 compounds among the EPA list; metals: sum of Hg, Cu, Cd, Pb, Zn.

Baltic Sea compared with Mediterranean sites, indicating higher pollution in the Nordic areas, which was in agreement with the results of chemical measurements. Among Mediterranean sites, Ebro exhibits the lowest AChE activity. This site is considered as a 'reference site' by the Spanish research group in Barcelona (Sole *et al*. 1995) for PAH and PCB contamination. Our results show that mussel PAH content here was the lowest among all the sites studied. However, the metal contamination is not significantly different from other sites on the Spanish coast and the Ebro river (one of the most important Spanish rivers which irrigates the agricultural areas associated with the use of pesticides, which are known to inhibit AChE). Biotic and abiotic factors can induce changes in biochemical parameters (Anderson and Forlin 1992). As reported by Viarengo *et al*. (1991), a general decrease in antioxidant defences during the winter periods was thus accompanied by an increase in MDA tissue levels, related to reproductive cycle and food availability (Hawkins and Bayne 1984). In the Baltic Sea CAT activity was higher in March than in October and November. In the Mediterranean Sea, the seasonal variation can only be observed in the site 'Planier' which was sampled twice. The CAT/MDA ratio was five times higher in summer than in autumn.

The use of a biomarker index facilitates the comparison between the different areas and sampling periods. In our approach the area studied may include a clean and a highly contaminated site in order to establish the limits of the scale, and the scale is adjusted for each cruise or sampling programme, thus, the seasonal variability is limited. Maximum biomarker index values (from 50 to 56) were obtained in Kiel and Toulon harbours and the Ebro delta. BI values from 40 to 49 were found in some sites in the vicinity of harbours or areas affected by industrial or urban wastes (Kiel, Warnemunde, Swinemunde, Cortiou, Carteau, Barcelona); BI values from 28 to 39 were found in sites more distant from pollution sources (Warnemunde 2, 3, 4, Swinemunde 2, 3, 4, Planier, Tarragona, Toulon out), or in harbours with only shipping or fishing activities (Ajaccio, Bonifacio, Porto Torres, Port Vendres in). BI values ranging from 21 to 27 were found in tourist areas. Finally, BI values lower than 21 were found in sites far from industrial, agricultural or urban activities (Fourmigue, Cala Monjoy, Calella, Porto Vecchio), or in the open sea (Swinemunde 5). Among the possible chemicals present in marine coastal environments, some metals and PAHs were measured in the mussels collected during our cruises. A tentative correlation analysis between BI and contaminants indicates a significant positive correlation with PAH content in transect studies from Baltic Sea. Previous studies reported a good correlation between BPH activity in the digestive gland and PAH content in sediments (Garrigues *et al*. 1990, Narbonne *et al*. 1991). No significant correlation was found in Mediterranean sites between BI and the measured contaminants. Sole *et al*. (1995) reported field studies in Spanish coast which indicated that despite a large variation in contaminant levels in mussels, the biomarker activities in digestive glands showed no positive relationship. However, data for other contaminants potentially present in coastal environments were not measured. Limited induction of BPH, GST and antioxidant activities have been considered of limited use as biomarkers in environmental monitoring (Sole *et al*. 1998). Calculation of a Global Biomarker Index based on a specific scale taking into account a multimarker response, seems to be able to translate scientific data to useful information to estimate the quality level of coastal environments. However, further experiments are needed to validate the BI approach.

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