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INTEGRATED BIOMARKER RESPONSE: A USEFUL TOOL FOR ECOLOGICAL RISK ASSESSMENT

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Abstract—A battery of biomarkers is often used to evaluate the effects of exposure to chemical contaminants and detect responses to environmental stress. Unfortunately, field application of biomarkers is subject to various constraints (e.g., the availability of living material) that can limit data acquisition and prevent the use of multivariate methods during statistical analysis. In these circumstances, a simple method is needed to summarize biomarker responses and simplify their interpretation in biomonitoring programs. The present study used star plots to display results for the panel of biomarkers used for each station and survey. Integrated biomarker response (IBR) was then computed as the star plot area. Star plots using IBR values instead of biomarker data make it possible to visualize between-site and/or between-survey differences for comparison with exposure conditions. This approach was applied to sites in the Baltic Sea and the Seine Estuary, English Channel. In both cases, IBR values were visually compared to polycyclic aromatic hydrocarbons (PAH) or polychlorobiphenyls (PCB) levels measured in mussel or fish tissues. The IBR, as an indicator of environmental stress, appears to be a useful tool for scientists and managers in assessing ecological risk.

Keywords—Biomarkers Graphic tool *Mytilus edulis* *Platichthys flesus* Coastal waters

INTRODUCTION

Biomarkers can provide valuable information in field or semifield testing and be used to measure a wide range of physiological responses to chemicals at the biochemical, cellular, or tissular level. Extensively studied in the field since the late 1980s [1–5], biomarkers are now considered to be useful tools for monitoring the biological effects of pollutants and environmental stress. Experience acquired with biomarkers in the marine environment [6] has led to the development and validation of analytical methods for fish and mussels, the most common target species. An intercalibration program initiated in 2000 [7] should confirm the efficiency of the analytical methods recommended for monitoring applications. However, validation of methods, though basic and necessary to the data-interpretation process, is not sufficient. The full potential of using a biomarker-based monitoring approach as a tool for environmental assessment is often critically limited by a lack of integrated statistical analyses. Improvement is needed in interpreting the variations among several biomarkers used for environmental diagnosis, which means that a methodology capable of evaluating the global variations of a battery of biomarkers is required. With the exception of two recent studies [8,9], few practical methods have been developed to determine global indices of environmental quality that take chemical and biological criteria into account.

The present study describes the use of star plots, a simple multivariate graphic method, to allow visual integration of a set of early warning responses measured with biomarkers. This method, combined with the computation of the star plot area giving the integrated biomarker response, was applied to recent field surveys in two different areas. Data were collected from the European Biological Markers of Environmental Contamination in Marine Ecosystems (1994–1998) program developed along the German Baltic coasts and from the French Seine Aval program carried out in the Seine Estuary within the English Channel (1997–1999). Validated biomarkers, commonly applied in field study using fish and mussel [4–8], were analyzed in two different target species: the mussel (*Mytilus edulis*) in the Baltic sea and the flounder (*Platichthys flesus*) in the Seine Estuary. Glutathione-*S*-transferase EC 2.5.1.18 (GST), a phase II metabolic effect, and neurotoxical effects acetylcholinesterase (AChE) and oxidative stress catalase (CAT), EC 1.11.1.6, were analyzed in mussels. Metabolic effects (dependent activities of cytochrome P450), ethoxyresorufin-*O*-deethylase (EROD), AChE, and genotoxic effects DNA adducts (ADDU) were measured in flounder tissues.

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MATERIALS AND METHODS

Sampling protocol

Mytilus edulis was collected in the Baltic Sea by grab or skin divers down to a depth of 40 m along the German coast. Three surveys were undertaken in March 1995, October 1995, and November 1996. For each survey, three transections were plotted from the harbors of Kiel, Warnemünde, and Pennemünde. Two stations were sampled for each transect presumably following pollution gradients (Fig. 1A). Gills and digestive glands were dissected and immediately stored in liquid nitrogen prior to laboratory analysis. At each station, 25 individuals were collected and five pools of five individuals made. Pools of mussel tissues were stored at −20°C for chemical analyses.

Platichthys flesus juveniles (one and two years old) were trawl-fished along 200 km in the Seine Estuary following a presumed pollution gradient (Fig. 1B) in September 1997. Prior to laboratory analysis, muscle and liver were dissected and stored in liquid nitrogen, and pooled livers prepared for chemical analyses were kept at −20°C. Biomarkers were analyzed in individual organisms, whereas chemical measurements were obtained from pools of 10 individuals. Both chemical and

biochemical analyses were performed on individuals of the same cohort.

Biochemical measurements

Biochemical analysis in mussels was determined according to the methods described in [8]. Both AChE and GST activities in gills were measured in vitro in the postmitochondrial fraction (S9), using the method of [10] for AChE and [11] for GST. In digestive gland, GST and CAT activities were measured in the cytosolic fraction. Enzymatic activities were expressed in relation to protein concentration, according to the method of [12]. The AChE activities in the S9 fraction of muscle [13] and EROD activities in the S9 fraction of liver [14] were determined in the flounder. The DNA adducts were measured in flounder liver [15] and expressed as the number per 10^9 nucleotides. Chemical analysis of PAHs was performed in mussels [16], and PAHs and PCBs were analyzed in flounder liver [17]. Both AChE and GST were expressed in nmol/min/mg protein, while CAT was expressed in μmol/min/(mg protein). For the Seine Estuary survey, GST measured in digestive gland and gills is noted, respectively, as GSTdg and GSTg.

Data processing

Data were first standardized, that is, processed to allow direct visual comparison of sampling stations (or sampling dates, i.e., surveys at a given station), for a given biomarker or of biomarkers for a given sampling station. Star plots were then used to represent the scores (standardized data) of a given biomarker obtained at each sampling station or the scores of the various biomarkers used at a given station. In the latter case, the IBR is the star plot area. For a given station of a given survey, the successive data-processing steps to the final score were as follows. The mean estimate (X) was computed when individual results were available; otherwise, the value from the pooled sample at each sampling station was used. For each biomarker, the general mean (m) and standard deviation (s) of X were computed for all stations and/or surveys, depending on the comparisons to be made. X was standardized to obtain Y, where $Y = (X - m)/s$. Then we computed $Z = -Y$ or $Z = Y$ in the case of a biological effect corresponding, respectively, to inhibition or activation. The minimum value (Min) for all stations and/or surveys for each biomarker was obtained and added to Z. Finally, the score (S) was computed as $S = Z + |Min|$, where $S \geq 0$ and $|Min|$ is the absolute value.

Star plots were then used to display biomarker results. If a station is to be characterized by the set of biomarkers measured, a star plot radius coordinate represents the score of a given biomarker at a given station. Let S_i and S_{i+1} be two consecutive clockwise scores (radius coordinates) of a given star plot, and let n be the number of radii corresponding to the biomarkers used in the survey. Thus, the area A_i obtained by connecting the ith and the $(i+1)$th radius coordinates can be calculated as

$$A_i = \frac{S_i}{2}\sin\beta(S_i\cos\beta + S_{i+1}\sin\beta)$$

where

$$\beta = \text{Arctan}\left(\frac{S_{i+1}\sin\alpha}{S_i - S_{i+1}\cos\alpha}\right)$$

α is $2\Pi/n$ radians, and $S_{n+1} = S_1$. Hence, the total area corresponding to a given station sampled at a given date, that is, the integrated biomarker response (IBR), is

$$IBR^{date}_{station} = \sum_{i=1}^{n} A_i.$$

When S_i and S_{i+1} are zeros, β cannot be computed, and A_i should be set to 0. In the case of four biomarkers, that is, $n = 4$ and $\alpha = \pi/2$, A_i simply reduces to $A_i = (S_iS_{i+1})/2$. The EXCEL software (Microsoft, Redmond, WA, USA) was used for all calculations and star plots.

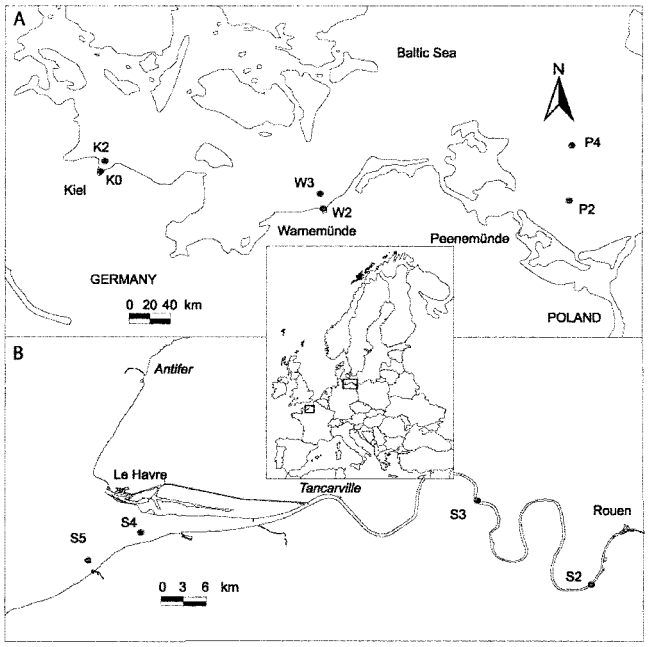

Fig. 1. Sampling station locations. (**A**) Baltic Sea; (**B**) Seine Estuary, English Channel.

Table 1. Mean estimates (from five pools of five individuals each), general mean (m) and standard deviation (s) for each biomarker (AChE = acetylcholinesterase; CAT = catalase; GST = glutathione-*S*-transferase measured in mussel digestive gland [suffix dg] and gills [suffix g]), and polycyclic aromatic hydrocarbon (PAH) concentration in Baltic sea, English Channel, mussel tissues

Survey	Station	AChE	CAT	GSTdg	GSTg	PAH
March 1995	K0	11.7	47.1	156.7	476.8	3,880
	K2	11.8	48.6	162.0	470.9	3,322
	W2	11.2	51.2	165.2	458.6	2,764
	W3	11.0	55.4	176.3	435.8	2,206
	P2	13.6	56.3	168.4	434.7	1,648
	P4	12.1	49.4	126.8	171.6	374
October 1995	K0	7.1	70.3	159.8	201.6	374
	K2	13.2	101.7	265.3	203.2	475
	W2	6.6	82.7	227.8	124.0	88
	W3	9.7	89.9	225.0	124.2	99
	P2	7.8	69.5	218.9	141.4	103
	P4	7.7	86.2	180.9	137.9	175
November 1996	K0	7.9	18.3	164.0	317.8	362
	K2	6.5	26.4	187.7	371.5	342
	W2	9.2	27.9	148.8	283.9	93
	W3	11.4	26.6	151.7	288.2	112
	P2	11.6	34.1	167.4	546.5	99
	P4	9.1	31.6	134.5	265.1	221
	m	10.0	54.1	177.1	303.0	
	s	2.3	24.7	35.8	141.2	

Table 2. Mean estimates (from 10 individuals), general mean (*m*) and standard deviation (*s*) for each biomarker (EROD = ethoxyresorufin-*O*-deethylase; AChE = acetylcholinesterase; ADDU = DNA adducts), and polycyclic aromatic hydrocarbon (PAH) and polychlorobiphenyl (PCB) concentrations in tissues of Seine Estuary, English Channel; flounder

	EROD	AChE	ADDU	PAH	PCB
S2	20.3	195.3	15.8	370	4,120
S3	19.2	295.0	10.3	417	2,734
S4	10.1	497.0	20.4	198	1,902
S5	19.6	377.0	7.3	78	5,633
m	17.3	341.1	13.4		
s	4.8	127.8	5.8		

Example: Computation of IBR at station W3 (Warnemünde Estuary) in March 1995

Biomarker mean estimates and the general mean and standard deviation for each biomarker are given in Tables 1 and 2 for the Baltic Sea and Seine Estuary surveys, respectively, together with contaminant data for the two sites. Results for the station W3 in March 1995 can be extracted from Table 1. Table 3 gives the successive results for data processing leading to the March 1995 station W3 scores (plotted in Fig. 2). The corresponding IBR value is then calculated as $IBR_{W3}^{03/95} = A_1 + A_2 + A_3 + A_4 = 4.68$, where $A_1 = (S_1S_2)/2 = (1.13 \cdot 1.50)/2 = 0.85$, $A_2 = 1.04$, $A_3 = 1.54$, and $A_4 = 1.25$.

RESULTS

Baltic Sea: Study on a large spatial scale

Collection of mussels in three Baltic Sea estuaries allowed comparison of biomarker variations in three sites with varied typology. As a result of the data standardization procedure described previously, star plots could readily be compared across stations and surveys (Fig. 3). The same biomarker GST could also be compared for different tissues (gills for GSTg and digestive gland for GSTdg). Multiple comparisons were made using two visual criteria; that is, the size and geometric form of the area polygons obtained with a combination of survey-station biomarkers were indicative of the spatiotemporal variability in exposure and the corresponding environmental stress. Possible comparisons and corresponding examples are given thereafter.

Comparison of a single biomarker for different surveys. The AChE areas showed lower inhibition in March than for the October 1995 and November 1996 surveys. The CAT activities showed areas with a similar pattern between March and October 1995, but the largest area obtained in October indicated higher protein expression. A very low CAT value for each station resulted in the quite reduced area obtained in November 1996.

Table 3. Example of score calculations: station W3 in the Baltic Sea, English Channel; March 1995 (AChE = acetylcholinesterase; CAT = catalase; GST = glutathione-*S*-transferase measured in mussel digestive gland [suffix dg] and gills [suffix g])

	AChE	CAT	GSTdg	GSTg
X	11.0	55.4	176.3	435.8
m	10.0	54.1	177.1	303.0
s	2.3	24.7	35.8	141.2
Y	0.45	0.05	−0.02	0.94
Z	−0.45	0.05	−0.02	0.94
Min	−1.58	−1.45	−1.41	−1.27
S	1.13	1.50	1.39	2.21

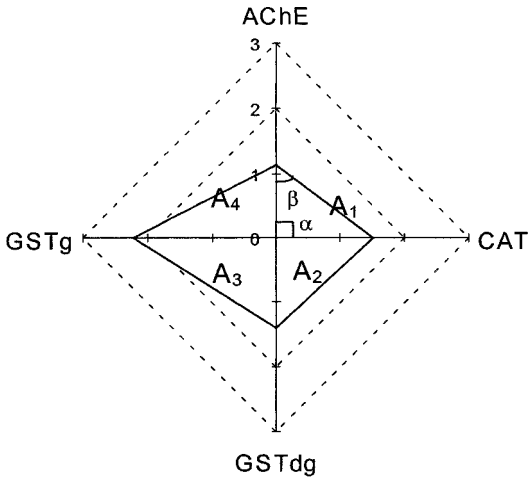

Fig. 2. Example of a biomarker star plot for station W3 (Warnemünde) in March 1995 (AChE = acetylcholinesterase; CAT = catalase; GST = glutathione-*S*-transferase measured in mussel digestive gland [suffix dg] and gills [suffix g]).

Comparison of a single station for different biomarkers and surveys. The values in October 1995 and November 1996 at station K2 showed contrary results for AChE but similar

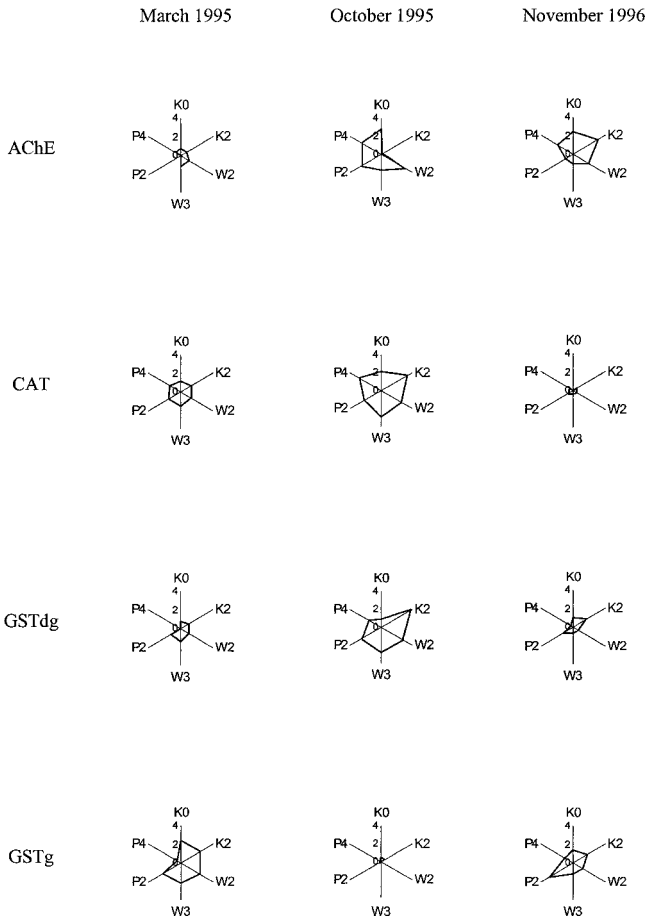

Fig. 3. Station star plots for each biomarker and survey in the Baltic Sea, English Channel (AChE = acetylcholinesterase; CAT = catalase; GST = glutathione-*S*-transferase measured in mussel digestive gland [suffix dg] and gills [suffix g]).

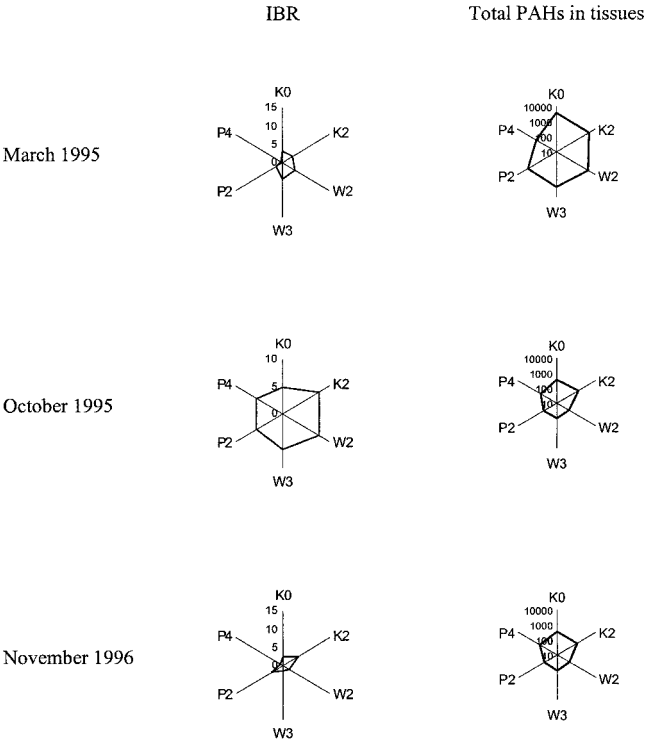

Fig. 4. Integrated biomarker response (IBR) and polycyclic aromatic hydrocarbon (PAH) star plots for the Baltic Sea, English Channel; PAH is expressed in μg/kg (dry wt).

trends for GSTdg. Station P4 showed lower values for GST in March 1995 compared to the other stations, while it presents comparable results for AChE and CAT in October 1995.

Comparison of a single survey for different biomarkers and stations. In March 1995, the values for GSTdg and GSTg showed rather similar between-station patterns. The same observation applies to a lesser extent to the comparison between AChE and GST across stations for the same survey.

All these combinations can provide visual spatiotemporal distinctions between AChE, CAT, and GST expression in mussels collected at the same station at different periods. Our ultimate goal was to integrate all this information into a global indicator. In Figure 4, the radius coordinates in left star plots are IBR values (Table 4), and the corresponding areas (Σ in Table 4) are intended to combine biomarker data at a higher level of integration than that achieved in Figure 3. These areas can be compared visually with the total PAH areas measured in tissues in log scale and selected as an indicator of chemical exposure. Area star plots respect the general patterns of data variability between stations and periods (surveys) and include all the combinations described previously (Fig. 3). This integrated graphic tool was constructed using a panel of biomarkers characterizing the same level of early biological change. The interpretation of area variations between surveys takes various biomarkers with the same biological significance

Table 4. Integrated biomarker response (IBR) for each station and survey; Σ = summed IBRs: $\Sigma = \Sigma_{k=1}^{6} IBR_k$

Survey	K0	K2	W2	W3	P2	P4	Σ
March 1995	3.0	3.2	3.9	4.7	2.2	0.4	17.4
October 1995	5.0	7.9	7.7	6.4	5.6	5.7	38.2
November 1996	2.4	5.0	1.9	1.3	3.7	0.7	15.0

Table 5. Scores (EROD = ethoxyresorufin-*O*-deethylase; AChE = acetylcholinesterase; ADDU = DNA adducts) and integrated biomarker response (IBR) for each station of the Seine Estuary, English Channel

Station	EROD	AChE	ADDU	IBR
S2	2.12	2.36	1.47	4.61
S3	1.88	1.58	0.52	1.91
S4	0	0	2.25	0
S5	1.97	0.94	0	0.67

into account. Integrated biomarker response values indicate that the highest protein expression occurred in October 1995 (Fig. 4). The March 1995 and November 1996 star plots, showing lower IBR values, are indicative of low mussel sensitivity as compared to October 1995. The panel of biomarkers used to compute IBR values indicates that mussel populations were more stressed at stations K2 during the October 1995 and November 1996 surveys, while P4 appears to be the less impacted station in March 1995 and November 1996.

The biological significance of the marker (protein activities) and the severity of the biological effect need to be related jointly to the exposure or dose level [18]. The IBR can be applied as a global index of environmental stress, but the classical challenge in ecotoxicology is to relate biological effects to chemical contamination levels in order to match a cause with an effect. To this end, star plots can be useful when the level of chemical contaminants is known. Figure 4 shows that the highest level of chemical exposure was obtained in October 1995. Except for station W2, the IBR area contour is very similar to that of PAH, indicating a higher level of contamination in the October and November surveys. Comparison of IBR and PAH star plots in March 1995 and November 1996 indicates that PAHs were probably not the main source of effects since IBR values differed, whereas PAH levels were quite similar. In general, good visual concordance does not appear to exist between IBR and PAH star plots.

Seine Estuary: Study on a local scale

Scores from the 1997 survey, with flounder as biological material, in the Seine Estuary (Table 5) were used as coordinates on star plot radii in order to represent biomarker data

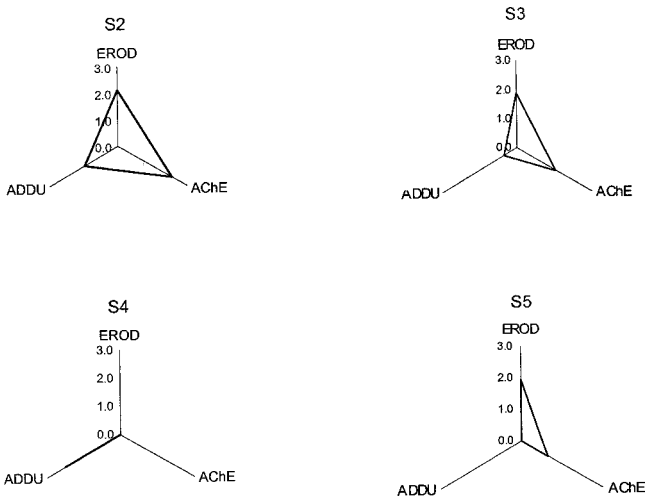

Fig. 5. Biomarker star plots for each station in the Seine Estuary, English Channel; EROD = ethoxyresorufin-*O*-deethylase; AChE = acetylcholinesterase; ADDU = DNA adducts.

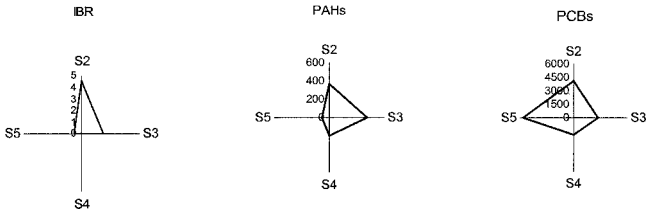

Fig. 6. Integrated biomarker response (IBR), polycyclic aromatic hydrocarbon (PAH), and polychlorobiphenyls (PCB) star plots for the Seine Estuary; PAH and PCB levels are expressed in μg/kg (dry wt).

graphically (Figs. 5 and 6). Corresponding areas, that is, IBR values (Table 5 and Fig. 6), for stations S4 and S5 (respectively, 0 and 0.67) located downstream are lower than those obtained at stations S3 (1.91) and S2 (4.61). The S2 would appear as the most impacted station by toxic effects, caused by a mixture of contaminants including PAH and PCB, the two measured organic contaminants. Both S2 and S3 show similar patterns for the three selected biomarkers, which is obviously not the case when S4 and S5 patterns are compared (Fig. 5). Both S4 and S5 (see locations on Fig. 1B) are subject to different hydrodynamic conditions [19]. The S4 is located in the highly turbid outflow of the Seine Estuary, while S5 lies downstream in Seine Bay, where high concentrations of PCBs can be found in the Seine River plume [20]. The S4 shows a high DNA adduct score (2.25) relative to the other stations but minimum values and null scores for ethoxyresoryfin-*O*-deethyolase (EROD) and AChE. Conversely, S5 is characterized by the lowest DNA adducts score but shows EROD and AChE scores similar to those of S3.

Figure 6 shows a visual reasonable agreement between the PAH and PCB concentration gradient measured in flounder and the upstream-to-downstream IBR variation (see values in Table 5). The PAH and PCB levels observed at S4 do not explain why the lowest IBR value was computed for this station. The very high PCB levels found at S5 do not seem to have influenced biomarker responses, especially DNA adducts, and the IBR value.

DISCUSSION

The application of IBR on a large spatial scale to Baltic Sea surveys, using data collected for different stations sampled several times at different sites, revealed the spatiotemporal interactions described previously. The IBR was also applied to more local surveys (the Seine Estuary) that involved sampling along a pollution gradient during a given period, using a smaller panel of biomarkers. In the Baltic Sea and Seine Estuary areas, contamination is known to be very diffuse and complex, that is, not characterized by a family of contaminants. Thus, the relation of individual biological effects to just a few chemical contaminants, even major and persistent ones such as PAHs or PCBs, would be a very difficult, if not impossible, task. A few measurements of one or two classes of contaminants are not adequate to account for biological effects in the Seine Estuary and in the Baltic Sea. For example, flounder with empty stomachs were collected at S4 [21], the station showing the lowest biomarker values and contamination levels relative to the other Seine Estuary stations. Thus, flounder feeding status could account for the low early warning signal expressed by EROD and AChE activities. The DNA adducts induced by adverse effects could be interpreted in relation to chronic exposure of flounder during their existence in S2 and S3. Most flounder collected at station S4 were juveniles (one or two years old) originating from the upstream estuary [21], while flounder collected at station S5 were two-year-old juveniles and three-year-old adults. As these fish lived in Seine Bay but not in the Seine Estuary, they were not exposed to contaminants to the same extent as juveniles living in the center of the estuary. Yet flounder collected in Seine Bay are influenced by the contaminated Seine plume. These physiological considerations could explain why early responses (EROD and AChE enzymatic activities) were expressed in S5 and not activated in S4, where DNA adducts could also be less sensitive to expected rapid physiological changes. Because of its location, S4 is subject to great hydrodynamic disturbances [21], especially marked salinity variations that certainly affect chemical toxicity and osmoregulation in fish [22].

A pool of available biomarkers would provide a more valid basis for interpretation of ecotoxicological surveys [8,9], allowing information to be summarized in the form of a multivariate data set. For instance, a global biomarker index was constructed on the basis of a large data set for Baltic Sea results in order to summarize standardized individual biomarker responses and classify sites [8]. The highest index, as globally in our study, was indicative of the most impacted area (the Kiel Estuary in the Baltic Sea). In another statistical procedure, linear discriminant analysis was used to classify sites in a large river–reservoir [9], allowing identification of the variables (e.g., EROD) most affecting discrimination. The main objective of our study was to develop an easy-to-use graphic means of summarizing available data. As in previous studies [8,9], it was assumed that a global response, in terms of a multimarker approach, is characteristic of environmental stress. Star plots, among other methods, are known to be adequate graphic tools for synthesizing multidimensional information. This type of plot not only serves as a visual representation but can also be used to indicate a global or integrated biomarker response on the basis of inner areas delimited by a broken line joining radius coordinates.

However, the relation between star plot features and corresponding environmental areas is highly dependent on a number of a priori choices. Our selected biomarkers correspond to the objectives of ecotoxicological study, as either early warning signals (EROD, GST, CAT, AChE enzymatic activities) or adverse effects (DNA adducts) of chemical exposure. As different biomarkers respond to different stressors, an advantage exists in using a set to assess the condition of target species and the quality of the environment. Obviously, successful application of IBR depends on a relevant choice of biomarkers in relation to the specific objectives of research or monitoring and the characteristics of the field site.

Interestingly, the star plot area in this study was not invariant with respect to biomarker position. If the sum of score values (radius coordinates) rather than areas had been considered, the graphics would have lost much of their interest. Another solution would have been to consider all possible permutations of biomarker position on the star plot, which would have indicated all possible areas from which a mean area, or IBR, could be estimated. However, a more pragmatic approach was to decide a priori on an order for a given study. Thus, two biomarkers closely related in terms of pure biological effect (EROD and GST activities analyzed, respectively, for determination of metabolic phases I and II) should be adjacent on the star plot. More weight can then be given to parallel results; that is, if two consecutive radii present high score

values, the area will not be negligible for contamination. For example, if GST results for digestive gland (GSTdg) and gills (GSTg) were not adjacent in November 1996 for station K2, the area score would be close to zero, which would not be consistent for a station showing coherent responses for the same biomarker measured in different parts of the animal. On the other hand, a single biomarker showing a nonzero score when all other biomarkers have zero scores would correspond to a null area, as in the case of the star plot for station S4 in the Seine Estuary (Fig. 5). In fact, some objective reason should exist for a biomarker position on the star plot. One possibility would be to order biomarker according to their ability to discriminate nonimpacted from impacted sites when demonstrated by previous studies using the same biological materials. Areas are also sensitive to the type of data processing used to obtain scores. The robustness of scores, as computed in this paper relative to other linear transformations, has been investigated for the Baltic Sea data set [23]. In this study, each biomarker estimate for a given station and a given survey was expressed as a percentage of the maximum estimated value across stations and surveys. Both the mean and the median were considered as estimators. No significant visual changes were apparent, and the scores seemed to provide a better indication of the very high survey-station interactions revealed as well by an analysis of variance (results not shown). Thus, the processing of information was certainly data-set sensitive, but to a limited extent because of the standardization procedure used. Using the percentage approach raises the question of defining a reference or background values and their natural variability. We are unfortunately lacking this information for most biomarkers. Once it is available and validated, it should be incorporated in data analysis, either exploratory or using statistical models. This addresses the more general issue of using the right scaling system, that is, a numeric way of helping in discriminating between natural variation and an actual anthropic impact. Taking into account the within-station variability was considered in [23] by weighing the previously mentioned percentages by the sampling standard deviation at a given station. This led to abnormally inflate the percentage values in case of a very homogeneous batch and to distort dramatically star plot visual aspect and then was not considered.

Environmental assessment is guided by societal concerns. The full potential of using a biomarker-based monitoring approach as a tool for environmental assessment is limited most critically by a lack of integrated statistical analyses. Biomarkers can be used as sensitive and cost-effective general indicators or as early signals of the presence of environmental stressors. Results obtained for the two field studies analyzed here suggest that the selection of an appropriate battery of biomarkers can avoid false-negative responses obtained with a single biomarker (showing no impact when an actual anthropic impact exists) and that careful selection of an appropriate combination of biomarkers can provide information about global adverse environmental effects. In the case of diffuse contamination, it seems more realistic to choose a battery according to a gradient of biomarkers extending from indices of exposure through effects of increasing severity to the endpoint. Biomarkers can provide informative data to corroborate and extend other indicators, such as chemical contaminants, physiological status, parasites, infectious agents, and many environmental factors. As habitat is a major determinant in exposure and its effects, the significance of a biomarker pool should be interpreted within the context of site typology and environmental factors. However, the interpretation of biomarker responses depends not only on biological and typological considerations but also on the socially determined characteristics of environmental quality appropriate to a particular ecosystem [24].

An obvious discrepancy exists between the well-known complexity of biomarker responses and the research of some heuristic, here graphic, methods to interpret molecular biomarker data, the limitations of which should be recognized: The danger exists of oversimplifying the interpretation of the data, especially by using absolute values without reference to within-station variability and other confounding factors. Thus, it should be stressed that our method should be considered as an exploratory tool, a first step in data processing, and should be combined with further going statistical analysis. As a final warning, it should also be emphasized that this method is appropriate only if an a priori justification exists for each biomarker used and if the physiological significance of the changes to each biomarker is well known.

CONCLUSION

Star plots were used in this study as one possible tool for visualization of biological effects. Data were processed to allow computation of an integrated biomarker response for all biomarkers measured at a given station. The IBRs could then be compared for different stations and surveys. This integrated approach takes the variations contained in the data set into account. Thus, star plots can be used as a useful graphic aid for exploratory analysis of data in a multibiomarker approach. As a compact means of representing data, these plots and IBRs could also be useful to managers and decision makers, for example, on maps.

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