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A novel "Integrated Biomarker Response" calculation based on reference deviation concept

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Abstract:

Multi-biomarker approaches are used to assess ecosystem health and identify impacts of environmental stress on organisms. However, exploration of large datasets by environmental managers represents a major challenge for regulatory application of this tool. Several integrative tools were developed to summarize biomarker responses. The aim of the present paper is to update calculation of the "Integrated Biological Response" (IBR) described by Beliaeff and Burgeot (Environ Toxicol Chem 21:1316–1322, 2002) to avoid weaknesses of this integrative tool. In the present paper, a novel index named "Integrated Biological Responses version 2" based on the reference deviation concept is presented. It allows a clear discrimination of sampling sites as for the IBR, but several differences are observed for contaminated sites according to up- and downregulation of biomarker responses. This novel tool could be used to integrate multi-biomarker responses not only in large-scale monitoring but also in upstream/downstream investigations.

Keywords: Biomarkers ; Data analysis ; Graphic tools ; Three-spined stickleback

1. Introduction

Recent publications argue for the application of biomarkers particularly in monitoring regimes defined by the European Water Framework Directive (WFD, Directive 2000/60/EC; Sanchez and Porcher 2009) and the Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC; Lyons et al. 2010). However, difficulties to analyze and integrate biomarker responses by environmental managers, decision makers and others non-specialists represent a major challenge to large scale deployment of these effect-based monitoring tools (Chèvre et al. 2003; Sanchez et al. 2011). To bridge this gap, several authors have developed integrative tools able to summarize response of a set of biomarkers in a single value and/or a graph (Narbonne et al. 1999; Beliaeff and Burgeot 2002; Chèvre et al. 2003; Broeg et al. 2005; Hagger et al. 2008). Among these indexes, the "Integrated Biological Responses" (IBR) described by Beliaeff and Burgeot (2002) that is the area of a star plot of standardized biomarker responses, is one of the most used in field and laboratory studies (Damiens et al. 2007; Arzate-Cardenas and Martinez-Jeronimo 2011; Serafim et al. 2011). Indeed, IBR can be calculated without specific software and combine a mathematical value and a graphical result to conserve specific response of investigated biomarkers. However, IBR suffers from two majors weak points, i) the IBR result is strongly dependent to the arrangement of the biomarkers on the star plot and ii) only up or down regulation can be considered. Recent evidences highlight that many biomarkers can be induced and inhibited according to organism exposure. For example, acetylcholinesterase activity, a well-known neurotoxicity biomarker historically described as inhibited by organophosphorous and carbamates (Payne et al. 1996) can be also induced by heavy metals (Barillet et al. 2007). Similarly, EROD activity, a biomarker of dioxin-like exposure is known to be induced by these pollutants but several studies report inhibition by environmental pollutants such as estrogens (Kirby et al. 2007). These complex biological responses are also reported in field studies and they must be considered in analysis of biomarker responses.

The aim of this work was to modify IBR calculation to avoid mistakes due to previously identified weak points. For this purpose, the concept of reference deviation based on the deviation between a disturbed and an undisturbed states, was used. This philosophy drives the assessment of water body ecological status described by the WFD and appears as a valuable argument to support the integration of biomarkers in the regulatory environmental monitoring programs. To evaluate the interest of this novel IBR calculation named "Integrated Biological Responses version 2" (IBRv2), it was applied in a case study based on the assessment of a set of biomarker responses in three-spined stickleback (*Gasterosteus aculeatus* L.) from several streams located in the North of France and characterized by various contamination levels. Briefly, a set of biochemical biomarkers including xenobiotic biotransformation activities in the liver (7-ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GST)), oxidative stress parameters in the liver (glutathione peroxidase (GPx), total glutathione content (GSH) and lipid peroxidation (TBARS)), neurotoxicity indicator (muscular acetylcholinesterase (AChE)) and endocrine disruption biomarkers (circulating vitellogenin (VTG) and spigginin the kidney (SPG)), was measured in three-spined sticklebacks from freshwater sites characterized by various levels of contamination (Sanchez et al., 2008a). Moreover, a basal line was established for the same biomarkers in both breeding and non-breeding fish living in an uncontaminated stream (Sanchez et al., 2008b).

2. Data processing

For the IBRv2 calculation, individual biomarker data (X_i) is compared to a mean reference data (X_0) and a log-transformation is applied to reduce variance.

$Y_i = \log(X_i/X_0)$ Equation 1

In a next step, the general mean (μ) and standard deviation (s) of Y_i were computed as previously described by Beliaeff and Burgeot (2002) and Y_i is standardized.

$Z_i = (Y_i-\mu)/\sigma$ Equation 2

To create a basal line centered on 0 and to represent biomarker variation according to this basal line, mean of standardized biomarker response (Z_i) and mean of reference biomarker data (Z_0) are used to define a biomarker deviation index (A).

$A = Z_i-Z_0$ Equation 3

To obtain an integrated multi-biomarker response named "Integrated Biological Responses version 2" (IBRv2), absolute value of A parameters calculated for each biomarker in each investigated site are summed.

$IBRv2 = \sum|A|$ Equation 4

For a single site, A parameters are reported in a star-plot to represent reference deviation of each investigated biomarker (Figure 1). The area up to 0 reflects biomarker induction and the area down to 0 indicates a biomarker inhibition.

IBR values were also calculated using the method previously published by Beliaeff and Burgeot (2002) and results of both methods were compared using linear regression.

3. Results

Results of IBRv2 are presented in the figure 2. Calculated values are between 1.1 and 10.3. More accurately, a clear discrimination of sampling sites according to environmental pressure status is observed. Low contaminated sites (VDV, LEZ and VDF) presented an IBRv2 values between 1.1 and 3.7 and high contaminated sites (VIL, RHO, ESC and REV) exhibited values between 5.8 and 10.3.

These results are compared with those of the original IBR method (Table 2) that are between 0.3 and 1.2 for low contaminated sites and between 3.3 and 8.6 for heavily contaminated rivers. Both models show similar pattern as indicated by a significant positive correlation between IBR and IBRv2 values is observed ($p<0.05$, $r^2=0.874$). It can separate the low and high impact sites. However, in this case study, intermediate sites (VIL and RHO) show a different result explained by EROD inhibition in VIL.

4. Discussion

The present study was designed to modify the Integrated Biological Response (IBR) previously developed by Beliaeff and Burgeot (2002) with two major objectives: i) to remove the dependency to the arrangement of the biomarkers on the star plot and ii) to discriminate induction and inhibition for each biomarker. On line with these objectives, a novel IBR calculation and representation were developed and named IBR version 2 (IBRv2). This novel version of IBR is based on the principle of reference deviation. For this purpose, a set of basal values was computed with the biomarker values measured in investigated sites. As indicated in the present paper, basal line can be established using reference values previously determined for a set of biomarkers measured in a sentinel fish species (Sanchez et al. 2008b). In this context, reference selection represents a major challenge for an accurate interpretation of integrated multi-biomarker responses. As previously described, application of IBRv2 in a large scale requires the establishment of a robust basal value for each selected biomarker. Compared to the IBR, it is a clear limit of this tool while basal lines are not available for many fish species. Indeed, basal values are dependent to sentinel species but also to various biotic and environmental factors such as sampling season or reproductive and nutritional status. Also, the novel IBR calculation cannot be considered as a universal tool for analysis of biomarker responses in large monitoring programs and requires complementary studies to determine basal values in relevant fish species (Viarengo et al. 2007). However, IBRv2 can be used without species limitation in upstream/downstream studies. In this case, biomarker values measured in organisms from the upstream site could be used to define basal line and responses recorded in downstream sites would be compared with upstream data integrating disturbances due to upstream environmental pressures (Sanchez et al., 2010). Similarly, a low contaminated site sampled in the same time could be used as reference in a large monitoring program but in this case, IBRv2 cannot be determined for this reference site. According to the reference deviation concept, IBRv2 values represent a sum of deviations between reference and measured values and not an area as described for the original IBR. Also, the result is not dependent to the arrangement of biomarkers in the star plot. Due to this modification, the range of result variation is different between both IBR versions (0 to 32 for IBR and 0 to 24 for IBR^2) and the site classification appears as modified for any sites such as VIL and RHO. This result is probably explained by the response profile of GSH content.

A noteworthy fact is that this novel multi-biomarker index is able to discriminate responses in low (VDV, LEZ and VDF) and high (VIL, RHO, ESC and REV) contaminated sites. Calculated IBR^2 value allows a good discrimination of sampling sites based on multi-biomarker responses with values between 1.1 and 3.7 for low contaminated sites and between 5.8 and 10.3 for heavily contaminated sites. As proposed by Beliaeff and Burgeot (2002), the IBRv2 value is associated to a star plot presenting the specific biomarker responses for each sampling site. Result examination confirms the real interest of this novel IBR calculation. Several biomarkers exhibit a response that can be induced or inhibited according to sampling site. This is true for EROD activity that is inhibited in fish from VIL site probably due to pesticide exposure (Table 1). A similar profile is also recorded for oxidative stress biomarkers and particularly for total GSH content. Sticklebacks from VIL and REV contaminated sites exhibited an increase of hepatic GSH and fish from RHO and ESC sites are characterized by a significant decrease of GSH content probably linked to contamination by heavy metals (Figure 2; Sanchez et al., 2008a).

To summarize, IBR calculation is modified to avoid two weaknesses of this tools. This modification is based on the application of the reference deviation concept widely used in ecological monitoring described by the WFD. Also, IBRv2 appears as a WFD compatible tool usable in European monitoring programs. However, as previously indicated, further studies are required to determine basal values of biomarkers for relevant sentinel fish species as previously defined (Sanchez et al., 2010) but also to define evaluation assessment criteria (EAC) et background assessment criteria (BAC) (ICES, 2011).

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Tables

Table 1. Multi-biomarker responses measured for basal line (REF) and investigated sites. <LOD : below the detection limit.

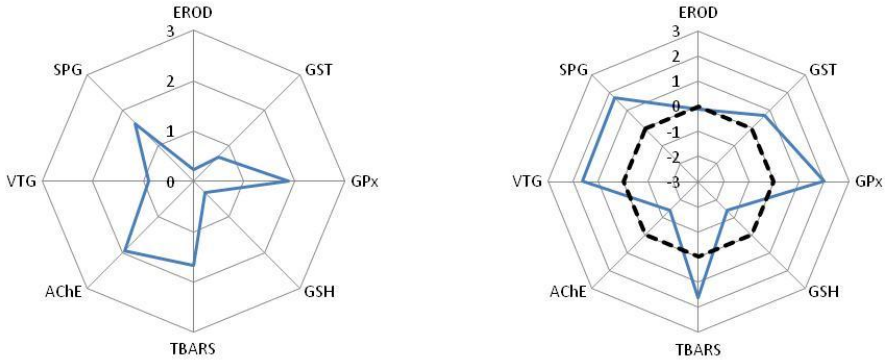
Sites	EROD	GST	GPx	GSH	TBARS	AChE	VTG	SP
	pmol/min/mg	U/g prot.	U/g prot.	µmol/g prot.	nmol/g prot.	U/mg prot.	µg/L	U/kidney
REF	6.2 ± 2.1	1.236 ± 396	95 ± 35	32.7 ± 15.9	49.5 ± 22	93 ± 26	<LOD	<LOD
REV	30.6±21.0	3197±890	67.2±45.9	40.2±16.0	162±86	41±11	21.8±17.2	76.5±25.8
LEZ	13.0±8.8	1662±278	83.2±34.1	26.1±11.1	54±31	90±15	<LOD	47.1±12.2
ESC	31.3±17.6	1433±416	85.8±46.6	7.0±4.8	126±59	67±11	213.4±212.7	159.7±57.9
RHO	4.8±4.1	1509±426	449.4±156.1	10.9±7.7	117±46	50±11	70.3±39.1	111.2±129.2
VIL	2.1±3.2	1585±222	552.2±170.0	38.8±15.3	100±50	32±9	<LOD	40.7±18.5
VDV	7.2±6.3	1024±167	103.6±60.1	25.2±11.7	66±31	86±8	<LOD	39.2±18.5
VDF	16.1±17.3	1386±256	108.9±68.6	28.9±9.9	77±30	102±18	<LOD	89.0±64.2

Table 2. Comparison between IBR and IBRv2 values calculated for a set of biomarkers measured in three-spined sticklebacks from rivers of the North of France (Sanchez et al. 2008).

Site	Contamination	IBR	IBRv2
VDV	Low	0.3	1.1
LEZ	Low	0.4	2.3
VDF	Low	1.2	3.7
VIL	High	5.0	5.8
RHO	High	3.3	7.8
ESC	High	7.6	9.4
REV	High	8.6	10.3

Figures

Figure 1. Theoretical comparison of “Integrated Biological Responses” (IBR) star-plot with the novel version developed in the present work and named “Integrated Biological Responses version 2” (IBRv2).



IBR = 3.3 **IBRv2 = 7.8**

Figure 2. “Integrated Biological Responses version 2” (IBRv2) values and associated star-plots for three-spined stickleback (*Gasterosteus aculeatus*) from 7 sampling sites located in the North of France. Site description and measured biomarker responses are presented by Sanchez et al. (2008a).

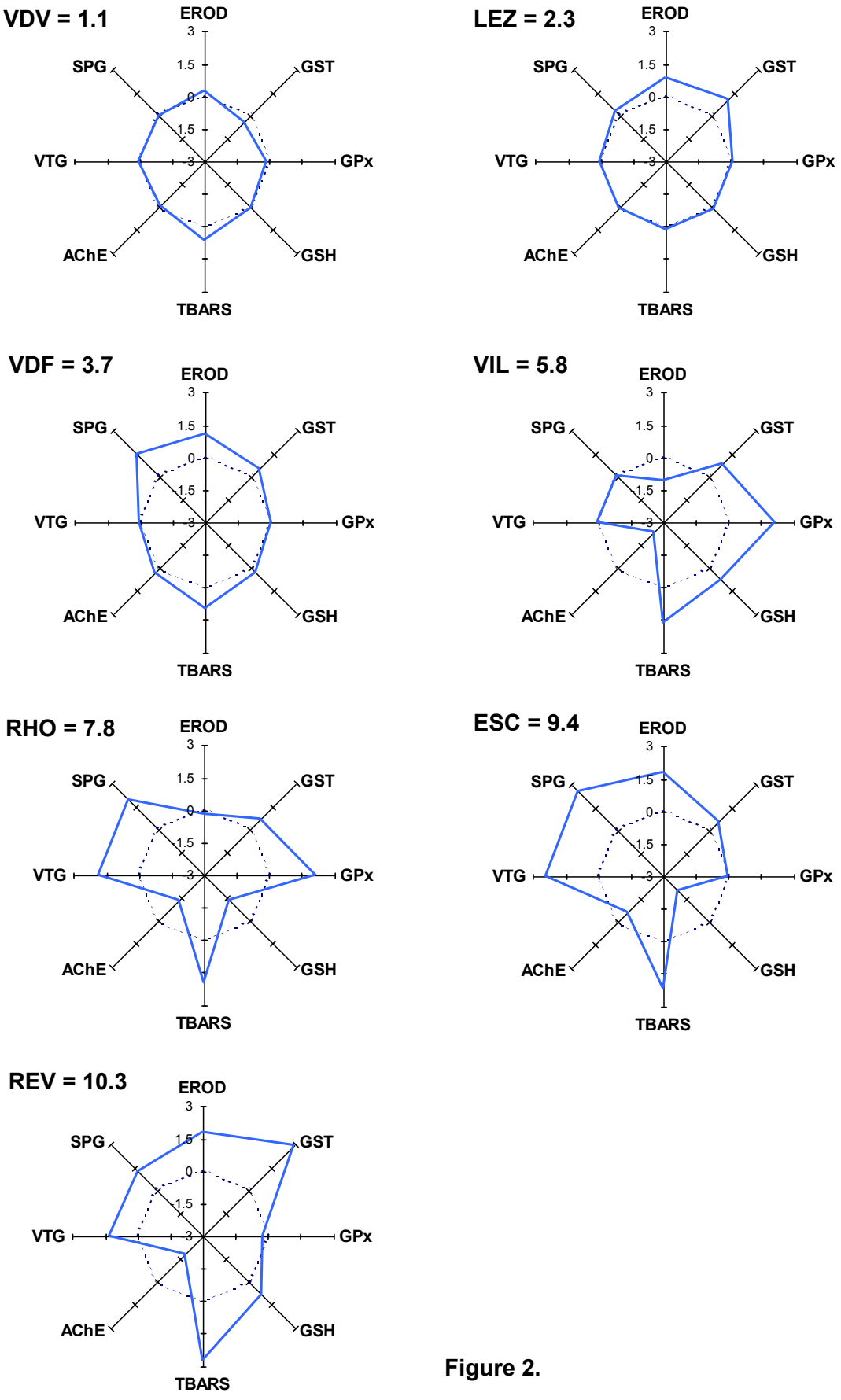

VDV = 1.1
LEZ = 2.3
VDF = 3.7
VIL = 5.8
RHO = 7.8
ESC = 9.4
REV = 10.3
EROD
GST
GPx
GSH
TBARS
AChE
VTG
SPG
3
1.5
0
1.5
3

Figure 2.