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Can biomarkers measure the environmental health of estuaries?

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Abstract

The European Commission's Water Framework Directive (WFD) has emphasised the need for biological-effects end points that can be used to classify the ecological health of aquatic ecosystems. Accepting the premise that a healthy ecosystem is reflected in the 'health' of the constituent biota, we have advocated the application of using a suite of biomarkers (a biological response that signals exposure to and/or adverse effects of potential chemical, physical or biological hazards) to measure environmental health through an integrated assessment of the health status of individual organisms (and thereby the ecosystem). As 95% of animal species are invertebrates (and include commercially-exploited species), we propose that it is reasonable to use them as surrogates of all coastal biota. We have developed a tool box of biomarkers (including molecular, cellular, physiological and behavioural endpoints) for a range of invertebrate species inhabiting different estuaries around the UK coastline. We have used the results in a pragmatic, weight-of-evidence, holistic approach to devise a Biomarker Response Index (BRI) (a relative set of criteria based upon a 'traffic-light system') to give a measure of the general health status of invertebrates. In this presentation, we report the underlying basis for the BRI approach and discuss the results from *Mytilus edulis* collected from different transitional water bodies (estuaries) along the southern coastline of the UK whose risks of failing the WFD has been classified with regards to point-source pollution. In eight of the ten transitional water bodies, mussels were healthier than predicted based on the risk classification for point source pollution from that particular estuary. Mussels from the other two water bodies showed a similar health status to that

predicted by the risk classification. Present results indicate that the BRI offers a potential measure of organism health that can be used in monitoring under the WFD to reduce uncertainty in defining risk classification and to provide evidence of impact.

Introduction

The protection, improvement and sustainable use of Europe's water supplies is a major goal of current European Water Policy. A key piece of legislation introduced to transform the way in which this is achieved across all European states is the European Union's Water Framework Directive (WFD) (2000/60/EC). The WFD is considered to be pioneering because it champions the ecosystem approach, that is, the integrated consideration of chemical and ecological status in defining water quality [with ecological status being an incorporation of biological, physico-chemical and hydro-morphological elements (Vincent, et al., 2002)]. The legislation requires the periodic assessment of all water bodies, including rivers, lakes, estuaries, coastal waters and groundwaters. Water bodies are then assigned to a classification system that grades their deviation from normality (high, good, moderate, poor, bad), with normality defined as a site with no, or very minor disturbance from human activities. The overall aim of the WFD is to achieve 'good' status for all waters by 2015.

The emphasis given by the WFD to ecological elements has been widely welcomed by scientists and managers, as it focuses management effort on the identification of impacted biota. However, ecosystems are complex and fluctuating entities, and the development of adequate ecological assessment and classification systems is one of the most technically challenging aspects of the legislation (EU, 2003). There are large sources of uncertainty associated with the monitoring of biological and physico-chemical elements, arising not only from the inherent variability of aquatic ecosystems, but also from the sampling process itself and the statistical definitions of quality boundaries (Carstensen, 2007; EU, 2003) and these have been the subject of considerable debate and study (summarised by Devlin et al., 2007). A key problem in constructing classification systems for biological parameters with confidence is in developing an understanding of spatial and temporal variability; for example, it may take many years for alterations in community structure to become apparent, leaving little opportunity for the timely remedial action required by the WFD to maintain water quality, and some considerable uncertainty in the definition of quality boundaries (Beltran, 2002, Carstensen, 2007).

The inclusion of whole organism assays and of biomarkers within the context of the WFD provides a potential means of tackling this uncertainty. Biomarkers, defined here as functional measures of exposure to stressors expressed at the sub-organismal, physiological or behavioural level (McCarthy and Munkittrick, 1996), have been used extensively as indicators of biological response in laboratory studies and in relation to individual contaminants or stressors (Huggett et al., 1992; Wilson and Suk, 2002). Their inclusion in field surveys of contaminated sites is increasingly being reported, where they offer the potential to assess the general health of organisms inhabiting contaminated ecosystems (Galloway et al., 2002, 2004a,b). Of particular relevance to the WFD, biomarkers are being developed that are inexpensive, easy to apply and provide a quick response, allowing rapid and cost-effective decision making (Galloway et al., 2004a,b; Allan et al., 2006). The issue of ecological relevance has been tackled by developing evidence-based approaches to monitor the risk to key components of the ecosystem, assuming that monitoring the adverse consequences for species occupying critical trophic levels provides insight into the integrity of the ecosystem as a whole (Galloway et al., 2004a,b, 2006).

Yet issues of how to construct classification systems and set quality boundaries for the use of biomarkers remain. To comply with the needs of a classification system such as the WFD, an index is required that can simplify the complex biological alterations measured by (often multiple) biomarkers into a single, predefined quality class. This leaves open for debate issues including the choice of reference and sampling site, quality control and replication, seasonal and temporal variability of the test species and the whole organism significance of small alterations to molecular receptors. Many alternative suggestions have so far been put forward including those that use simple numerical grading indices constructed using univariate or multivariate methodologies, ranking systems and discrimination methods (Adams et al., 1993; Narbonne et al., 1999; Blaise et al., 2002; Chevre et al., 2003a; Aarab et al., 2004; Bodin et al., 2004; Broeg et al., 2005). For example, Adams et al. (1993) described a rapid health assessment index (HAI) for fish, based on field necropsy and histological changes, in which arbitrary-numerical values were assigned to each abnormal condition based on the severity or damage incurred by each organ or tissue type, and then summed to produce an HAI value for each fish. Aarab et al. (2004) described a scoring system, again for fish, in which a multi-marker pollution index was constructed by combining the discriminatory power (calculated from discriminant analysis of field survey results) and mean value of each biomarker. In this case, biomarkers were chosen to reflect the specific molecular mechanism of action of particular toxicants, whereas in the 'bioeffect assessment index' proposed by Broeg et al. (2005), only biomarkers of general toxicity were included, to screen for disturbance across different levels of biological organisation, with the intention of highlighting impacted areas for further study.

Whilst these studies highlight the potential usefulness of a quantitative biomarker index in classifying biological quality, there has, until now, been little opportunity to compare how such an index might meet the specific needs of the WFD and accompanying legislation. The Directive's provision to protect the water environment from especially dangerous chemical substances is taken forward under the Dangerous Substances Directive (DSD, 76/464/EEC). To meet this legislation, the Environment Agency of England and Wales is required to classify every transitional water body and coastal region in England and Wales for its risk of failing the WFD due to point source pollution (metals and dangerous substances). The classification combines knowledge of permitted discharges, the presence of alien species and specified hydro-morphological factors (www.environmentagency.gov.uk). Each water body then receives a quality rating to one of four classes; at risk, probably at risk, probably not at risk and not at risk. The inclusion of biological data to quantify the extent of impact to biota inhabiting each transitional water body should in theory increase the evidence base, and hence the certainty, of this risk-based analysis (UKTAG, 2005).

The aim of this study was to determine the viability of constructing a biomarker response index (BRI) and combining it with the Environment Agency assigned risk classification for point source pollution for transitional water bodies in the South West of England. Ten estuaries were chosen, classified by the Environment Agency DSD procedures to be at varying degrees of risk from anthropogenic impact, largely from heavy metals, petroleum derived organics and pesticides. The testing regime included biomarkers of exposure (to organophosphorous pesticides and metals), and of sublethal effect at the molecular, cellular and physiological level (genetic damage, immune function, antioxidant status, cell viability, heart rate, feeding rate). The common blue mussel *Mytilus edulis* was selected for study as its sessile, filter-feeding habit, relatively low metabolic transformation rate and propensity to bioaccumulate organic pollutants make it a useful bioindicator species. A biomarker response index (BRI) was constructed to grade the level of biological impact, and the resulting risk classification for each estuary compared with the EA assigned risk from point source pollution. The implications and potential of this kind of analysis for environmental decision making are discussed.

Methods

Study sites

The ten transitional water bodies chosen for inclusion in the study were located in the south-west coast of England (Figure 1). Risk assessments for point source pollution were assigned to each estuary by the Environment Agency following the UKTAG Technical Guidance Document (UKTAG, 2007) (Table 1). The sampling site for each estuary was chosen using the following criteria (a) near the mouth of the estuary, (b) below high-water mark and (c) mussels were present at each site.

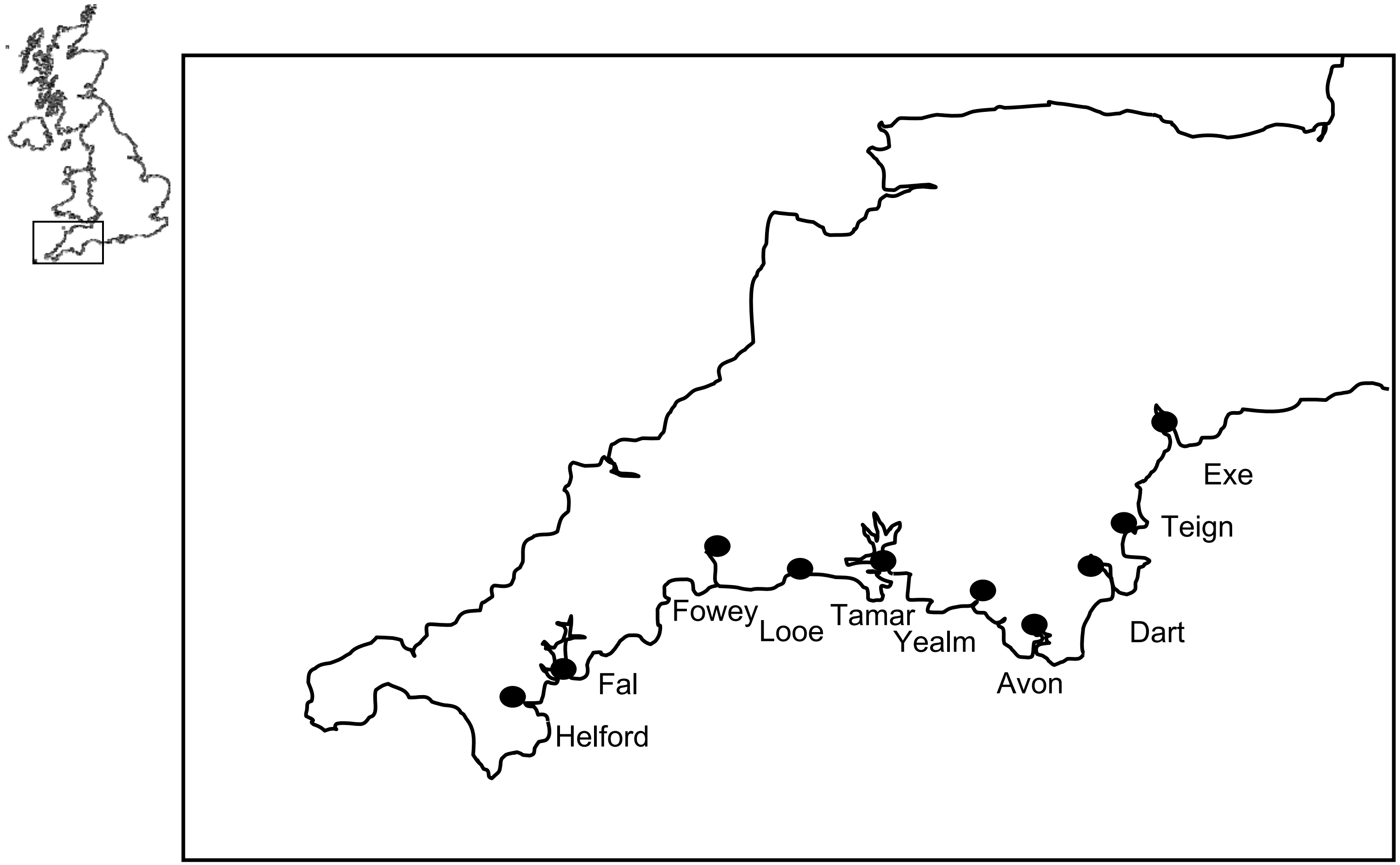

Figure 1. Locations of the ten transitional water bodies located in South-west England.

Table 1. Risk assessment due to point source pollution assigned to ten transitional water bodies in South-west England **(www.environmentagency.gov.uk).**

Transitional water body	Habitat type	Latitude and longitude	Risk assessment classification	Metal pressures *	DSD failures**
Avon		50°16'44" N, 3°52'14" W	Probably not at risk	4	0
Fowey		50°20'06" N, 4°38'02" W	Probably not at risk	2	0
Helford		50°05'37" N, 5°07'50" W	Probably not at risk	4	0
Exe		50°37'99" N, 3°25'33" W	Probably at risk	4	0
Teign		50°32'24" N, 3°30'02" W	Probably at risk	4	0
Looe		50°21'09" N, 4°27'18" W	Probably at risk	4	0
Dart		50°24'25" N, 4°12'23" W	At risk	4	7 (List 1) 4 (List 2)
Yealm		50°18'49" N, 4°03'09" W	At risk	4	3 (List 1) 1 (List 2)
Fal (Carrick Roads)		50°09'53" N, 5°04'22" W	At risk	1	22 (List 1) 7 (List 2)
Tamar (Plymouth sound)		50°24'25" N, 4°12'23" W	At risk	1	9 (List 1) 6 (List 2)

***Metal pressure scale 1-4**. 1 = high load, 2 = moderately high load, 3 = moderately low load, 4 = low load.

** **Dangerous Substances Directive (DSD) (76/464/EEC)** List I covers those compounds classified as persistent, bio-accumulating or toxic. List II covers substances whose effects are not classified as persistent, bio-accumulating or toxic, but for which significant concern exists over their potentially harmful effects. The quoted number indicates the number of compounds for which environmental quality standards are likely to be breached based on consented discharges.

Sample collection and preparation

Each transitional water body was visited once over a four-week period in January 2006 and eight blue mussels, *Mytilus edulis* (4-6cm shell length), were collected by hand at low tide from similar locations at the estuary mouth region; limiting sampling time reduced viability in mussel reproductive state and variability in environmental conditions such as temperature and salinity. Mussels were transported immediately to the laboratory and maintained at 15^{o}C in aerated water collected from their site of origin (salinity 32-36); measurement of physiological condition (feeding/clearance rate and heart rate) was carried out immediately and other biomarker endpoints were analysed within 24h of returning to the laboratory.

Biomarkers

To assess the general health of the animals, a suite of biomarkers was selected to measure a range of biological parameters at different levels of biological organisation. Heart rate and feeding/clearance rate of mussels were taken as physiological responses of general condition. Heart rate was measured following a short acclimation period (1-2h) using the Computer-Aided Physiological Monitor (CAPMON) system (Depledge and Anderson, 1990). Feeding/clearance rate was determined as described by Widdows et al. (2002).

Following the physiological measurements, haemolymph was collected from the adductor muscle of each mussel and used for several endpoints. Antioxidant activity was measured using the ferric reducing ability of haemolymph (FRAP) assay (Benzie and Strain, 1996), together with total haemolymph protein concentrations (Bradford, 1976); at a cellular level, the neutral red retention assay was carried out as a measure of lysosomal damage (Galloway et al., 2004a,b); phagocytosis activity was calculated to indicate immunocompetence (Pipe et al., 1995); the micronucleus assay was applied to determine DNA damage (Countryman and Heddle, 1976); and acetylcholinesterase activity (exposure biomarker of organophosphate pesticides and carbamates) was determined (Ellman et al., 1961). The length, width and tissue weight of the mussels were recorded as standard parameters; gills were dissected and used for analysis of the exposure biomarker metallothionein as described in Viarengo et al. (1997). All biomarkers used in the current study have been optimised and validated in laboratory and field exposures (Galloway et al., 2002, 2004a,b, 2006; Brown et al., 2004).

Biomarker Response Index (BRI)

The Biomarker Response Index (BRI) evolved from the Bioeffects Assessment Index (BAI) and the Health Assessment Index (HAI) developed previously for fish (Adams et al., 1993; Broeg et al., 2005). To enable comparison of the different biological endpoints, biomarker endpoints were ranked numerically to represent varying degrees of severity from normal reference responses, as recommend under the WFD for ecological and chemical parameters. The baseline data (a compilation of previous laboratory and field studies) were divided into four categories: those that had slight alteration from baseline responses were assigned a numerical rank of four, moderate alterations were assigned three, major alterations a two and severely altered responses were assigned a one.

The biomarker ranks were also weighted to account for important differences in responses at different levels of biological organisation. The biomarker ranks were weighted as follows: physiological = 3, cellular = 2 and molecular = 1. The final BRI value was calculated using the following equation to provide a BRI value of 1-4:

Biomarker Response Index (BRI = ∑ (biomarker$_1$ rank x biomarker$_1$ weighting) + (biomarker$_2$ rank x biomarker$_2$ weighting)$_n$ / ∑ (biomarker$_1$ weighting) + (biomarker$_2$ weighting)$_n$

The BRI was then assigned a biological status (Table 2) to indicate the degree of alteration from normal/reference responses in alignment with the categories under the Water Framework Directive for ecological and chemical assessment.

Table 2. Biological health status of organisms based on their BRI.

Biomarker Response Index	Biological status and colour code	
3.01-4.0	No or slight alteration from normal response	Green
2.76-3.00	Moderate alteration	Yellow
2.51-2.75	Major alteration	Orange
0-2.5	Severe alteration	Red

Results

Table 3 shows the biomarker results from the different estuaries and the BRI values resulting from these measurements are illustrated in Table 4. The risk classification determined by the Environment Agency (Table 4) is based upon point source pollution (www.environmentagency.gov.uk). There are differences between the overall health status of these estuaries based on the biological end points from the mussels (BRI) and their risk classification based on point source pollution. Based on the BRI, 8 out of the 10 water bodies are designated as being healthier than predicted under the WFD classification. The BRI values ranged from the Tamar, allocated the lowest value of 1.85, to the Exe Estuary which scored a BRI of 3.85, making this the healthiest estuary sampled during the current study. For two estuaries, the Tamar and the Helford, BRI scores corresponded with their risk classification (Tamar Estuary - at risk from point source pollution and severe alterations in biological response; Helford Estuary - probably not at risk from point source pollution and only moderate alterations in biological responses).

Table 3. Summary of biomarker responses according to site of collection; values are mean ± two standard errors (n= 8).

	Physiological		Cellular /Molecular					Exposure	
	Feeding rate (Litres/hour)	**Heart rate** (Beats/min)	**Micronucleus** (number/1000 cells)	**Cell viability** (% stability)	**Phagocytosis** (particles 10^7/mg protein)	**Protein** (mg/ml)	**Antioxidant status** (Δ absorbance/mg protein)	**Acetylcholinesterase activity** (μmol AChE/min/mg protein)	**Metallothionein** (μg/g wet weight tissue)
Avon	2.30 ± 0.15	31.47 ± 2.66	1.0 ± 0.65	67.50 ± 20.61	47.5 ± 9.99	0.79 ± 0.34	15.44 ± 3.53	63.02 ± 14.39	180.94 ± 55.69
Fowey	2.52 ± 0.24	30.95 ± 3.06	1.0 ± 0.65	30.60 ± 9.89	35.1 ± 4.03	1.406 ± 0.31	11.05 ± 4.66	17.43 ± 6.12	138.31 ± 53.11
Helford	1.16 ± 0.57	28.84 ± 2.14	0.875 ± 0.59	32.50 ± 16.36	41.2 ± 6.39	1.818 ± 0.54	11.01 ± 4.64	41.96 ± 8.92	113.27 ± 59.40
Exe	2.31 ± 0.65	29.74 ± 2.60	0.875 ± 0.45	72.50 ± 10.88	35.4 ± 7.47	0.922 ± 0.26	17.65 ± 6.58	67.45 ± 16.99	92.73 ± 34.92
Teign	2.96 ± 0.41	28.27 ± 4.09	1.0 ± 0.53	64.38 ± 10.25	32.7 ± 7.44	0.558 ± 0.12	10.17 ± 3.68	51.82 ± 12.06	55.99 ± 13.20
Looe	1.84 ± 0.42	34.45 ± 1.84	1.25 ± 0.90	43.75 ± 12.06	26.5 ± 2.66	1.322 ± 0.41	9.36 ± 3.65	34.37 ± 5.67	107.05 ± 15.03
Dart	0.92 ± 0.44	31.39 ± 4.26	0.857 ± 0.59	74.40 ± 12.31	36.9 ± 8.30	0.838 ± 0.30	14.28 ± 6.82	47.32 ± 14.18	133.39 ± 49.50
Yealm	2.56 ± 0.42	33.18 ± 2.46	2.0 ± 0.81	43.57 ± 15.50	42.9 ± 4.37	0.61 ± 0.26	14.59 ± 4.43	51.3 ± 11.53	42.71 ± 13.20
Fal	2.41 ± 0.57	32.17 ± 3.23	0.833 ± 0.75	44.30 ± 14.44	37.9 ± 12.08	1.367 ± 0.65	9.89 ± 1.89	17.88 ± 4.19	156.42 ± 37.34
Tamar	1.47 ± 0.49	40.37 ± 3.15	2.75 ± 1.45	43.75 ± 7.96	50.4 ± 6.11	0.439 ± 0.14	23.33 ± 7.38	49.86 ± 22.28	67.41 ± 24.36

Table 4. Final Biomarker Response Index (BRI) and overall health status of Mytilus edulis from transitional water bodies in South-west England. The overall health rank corresponds to 4= slight alteration from normal responses (biomarker value 3.01-4.0), 3= moderate alteration (biomarker value 2.76-3.00), 2= major alterations (biomarker value 2.51-2.75) and 1= severely altered responses (biomarker value 0-2.5). The risk classification rank ***was*** determined by the Environment Agency of England and Wales (EA) and corresponds to 4= Not at risk, 3= Probably not at risk, 2= Probably at risk and 1= At risk from failing the WFD based on point source pollution (www.environmentagency.gov.uk).

	Final Biomarker Response Index (BRI)	Overall Health Rank (biomarker rank)	Risk Classification Rank for point source pollution (EA determined)	Difference between overall health rank and risk classification rank
Exe	3.85	**4**	**2**	Healthier
Teign	3.64	**4**	**2**	Healthier
Dart	2.92	**3**	**1**	Healthier
Avon	3.21	**4**	**3**	Healthier
Yealm	2.85	**3**	**1**	Healthier
Tamar	1.85	**1**	**1**	Same
Looe	2.78	**3**	**2**	Healthier
Fowey	3.21	**4**	**3**	Healthier
Fal	3.14	**4**	**1**	Healthier
Helford	2.92	**3**	**3**	Same

Discussion

During the current study, baseline data for each of several biomarkers at different levels of biological organisation were used to establish a Biomarker Response Index whereby four categories of impact were identified to parallel risk classifications described in the WFD. The use of different univariate and multivariate methodologies, metrics and indices to measure anthropogenic impact has increased dramatically over recent years and various studies have described the development of biomarker-based indexes (Narbonne et al. 1999; Beliaeff and Burgeot 2002; Chevre et al., 2003a,b). Bodin et al. (2004) developed an integrated biomarker response (IBR) index to compare the physiological state of native and transplanted mussels from two polluted coastal areas in southern France. Similarly, Blaise et al. (2002) illustrated how a biomarker index could distinguish both spatial and temporal variations in biomarker responses in soft-shell clam (*Mya arenaria*) populations from anthropogenic-contaminated sites by using discriminant analysis. The majority of examples using multi-biomarker indexes highlight how they can successfully determine differences in responses between polluted sites; however, the real merit of using biological indices, in our opinion, is to translate and simplify complex biological alterations into a resource that can be used to inform and drive environmental monitoring and legislation. Few studies have attempted to use biomarker indexes in this context. Examples of how biomarker-based indices can be integrated into environmental management have been demonstrated as part of the European BIOMAR programme. For example, Narbonne et al. (1999) developed a classification of water quality in European coastal sites, ranging from class 1 (clean areas) to class 5 (highly polluted areas), using a global biomarker index for Baltic mussels. Aarab et al. (2004) also used a scoring-based biomarker approach to create a global index (the multi-biomarker pollution index) which had previously been applied in the European BIOMAR programme. This latter scoring index was based on fish collected from eleven sites in rivers in south-west France and was initiated to complement freshwater monitoring programmes carried out by the Water Agency Adour-Garonne (Aarab et al. 2004). However, the selected biomarkers reflected early molecular mechanisms of action of contaminants (i.e. phase I and II of drug metabolism, oxidative stress and neurotoxicity), whereas for biological-effects monitoring to be ecologically meaningful, it is generally agreed that a set of biomarkers at different levels of biological organisation should be chosen. Indeed, Broeg et al. (2005) argued that this same approach should also be considered when creating a biomarker assessment index (BAI). The latter authors demonstrated that, by using a set of rapid and cost-effective biomarkers at various levels of biological organisation, a reliable database for the assessment of life quality of fish located in the German Bight could be achieved (Broeg et al., 2005). The approach Broeg et al. (2005) used to create their BAI was based on a quantified health assessment index (HAI) devised by Adams et al. (1993) for the rapid evaluation of fish condition in the field. To calculate the HAI, all variables were assigned a numerical value which was based on the degree of severity of the response compared with normal responses. The main objective of creating the HAI, was to provide a simple and rapid assessment of on organism’s general health. Although the ranges of values used to calculate the HAI were subjective, the authors argued that the risk of error was minimised by the integration of numerous biomarkers at differing levels of biological organisation. In addition, by using a suite of biological responses, a weight-of-evidence approach can be adopted that allows for discrimination of clean and healthy sites (Galloway et al., 2006).

What is vital in the development of any new tool for environmental monitoring is the reliability and reproducibility of baseline data that can be used to set appropriate standards. With reference to the WFD, it has been postulated that a reference site for any study should be a pristine site where no, or only very minor disturbances, from human activities has occurred. However, one of the problems in defining reference conditions is the general absence of non-impacted sites (Borja et al., 2004). Hence, the WFD has identified four options for deriving reference conditions: (1) an existing undisturbed site or a site with only minor disturbance; (2) historical data and information; (3) models; (4) expert judgement (Vincent et al., 2002). Furthermore, because natural variability exists even in reference conditions, Borja et al. (2004) suggested that, for most parameters, the defining criteria should be expressed as ranges. Muxika et al. (2007) also emphasised that because the WFD looks for human-induced changes it must, therefore, assume natural variability in the methodology used, as well as in the reference conditions.

In the present study, a suite of biological endpoints formed the basis for a biomarker-based index (BRI) which has been applied to ten estuaries in south-west England. Of the ten, the Tamar Estuary was identified as requiring further investigation in terms of consented loads and point source pressures. Although the animals in this estuary displayed a severe reduction in health compared with baseline control responses, there was no evidence that the effects were due to either pesticides (OP and/or carbamates) or metals (the biomarkers of exposure for theses two classes of contaminants were not elevated in mussels inhabiting this estuary). Other potential sources of contamination in the Tamar Estuary, which may account for the severe alteration in the general health of the mussels inhabiting this estuary, include tributyltin, PAHs or radioactive material, as the Tamar has a long history of waste disposal from the Royal Devonport dockyard (Bryan and Langston, 1992; Lindsay and Bell, 1997).

Mussels from the Fal Estuary, which is industrially and recreationally similar to that of the Tamar, however, showed no significant variation in general health from normal baseline responses. Exposure biomarkers showed that mussels from the Fal experienced exposure to high concentrations of pesticides (OP and/or carbamates) and metals (as illustrated by severe reduction in esterase activity and increase in metallothionein concentrations). The Fal Estuary has been studied extensively due to its historical link with metal mining activities (Bryan and Hummerstone, 1973a, b, c; Bryan and Gibbs, 1983; Bryan and Langston, 1992), and the uniqueness of its inhabitants to be able to tolerate extremely high levels of metals. Species such as the alga *Fucus vesiculosus* (Bryan and Hummerstone, 1973c), the ragworm *Nereis diversicolor* (Bryan and Hummerstone, 1973,a,b; Mouneyrac et al., 2003) and the shore crab *Carcinus maenas* (Bryan and Gibbs, 1983) have developed adaptations to extremely elevated heavy metals. The involvement of adaptation in ERA is not fully explored; for example, an ecosystem may be under significant chemical pressure but the organisms inhabiting the environment do not show signs of impact. It may be argued for the Fal Estuary that, although the mussel's cellular and physiological responses were within a normal range, there will be some metabolic cost to this adaptation to maintain a general status of good health, such as a reduction in energetic budget, reproductive degradation or survival potential (Calow and Sibly, 1990; Hollaway et al., 1990; Durou et al., 2005). In environmental management, it is difficult to account for these specific, highly-stressed environments where adaptation may have taken place to a single stressor. It may be simpler to accept that if an ecosystem is sustaining the health of its organisms, and hence its community, then there is no need for further monitoring or risk assessment. This assumption is somewhat mitigated, however, by the fact that organisms from these specific environments are often less adaptable to additional pollution insults (Mouneyrac et al., 2003), and hence may actually require extra vigilance in terms of monitoring in order to prevent a sudden surprise deterioration in ecosystem health.

As mentioned previously, mussels from 8 out of the 10 estuaries achieved a healthier status based on the BRI than predicted based on the risk assessment classification. One possible explanation for this apparent discrepancy is that the point-source pressures were not exceeded at the time of sampling. Within the WFD, the proposed procedure for determining the likelihood of transitional and coastal waters failing to achieve “good status” objectives due to hazardous substances incorporates recent compliance with EQS as well as an assessment of the pressure posed by licensed point - source discharges of metals. Thus, although part of the risk classification system is based on annual inputs of consented loads which may have not been relevant at the time of sampling due to timing of release, this also may be true of the chemicals that had previously failed EQS levels. In addition, as the study was carried out during the winter months, there is a possibility that the increase in flow rates and dilution factors due to heightened rainfall may have reduced the biological responses. However, the most likely explanation for the differences in biological responses and risk classifications is that the risk assessments were over cautious; although this may only be proven after a larger scale monitoring programme has been carried out.

In conclusion, during the current study we have shown that a biomarker-based index (BRI) has the potential to be used in monitoring under the WFD. Annex V of the WFD highlights the need for biological elements, as well as physiochemical and hydromorphological components, for the determination of good ecological status for different water bodies. Thus, for the first time, the inclusion of biological effects into ERA may allow for a more holistic (and more meaningful) way of assessing contaminant effects on the aquatic environment.

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