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The use of biomarkers as integrative tools for transitional water bodies monitoring in the Water Framework Directive context — A holistic approach in Minho river transitional waters

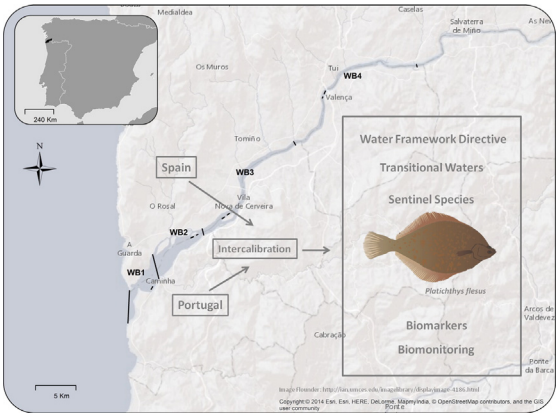
R. Capela [a,*], J. Raimundo [a,c], M.M. Santos [a,b], M. Caetano [a,c], C. Micaelo [a,c], C. Vale [a,c], L. Guimarães [a], M.A. Reis-Henriques [a,*]

[a] CIIMAR/CIMAR – Interdisciplinary Centre for Marine and Environmental Research, University of Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal
[b] FCUP – Department of Biology, Faculty of Sciences, University of Porto, Portugal
[c] IPMA – Portuguese Institute of the Sea and Atmosphere, Lisbon, Portugal

HIGHLIGHTS

- We need to establish biological monitoring in transitional waters.
- Biomarkers can be used as whole tools to monitor ecological status in estuaries.
- We studied patterns of biomarker response in juvenile flounder in the Minho estuary.
- Juvenile flounder showed a robust biomarker response over physical trait gradients.
- The use of juvenile flounder instead of adults eliminated confounding factors.

GRAPHICAL ABSTRACT



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ABSTRACT

The Water Framework Directive (WFD) provides an important legislative opportunity to promote and implement an integrated approach for the protection of inland surface waters, transitional waters, coastal waters and groundwaters. The transitional waters constitute a central piece as they are usually under high environmental pressure and by their inherent characteristics present monitoring challenges. Integrating water quality monitoring with biological monitoring can increase the cost-effectiveness of monitoring efforts. One way of doing this is with biomarkers, which effectively integrate physical–chemical status and biological quality elements, dealing holistically with adverse consequences on the health of water bodies. The new Marine Strategy Framework Directive (MSFD) already incorporates the biomarker approach. Given the recent activities of OSPAR and HELCOM to harmonize existing monitoring guidelines between MSFD and WFD the use of similar methodologies should be fostered. To illustrate the potential of the biomarker approach, juveniles of flounder (*Platichthys flesus*) were used to evaluate the quality of the

* Corresponding authors.
E-mail addresses: rcapela@ciimar.up.pt (R. Capela), mahenriq@icbas.up.pt (M.A. Reis-Henriques).

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Minho river-estuary water bodies. The use of juveniles instead of adults eliminates several confounding factors such changes on the biological responses associated with reproduction. Here, a panel of well-established biomarkers, EROD, AChE, SOD, CAT, GST, LPO, ENA and FACs (1-Hydroxyrene) were selected and measured along with a gradient of different physical conditions, and integrated with trace elements characterization on both biota and sediments. In general, a clear profile along the water bodies was found, with low seasonal and spatial variation, consistent with a low impacted area. Overall, the results support the use of both the battery of biomarkers and the use of juvenile flounders in the monitoring of the water quality status within the WFD.

1. Introduction

The Water Framework Directive 2000/60/EC (WFD) was approved in 2000, by the European Union (EU) members, with the purpose of establishing a framework for the protection of inland surface waters, groundwater, transitional waters and coastal waters. It constitutes a new view of the water resources management in Europe, that for the first time is mainly based on biological elements (ecosystems at the center of management decisions), (Borja, 2005).

For the directive implementation, new specific measures for the control of the ecological status and the development of an integrated EU water policy are required. Implicitly, the EU acknowledges the problems of judging the system's chemical and ecological quality and has delegated this to the member states although there are Common Implementation Strategies to ensure a comparable approach (de Jonge et al., 2006). It requires the monitoring and classification of all European surface and ground waters on several biological and physical–chemical criteria in order to ensure a 'Good Ecological Quality' of all water bodies (WFD 2000/60/CE). Therefore, the criteria harmonization becomes a crucial factor in its implementation and constitutes a complex call for the member states, as it also requires a close cooperation for a homogeneous, harmonic and coordinated effort, in order to interpret and apply the directive basis in the same way (Borja, 2005; Borja and Dauer, 2008; Van Hoey et al., 2010).

This directive provides an important legislative opportunity to promote and implement an integrated approach to risk assessment of chemicals. In this context, the transitional waters constitute a central piece; they are usually under high environmental pressure and by their inherent characteristics present several monitoring difficulties. Those areas have a relatively lower amount of studies performed within this context compared to the other water resources (Martinez-Haro et al., 2015). Another central question is whether the present monitoring can only detect large statistically significant changes and unusual occurrences or whether it has the ability, in the dynamic estuarine and coastal systems, to measure and detect subtle changes.

Given the budget limitations, a cost-effective monitoring approach will have to be set in place, so in general we need to integrate water quality monitoring and biological monitoring with applied system research (de Jonge et al., 2006). The integrated risk assessment for environmental and ecological issues may be the answer to that. It can provide early signs of previously unidentified risks, with both human and wildlife species in the role of sentinel species (Valavanidis and Vlachogianni, 2010). Biomarkers can offer a good connection between both classical (physical–chemical) and biological approaches, dealing holistically with the adverse consequences on health status caused by the possible exposures. In recent years OSPAR and ICES have increased their focus on the integration of biological and chemical data (OSPAR Commission, 2015), and the Marine Strategy Framework Directive 2008/56/EC (MSFD) already incorporates biomarker responses as early warning signals of pollution in order to anticipate potential impacts at higher levels of biological organization (Allan et al., 2006; Sanchez and Porcher, 2009). Furthermore, the estuarine areas due to their inherent characteristics present several additional challenges if we aim to perform a correct impact evaluation even by traditional approaches. The acquired information can be relevant to the future application of the proposed tools within the WFD context, as suggested in the few available studies (Serafim et al., 2012; Basset et al., 2013; Chapman et al., 2013). The integration of such data forms an important methodology, linking contaminants and ecological responses aiming at assessing the overall quality of the marine environment. Given the connectivity between both directives (WFD and MSFD) the use of similar monitoring methodology should be fostered. Over the past decades, a wide range of biomarkers have been employed in monitoring as early-warning indicators of contamination and ecosystem health (Giltrap et al., 2013). Combining the use of biomarkers with chemical determinations and ecological evaluation offers a huge scope to extend their use to reduce uncertainty in the risk assessment of hazardous substances (Hagger et al., 2008).

Biomarkers can be considered 'functional measures of exposure to stressors expressed at the sub-organismal, physiological or behavioural level' (Galloway, 2006). However, their use needs to be accompanied by an understanding of the significance of these measurements to ensure the adequate and reliable interpretation of results by water quality managers (Allan et al., 2006).

The current work aims at establishing and applying a methodology that is able to provide information that may allow the classification of the international transitional water bodies of the Minho river-estuary within the WFD context. The magnitude of river discharges as well as tidal and seasonal fluctuations pose several difficulties on settling proper monitoring programs and are particularly challenging on the managing perspective (Ferreira et al., 2006a, 2007).

The main objective of the presented work was to validate biomarkers, which are sensitive, easy to apply and cost-effective to use in the ecological state evaluation of transitional water bodies in a practical monitoring situation. Biomarkers can provide valuable information both for establishment of reference conditions and the environmental quality assessment of the Minho river transitional waters.

A set of tools based on ecotoxicological biomarkers was validated with the purpose of fostering its integration in the WFD. The WFD is a 'dynamic Directive' which allows further incorporation of new methodologies, changes in the previous definitions and classification of sites, etc., because any increase in knowledge should feedback into any further assessments (Borja, 2005). As it will be shown ahead, the practical application of the described methodology could be pertinent in order to provide new insights of the biomarkers use within this context. For the present study, flounder (*Platichthys flesus*) juveniles were selected as model organism because of their presence throughout the whole study area. The flatfishes of the Pleuronectidae family are a worldwide spread group of fishes, occurring in fresh, brackish and marine waters. The population in the Minho estuary is largely dominated by juveniles being the upstream zones (freshwater) of the estuary preferred by the species (Souza et al., 2013) allowing the results validation on all the sampling points of a wide range of hydrographical and geomorphologic characteristics. Several studies propose this species as a primary species for monitoring programs (Davies and Vethaak, 2012; Giltrap et al., 2013; Nunes et al., 2014). However, all have used adult animals. Here, we propose the juveniles as study model, in order to eliminate several confounding factors (maturation and physiological status and spawning