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Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers

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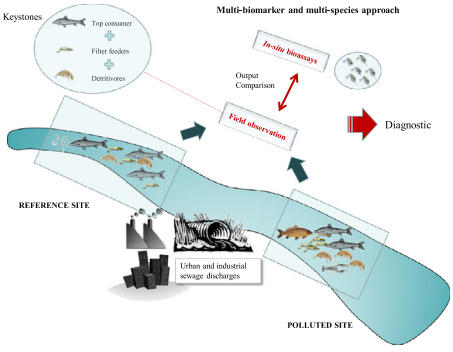
HIGHLIGHTS

- Biomarkers can assess the effects of multiple stressors on Mediterranean rivers.
- Biomarker studies need to increase the ecological realism.
- Multi-species and multi-biomarkers can help predict ecosystem impairments.
- A proposal to select biomarkers in Mediterranean rivers is outlined.
- Biomarkers should be incorporated to the EU's Water Framework Directive.

GRAPHICAL ABSTRACT



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ABSTRACT

Mediterranean rivers are probably one of the most singular and endangered ecosystems worldwide due to the presence of many endemic species and a long history of anthropogenic impacts. Besides a conservation value per se, biodiversity is related to the services that ecosystems provide to society and the ability of these to cope with stressors, including climate change. Using macro-invertebrates and fish as sentinel organisms, this overview presents a synthesis of the state of the art in the application of biomarkers (stress and enzymatic responses, endocrine disruptors, trophic tracers, energy and bile metabolites, genotoxic indicators, histopathological and behavioural alterations, and genetic and cutting edge omic markers) to determine the causes and effects of anthropogenic stressors on the biodiversity of European Mediterranean rivers. We also discuss how a careful selection of sentinel species according to their ecological traits and the food-web structure of Mediterranean rivers could increase the ecological relevance of biomarker responses. Further, we provide suggestions to better harmonise ecological realism with experimental design in biomarker studies, including statistical analyses, which may also deliver a more comprehensible message to managers and policy makers. By keeping on the safe side the health status of populations of multiple-species in a community, we advocate to increase the resilience of fluvial ecosystems to face present and forecasted stressors. In conclusion, this review provides evidence that multi-

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biomarker approaches detect early signs of impairment in populations, and supports their incorporation in the standardised procedures of the Water Frame Work Directive to better appraise the status of European water bodies.

1. Introduction

Mediterranean rivers are one of the most vulnerable ecosystems worldwide due to a long-history of anthropogenic impacts, including habitat degradation and species introductions (Bonada and Resh, 2013). The conservation concern is particularly great in these systems because of their high percent of endemic species (Maceda-Veiga, 2013; Quiñones and Moyle, 2015), and the ramifications that biodiversity loss can have for ecosystem function and support services (Hooper et al., 2005). Water pollution is still one of the major threats to Mediterranean rivers despite a marked investment in sewage treatment plants in recent decades (Prat and Munné, 2000; Sabater et al., 2009). This is attributed, in part, to the reduced dilution ability of these rivers due to climate and water abstractions (Prat and Munné, 2000). Other pollution sources such as agricultural run-off and some illegal spills further increase the environmental concern of pollutants (López-Doval et al., 2013; Sabater et al., 2009). In some Mediterranean rivers, the risk posed by chemicals to the biota is particularly worrisome due to a long list of regulated and emerging pollutants, including pharmaceuticals and personal care products (Gorga et al., 2015; Von der Ohe et al., 2011). Since this risk may increase under forecasted global change scenarios (Petrovic et al., 2011; Vörösmarty et al., 2010), understanding how pollutants affect the biota of Mediterranean rivers is central to adapt policies and develop effective management strategies.

The use of sentinel species (bio-indicators) has been traditionally used in studies of bio-monitoring, including environmental risk assessment (Friberg et al., 2011). Whilst chemical surveys only identify a fraction of environmental pollutants, often those included in 'priority lists', the use of bio-indicators enables assessing the overall effects of measured and unmeasured pollutants interacting with natural and other anthropogenic stressors in the complexity of natural systems (Birk et al., 2012). Driven by the requirements of international legislation, such as the EU's Water Framework, different taxonomic groups (e.g. algae, macrophytes, macro-invertebrates and fish) are used to determine the ecological status of water bodies across member states (Birk et al., 2012; Friberg et al., 2011). By virtue of their different life-span bio-indicators track environmental variations at variable temporal scales, ranging from weeks (invertebrates) to years (fish) (Barbour et al., 1995; Muñoz et al., 2012). Also, since bio-indicators differ in ecological traits such trophic position, they can inform if pollutants have permeated upwards the food-webs (bio-accumulation and bio-magnification risks) (Monroy et al., 2014). Besides a direct interest for policy-making, these foregoing traits enable using bio-indicators as an umbrella for protecting other taxa (Ormerod et al., 2010); a feature particularly relevant in monitoring heavily human-disturbed but ecologically unique systems like Mediterranean rivers (Bonada and Resh, 2013).

Traditionally, bio-monitoring of running waters has been made with community-based measures, named multi-metric indices (MMIs), being macro-invertebrates and fish the dominant taxa (Friberg et al., 2011). Since first attempted by Karr (1981), many MMIs have been developed worldwide with more than 300 available in Europe (Birk et al., 2012). These procedures based their diagnostics in comparing community features (metrics) between tested sites in relation to those predicted or observed in a set of reference sites (Dallas, 2013; Hawkins et al., 2010). Despite their relatively easy applicability, a major caveat is that MMIs do not identify causes of impairment (Muñoz et al., 2012), and even eclipse biotic and abiotic impacts such as habitat degradation and introduced species (Benejam et al., 2009; Maceda-Veiga et al., 2014). Also, MMIs typically fail to detect subtle effects at the individual level (Damásio et al., 2007; Muñoz et al., 2012), and then when detrimental effects are visible at the community level it can be too late to undertake conservation measures and to prevent local extinctions (Clements and Rohr, 2009). In species poor systems, such as the fish communities of Mediterranean rivers, the diagnostic ability of MMIs is particularly limited given the reduced set of metrics available (Aparicio et al., 2011; Figuerola et al., 2012). Thus, practitioners need more specific, sensitive and complementary diagnostic procedures, especially because Mediterranean rivers harbour markedly threatened fish (Quiñones and Moyle, 2015), arthropods (Pedraza-Lara et al., 2010) and molluscs species (Lopes-Lima et al., 2014).

Biomarkers, defined here as any measurable molecular, cellular, histological, physiological or behavioural response (Depledge, 1993; Fig. 1), have been adopted as 'early-warning signals' of individuals' imbalance (Galloway, 2006; Wu et al., 2005). Often on a stressor-specific basis, biomarkers have improved our mechanistic understanding of how multiple stressors affect aquatic biota (e.g. Faria et al., 2010a; Kidd et al., 2007). However, the ecological relevance of biomarkers – linkage between responses from (sub-) individual to population and ecosystem levels – is often unclear (Friberg et al., 2011). This may explain why the use of biomarkers is still not extended in biomonitoring schemes (Amiard-Triquet et al., 2012). This critical review aims to increase the ecological relevance of biomarker approaches in European Mediterranean rivers via three aspects: selection of bioindicator species of macro-invertebrates and fish according to their ecological traits and the food-web structure of Mediterranean rivers, selection of biomarkers according to its specificity and ecological relevance, and selection of appropriate experimental designs and data treatments to increase the diagnostic ability and ecological relevance of biomarker outputs. Finally, we outline future research lines that may contribute to a more systematic adoption of biomarker approaches in the biomonitoring schemes of European Mediterranean rivers.

2. Macro-invertebrates and fish as bio-indicator species

Several traits justify the use of macro-invertebrates and fish as sentinels in bio-monitoring, including their relatively well-known taxonomy, functional role and the goods they provide to society (e.g. fisheries) (Friberg et al., 2011). However, this perspective is not exempt of a debate on whether ecosystems or particular taxa should be the target in bio-monitoring and conservation (e.g. Friberg et al., 2011; Lindenmayer et al., 2007). Despite criticisms and caveats, it is well-established that the loss of keystone species results in multi-trophic impacts influencing ecosystem processes (Rodríguez-Lozano et al., 2015; Rohr et al., 2006). However, we are still far from fully understanding the biodiversity-ecosystem functioning relationship and predict the consequences of changes in species assemblies (Ings et al., 2009). Thus, the precautionary principle recommends species conservation regardless of its functional role. This is essential nowadays given that species loss occurs at alarming rates (Dudgeon, 2013), and recent evidence suggests that the biodiversity level required to sustain freshwater ecosystem functions may have been underestimated in a changing environment (Perkins et al., 2015). Clearly, assessing and restoring whole-ecosystem processes can provide multiple benefits, including species conservation (Lindenmayer et al., 2007). However, policy-makers also need to show society visible pieces of ecosystem health, and by virtue of their charismatic nature macro-invertebrates and fish meet this requirement.

Albeit challenging, in-deep knowledge of the community structure and species ecology can help select *the best* set of bio-indicators and hence increase the ecological relevance of bio-monitoring outputs

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