Project context and objectives

The Water Framework Directive (WFD) is the most important piece of water legislation in Europe. It aims at ensuring the 'good water status' of EU water bodies and thus includes both its good chemical and ecological status. To achieve and assess a 'good ecological status', the WFD advocates the integration of various lines of evidence, and demands a set of low-cost tools and techniques to deliver appropriate data. The WFD accounts for chemical and ecological evaluation, but there are no requirements regarding the establishment of cause-effect relationships in the assessment of environmental quality. By incorporating bioassays and biomarkers to ascertain the cause-effect of a failing ecological status in a water body the assessment of water quality could be reinforced.

QWATER aimed to gauge the ecological relevance of integrating short-term toxicity bioassays and biomarkers into quality elements in the WFD, as these may contribute to our ability to assess and manage EU water bodies. The project-working hypothesis was that '**bioassays and biomarkers may contribute significantly to our ability to more comprehensively assess and manage water bodies in compliance with the WFD**'. To assess this hypothesis, ecologically relevant in situ cost-effective toxicity bioassays were first developed and validated in order to use in a battery of in situ bioassays using representative species for several key functions in the ecosystem. Biomarkers, determined on the individuals used for the bioassays, were also integrated. Then, modelling tools were used to verify whether the integration of 'quality elements' (bioassays and biomarkers) did (or did not) strengthen the robustness of the standard Ecological Quality Status approach used to assess water quality.

Project results and outcomes

The outcomes of the project can be broken down into two main areas, namely development of in situ bioassays for ecological relevant estuarine species, and application, evaluation and integration of a battery of in situ bioassays and biomarkers, along with traditional biological community assessment, to assess water quality in estuaries.

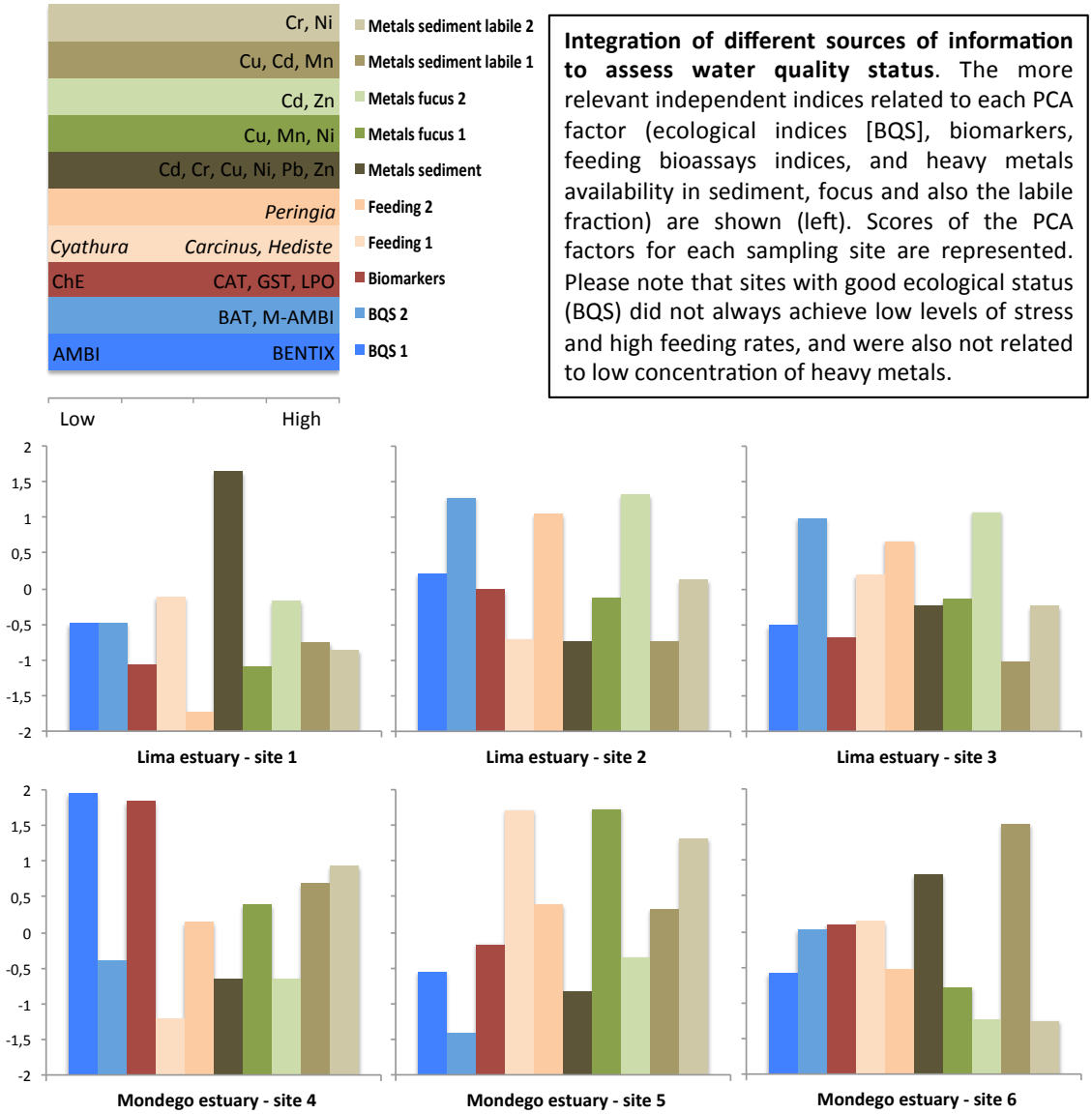
i) Two new in situ bioassays based on the postexposure feeding response of *Cyathura carinata* (benthic omnivorous isopod; published in **http://dx.doi.org/10.1016/j.envres.2014.07.013**), and *Echinogammarus marinus* (macroalgae-associated omnivorous amphipod; manuscript under review) were developed and fine-tuned under laboratory and field conditions. Results showed that bioassays with these species are potential useful cost-effective tools for estuarine sediment toxicity assessments since they successfully discriminated between polluted and unpolluted estuaries. Additionally, a study to develop a rapid an accurate methodology to quantify the filtering rate of *Cerastoderma edule* (filtering bivalve), and to check its sensitivity after the exposure to a reference toxicant (cadmium-Cd) was developed under laboratory conditions (manuscript in preparation). This study was the first step in developing an in situ bioassay with this species. Finally, two further studies with *C. carinata* and *Peringia ulvae* were performed to complete the assessment of the bioassays endpoints sensitivity. On the one hand, *C. carinata* was used to evaluate the recoveries of feeding rates after being exposed to sublethal concentrations of Cd under laboratory conditions. Results showed that inhibitions in the feeding rates after exposure represented a stable response since no evidence of recovery was detected in 24h after the exposure period (published in **http://dx.doi.org/10.1016/j.envres.2014.12.023**). On

the other hand, *P. ulvae* was used to study habitat selection patterns under patchily contaminated sediment. Results showed a trend to avoid contaminated patches in the immediate and very short term. However after 24h exposure, organisms exposed to a high level of contamination seemed to have lost the ability to move and avoid contaminated patches (manuscript under review). Therefore, avoidance response can be used as an endpoint for developing a bioassay with this species.

ii) To face the second objective, firstly, we reviewed studies providing an ecological water status assessment through more conventional community based approaches, in which bioassays and/or biomarkers were also applied to complement the evaluation, that is, studies that have attempted to integrate ecotoxicological approaches and community based ones in the assessment of surface water bodies. From the studies emerged that investigations at community level appear suitable for the assessment of ecological quality, whereas bioassays/biomarkers appear to be specially useful to investigate the causes of ecological impairment, allowing a better understanding of the cause–effect-relationships and to work as early warning, rapid evaluation and cost effectiveness systems of ecosystem disturbance. In this sense, community level responses and ecotoxicological approaches seem to be complementary reinforcing the need to use combined approaches of different disciplines in order to achieve the best evaluation of the ecosystem community health (published in **http://dx.doi.org/10.1016/j.ecolind.2014.07.024**).

In QWATER, a battery of five in situ bioassays was applied simultaneously in six different sampling sites within the estuaries of Mondego and Lima rivers. The battery was composed by ecological relevant species of the different trophic levels of estuaries: the crab *Carcinus maenas* (predator omnivorous), the snail *P. ulvae* (decomposer), the polychaete *Nereis diversicolor* (scavenging omnivourous), the isopod *C. carinata* (benthic omnivorous), and the amphipod *E. marinus* (macroalgae-associated omnivorous). Biomarkers (i.e., the enzymes Cholinesterase, Glutathione S-transferase, and Catalase, and lipid peroxidation) for a set of the individuals used in the battery were determined as another line of evidence in collaboration with the group headed by Prof. Dra. Guilhermino, University of Porto. Additionally, the chemical characterization of a set of metals (Cd, Cr, Cu, Mn, Ni, Pb and Zn) in samples of sediment and algae, and the proportion of the labile fraction of metals in sediments from each deployment site were studied. The chemical characterization was carried out in collaboration with Dr. Mark Taggart, from the University of the Highlands and Islands, UK, and Dra. Natalia Ospina-Alvarez from the University of Warsaw, Poland. Finally, the ecosystem status of the selected sites was addressed based on ecological indices (e.g., BAT, BENTIX, M-AMBI). Therefore, different sources of information, ecotoxicological (bioassays and biomarkers), chemical and ecological indices, were available for the integration.
Principal component analyses were performed independently for the indices of each source of information, in order to improve the interpretation of the resultant PCA-factors in biological terms. The values of the PCA-factors for each sampling site were extracted and comparatively assessed. Results shown some discrepancies in the water quality determined from each independent factor, i.e., the sampling sites were not equally ordered by all factors (see below). Therefore, only by interpreting the values of all PCA-factors together is possible to perform a complete assessment of water quality status. For instance, ecological indices highlighted one of the sampling sites in the Mondego as the best water quality. However, bioassays and biomarkers derived indices showed a high level of stress at that site, which was reinforced by high levels of

pollutants from the chemical indices. Thus, the QWATER working hypothesis about that the ecotoxicological indices are able to significantly contribute to the assessment and managing of water bodies in compliance with the WFD could be validated (manuscript in preparation).



Integration of different sources of information to assess water quality status. The more relevant independent indices related to each PCA factor (ecological indices [BQS], biomarkers, feeding bioassays indices, and heavy metals availability in sediment, focus and also the labile fraction) are shown (left). Scores of the PCA factors for each sampling site are represented. Please note that sites with good ecological status (BQS) did not always achieve low levels of stress and high feeding rates, and were also not related to low concentration of heavy metals.

Potential Impact

The European regulatory authorities are presently in the phase of implementing the WFD based on community level approaches all over Europe. First results from WFD will be available in the next years. Interestingly, bioassays developed here and biomarker, are available tools to be introduced as new feasible, cost-effective and sensitive protocols in the WFD. It might then become pertinent to stand for the combined/complementary use of ecological indices, biomarkers, and bioassays in the assessing the ecological quality status of water bodies (see also **http://dx.doi.org/10.1016/j.ecolind.2014.07.024**). Overall, this Marie Curie project allowed us to efficiently contribute to national and international efforts focused in evaluating the water quality in European water bodies.