Water Framework Directive Implementation: Intercalibration Exercise for Biological Quality Elements – a Case Study for the South Coast of Portugal

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ABSTRACT


This work summarises the Intercalibration Exercise (IE) required for the Common Implementation Strategy of the Water Framework Directive (WFD; 2000/60/EC) that was carried out in Portugal, and applied to a coastal region. The WFD aims to achieve good ecological status for all waters in the European Community by 2015. The Ecological Status of a water body is determined using a range of Hydromorphological and Physico-Chemical Quality Elements as well Biological Quality Elements (BQE). In coastal waters, the Biological Elements include Phytoplankton, Other Aquatic Flora and Benthic Invertebrate Fauna. Good cooperation with the other Member States allowed the IE to proceed without a complete data set, and Portugal was able to intercalibrate and harmonise methods within the North East Atlantic Geographical Intercalibration Group for most of the BQE. The appropriate metrics and corresponding methods were agreed under the framework of the RECITAL (Reference Conditions and Intercalibration) project, funded by the Portuguese Water Institute, INAG. Some preliminary sampling was undertaken, but not sufficient to establish the Reference Conditions.

The study area was a coastal lagoon in the southern part of Portugal. The focus was on the Phytoplankton Quality Element, but other BQE were also taken into account. Two sampling stations in Ria Formosa coastal lagoon were considered in this exercise: Ramalhete and Ponte. The metrics adopted by the Intercalibration Exercise groups were applied enabling the classification for the two stations of Good/High Status for the majority of the BQE parameters.

ADDITIONAL INDEX WORDS: Phytoplankton, Coastal Lagoon, Ecological Status

INTRODUCTION

The Ria Formosa is a mesotidal coastal lagoon in the southern part of Portugal (Fig. 1) which is 55km long, 6 km at its widest, and has an area of 150 km², with an average channel depth of 3.5 m. It is separated from the ocean by five barrier islands and two peninsulas, and the water exchange with the sea is achieved through 6 inlets, 4 of them natural and 2 artificial. Only one small freshwater input is permanent throughout the year. All the other freshwater inputs are torrential streams which only flow into the lagoon when there is heavy rainfall. As the salinity gradient is insufficient to be considered a transitional water, the Ria Formosa is categorised as a coastal water. Because of its biodiversity value, the coastal lagoon and part of the hinterland has been classified as a national park, as well as a site for the NATURA 2000 network and for the RAMSAR convention. It is also under the protection of the Habitats and Birds Directives. The Ramalhete and Ponte stations in the Ria Formosa are registered as Water Framework Directive intercalibration sites Nº 1261, Site Code C3979 and Nº 1259, Site Code C3978, respectively (R and P in Fig 1; more details and references in LOUREIRO et al., 2006).

The Water Framework Directive (WFD) established a framework for European Community action in water policy and management concerns. An intercalibration exercise (IE) was...
foreseen and planned (Annex V of the WFD) as a means of fulfilling its key objective of achieving a good water status for all community waters by the year 2015. The IE was aimed at consistency and comparability of the classification results of the monitoring systems operated by each Member State for the Biological Quality Elements (BQE). The IE should establish values for the boundary between the classes of high and good status and also for the boundary between good and moderate status (EP, 2000). The IE was carried out at the Geographical Intercalibration Group (GIG) level rather than within (eco) regions. On this basis, Portugal belongs to the North East Atlantic (NEA-GIG), which comprises the area from northern Norway to the Strait of Gibraltar. Good cooperation with other Member States allowed the Intercalibration Exercise to proceed unhindered by the shortage of appropriate data, and Portugal was able to intercalibrate and harmonise methods within the NEA-GIG group for most of the BQE (EC, 2005). The appropriate Metrics and corresponding methods were agreed for Coastal Waters (CW). The intertidal habitats of Transitional Waters were not considered at this stage. Some preliminary sampling was undertaken, but not sufficient to allow the establishment of Reference Conditions or the Intercalibration Exercise. As a consequence, it was decided that it was unrealisic to proceed with the IE of Transitional Waters within the time frame for the other water types, and this would be part of the second round of Intercalibration.

**Biological Quality Elements (BQE)**

A good Ecological Status (ES) of a water body is achieved through the fulfillment of the reference conditions which should be set for a number of Hydromorphological and Physico-Chemical Quality Elements as well as BQE. For CW, the BQE include Phytoplankton, Other Aquatic Flora and Benthic Invertebrate Fauna, and several indices must be considered for each of these.

**Phytoplankton**

Phytoplankton is the community of microscopic photosynthetic protozoa that float in natural waters. It depends upon many environmental conditions for growth, so it can be a sensitive indicator of change. Proliferation of certain taxa, “blooms”, may be a symptom of eutrophication (Cloern, 2001). The indices to assess Phytoplankton are biomass, composition and abundance.

Generally, biomass is determined by weighing, but due to the difficulty of isolating their contribution to the total plankton biomass, chlorophyll-a concentration is used as an indirect or proxy measurement. Abundance can be estimated by cell counts. Though composition is a complex index, it can be estimated by counting cells which can form nuisance blooms, such as Phaeocystis pouchetti, as an indication of poor water quality.

**Other Aquatic Flora**

Submerged Aquatic Vegetation (SAV) is a BQE for Coastal Waters. SAV plants are naturally present in diverse type of habitats in CW, and as primary producers, they are of great importance to the basic functioning of ecosystems. Plants depend on physico-chemical conditions (e.g. light, temperature, nutrients) that may change considerably due to anthropogenic causes, and consequently also induce important modifications along the food chain. Alterations of the morphology, water quality, salinity, and of the hydrodynamics of a system may contribute to loss of habitat and a shift in primary producers. Some ephemeral species, taking opportunistic advantage of environmental changes, might bloom and directly limit the growth of perennial ones. SAV is a sensitive indicator of environmental conditions. Macroalgae, seagrass, and the salt marsh vegetation may all be used for evaluation purposes in CW. Marine macroalgae are usually selected to evaluate the status of rocky shore, while seagrass and salt marsh plants are often excellent indicators for soft bottoms. The indices used for this evaluation process are abundance and composition.

**Benthic Invertebrates**

Macrobenthos is a BQE for Coastal Waters. Macrobenthos has a central role in marine and estuarine ecosystems. It responds rapidly to anthropogenic and natural stress (Pearson and Rosenberg, 1978; Dauer et al., 1993). Due to their limited mobility, benthic communities are sensitive to local disturbance, and due to their permanence over seasonal time scales, they integrate the recent history of disturbances that might not be detected in the water column (Bettencourt et al., 2004). Different species, exhibiting different tolerances to stress, are found in benthic communities (Dauer et al., 1993), which covers the WFD requirement of integrating sensitive species. The indices for the evaluation of the ES are composition (proportion of disturbance-sensitive taxa within the biological community) and abundance (species richness).

**Metrics**

For the IE, a metric had to be chosen within the same NEA-GIG for each index of each BQE. Most of the work in the intercalibration process has been centered on the phytoplankton and its associated metrics. For Portugal, it was agreed that the composition index for phytoplankton did not need to be assessed (cell counts of Phaeocystis spp.), due to the low frequency of Phaeocystis blooms in Portuguese coastal waters. Although, biomass and abundance should be assessed and integrated in order to obtain the final result for the classification of the status of the water bodies in study.

**METHODS**

In the field, water samples for Biomass estimation were collected using a Niskin bottle. For abundance of microphytoplankton, the water samples were passed through a plankton net with a mesh size of 200 µm and were fixed subsequently with either Lugol or gluteraldehyde solutions. In the laboratory, the water samples for the chlorophyll-α (Chl-α) determination were filtered under gentle vacuum using GF/F 47mm diameter filters. The Chl-α extraction was made with 90% acetone (v/v), and the determination was made by

### Table 1: Metrics for Phytoplankton, Other Plants and Benthic Invertebrates in Portugal.

<table>
<thead>
<tr>
<th>Index</th>
<th>Metric</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytoplankton</strong></td>
<td></td>
</tr>
<tr>
<td>Biomass</td>
<td>Concentration of Chl a</td>
</tr>
<tr>
<td>Abundance</td>
<td>Total cell counts of microphytoplankon (&gt;20µm)</td>
</tr>
<tr>
<td>Composition</td>
<td>Occurrence of Phaeocystis spp. (not applicable to Portugal)</td>
</tr>
<tr>
<td><strong>Other plants</strong></td>
<td>(Macroalgae)</td>
</tr>
<tr>
<td>Abundance</td>
<td>Cover presented by opportunistic macroalgae</td>
</tr>
<tr>
<td>Composition</td>
<td>Presence/absence of species from a selected reduced species list in CW</td>
</tr>
<tr>
<td><strong>Benthic Invertebrates</strong></td>
<td></td>
</tr>
<tr>
<td>Abundance</td>
<td>P-BAT (combination of Shannon-Wiener index with Margalef Index)</td>
</tr>
<tr>
<td>Composition</td>
<td>AZTI Marine Biotic Index (AMBI)</td>
</tr>
</tbody>
</table>
spectrophotometry at the specific wavelengths 663 and 750 nm (Parsons et al., 1984). For the abundance of the microphytoplankton, counts were made under an inverted microscope. The statistical analysis followed Devlin et al. (2005) and subsequent decisions on the NEA-GIG (EC, 2005). These specify that biomass estimation should be on the basis of the creation of box-plots that define the median, quartiles and the spread of data. The data should include surface samples and summarise a 6-year period, when available. The important values for the tool are the median and the 90th percentile. For the abundance, the data should be selected as the frequency of microphytoplankton taxa cell counts above 100,000 cells per ml.

RESULTS AND ANALYSIS

Reference Values

The classification of the water bodies requires reference values as thresholds for the Chl-a metric and for cell counts. The reference value for Chl-a was calculated based on de OSPAR recommendation that 50% is adequate to describe a slight to moderate deviation from the reference level (OSPAR, 2003). The data used to validate the phytoplankton biomass has been provided by the Instituto de Investigaciónes Mariñas – CSIC and Instituto Español de Oceanografía – Centro costero de La Coruña. The calculated values of the 90th percentile are 4.73 g l-1 at the La Coruña station (sampled monthly, from 1992 to 2006) and 10.55 g l-1 at the Vigo station (two samples per week, from 1987 to 1996). According to these data, the Chl-a thresholds proposed for the Iberian upwelling coast are 8 g l-1 for the limit between high and good status, and 12 g l-1 for the limit between good and moderate status. The data used for phytoplankton abundance has been provided by the Instituto Español de Oceanografía. Phytoplankton cell counts in the Ria de La Coruña have been carried out from 1992 to 1998. The threshold is then fixed at 100,000 cells m-1 and for a water body with single taxa counts of less than 20%, 20-39% and 40-69% exceeding this threshold are considered to be in high status, good and moderate status, respectively.

Results for Phytoplankton in Ria Formosa

All the data for Chl-a from 1999 to 2006 has been collected for the two intercalibration sites, Ramalhete and Ponte, in the Ria Formosa. Fig. 2 shows the box-plots where the upper quartile is represented by the 90th percentile. The two lines represent the boundaries for the high/good (H/G) and good/moderate (G/M) status.

There were some gaps in data for the metric of abundance (cell counts) at the two intercalibration sites for, but a reasonable amount of data (102 samples from 2002-2005) were available for the two intercalibration stations. The results showed that from the 102 samples, 22 of them exceeded the threshold value (10^3 cells. m⁻³).

For the phytoplankton BQE estimate in this study, there is a different results for the two indices. For the biomass, both the stations are in a High State, as the 90th percentiles for Chl-a concentration, are much lower than the threshold of 8 μg l⁻¹. For the index of abundance, both the stations are in a Good State, as only 21.56% of the samples exceed the threshold.

DISCUSSION

The intercalibration exercise was based on a series of terms of reference: first the Member States established a network of intercalibration sites representing boundaries between quality classes High-Good and Good-Moderate; then the IE grouped the common types shared by Member States of the same Geographical Intercalibration Group and focused on a specific pressure, which was eutrophication, and, for that reason, the Biological Quality Elements were assessed. A full knowledge about all the indices of the three different BQE should be understood in order to classify one water body in High, Good or in Moderate Status. In this work, Phytoplankton BQE was tested with all the data that was available in their two different indices: biomass and composition. The biomass index classifies both the Ponte and Ramalhete stations as being in High Status, while the index for abundance puts the region where the two stations are as being in Good Status. Loureiro et al. (2006) suggested that the boundary conditions for Ponte and Ramalhete over the 2001-2002 period that they studied, based mainly on their study of Physico-chemical and Phytoplankton Quality Elements, as high/good and good/moderate respectively.

In order to have a more realistic perception of the Ecological Status of the Ria Formosa, it is necessary to have information about the other BQE before making a final statement about this Status: these include Other Plants (seagrass, macroalgae and salt marshes) and Benthic Invertebrates. Three different studies have recently assessed these two BQE in the same study area, taking into account the requirements for the WFD.
Parvatkar (2008) assessed the macroalgae at both the stations. This work occurred from October 2007 to April 2008, and followed the Scanlan et al. (2007) method of site classification using as basic parameters: the total available intertidal area for macroalgae growth (in hectares), aerial coverage (% of the patch area covered), percentage cover, and biomass (in g wet weight m-2). The dominant alga, which has been used for the classification is the genus Enteromorpha, which is now included in the genus Ulva. Two approaches have been followed. The first using Ulva spp. to integrate both biomass and percentage cover, according to the decision table of Scanlan et al. (2007). The results for this approach shows the Ecological Quality Status at Ramalhete ranges from Good (in December and February) to High (in November, January, March and April); but at Ponte it ranges from Poor (January and February) to Moderate (November, December and March) and to high (April). The second approach is based on data for % cover of Ulva spp. according to the decision table of Scanlan et al. (2007) adapted by Patrício et al. (2007).

With this latter approach, the status for Ramalhete does not alter with the status obtained by the biomass approach but, in contrast, the situation is worse for Ponte where the status ranges from Bad (January and February), Poor (November, December and March) and High (April). For Macroalgae, there is a large variation in algal distribution for both space and time and Patrício et al. (2007) recommends that a multiyear monitoring of data and human activities should be taken into account when considering macroalgae as a water quality assessment tool.

Marin (2008) has focused on the application of seagrass to be used as a BQE in the same study area. This parameter is also included in the Other Plants BQE, although Portugal did not participate in the IE for this parameter in CW. The taxonomic composition and seagrass abundance (bed area extent and shoot density) are the two metrics assessed in this study. These metrics are scored by two methodologies (Foden and Brazier, 2007): 1) GPS or aerial photography combined with ground truth data (Short et al., 2006) are used to estimate taxonomic composition and seagrass abundance over a given area, for comparison with reference conditions; 2) The metrics are scored and compared with reference conditions over a time series, depending on the available data for each parameter, with data for example from 1994-2008 for taxonomic composition, from 2007-2008 for the bed extent metric, and from 1998-2008 for the shoot density metric. High Status is the mean results for the two stations, although Ramalhete has a slightly better score, as the ratio between the actual level of a biological indicator and the reference level of the indicator was closest to 1 (0.97 for Ramalhete; 0.90 for Ponte).

Furtado (2008) has assessed the benthic invertebrates as a BQE for a region close to Ramalhete and along the Ria Formosa. In this study, a complete dataset of 133 sampling observations of macrobenthic invertebrates over 12 years has been analyzed. Several univariate and multivariate indices have been applied to score the composition and abundance metrics. The results for Ramalhete station show a mean of Good Status, where the P-BAT index for abundance is greater than 0.59, and where the AMBI tool (Muxika et al., 2003) for species composition is between 1.2 and 3.2.

Table 2 integrates all the current data on BQE for the two stations in the Ria Formosa and summarises their current status under the terms of the WFD.

**CONCLUSIONS**

The ecological status of two stations in the Ria Formosa Coastal Lagoon was evaluated mainly on the phytoplankton BQE, but also integrating this result with recent assessments made in the same study area for other BQE including Other Plants and Benthic Invertebrates. The evaluation was made according to the guidelines agreed in the NEA-GIG, based on the requirements in Annex V of the WFD for the IE. The two stations in the study were chosen as they are already registered in NEA-GIG as intercalibration sites for this coastal system. The phytoplankton BQE status was classified according to the evaluation of two indices: biomass and abundance, with Chl-a concentration and cell counts as the respective metric. Both stations presented Good to High Status. When the integrative assessment was taken into account for the other BQE, both stations were classified mainly as being Good to High Status at the two stations, except for the macroalgal index at the Ponte station, which varied from Poor to Moderate, depending on the classification tool.

In this study, as well as in the global exercise at Community level, one of the major obstacles for intercalibration process was the limited availability of data. The IE was based on results from monitoring systems still under development, with limited data available and practically no possibility to collect additional data. The intercalibration network included sites impacted by the most widespread pressure, eutrophication. Therefore, only those parts of the classification systems targeted to detect impacts of eutrophication were intercalibrated for the selected Quality Elements.

**LITERATURE CITED**


ACKNOWLEDGEMENTS

This work was partially funded by the Portuguese Water Agency- Instituto da Água (INAG) project for Reference Conditions and Intercalibration (RECITAL).