# Service Tender for the Provision of Scientific Expert/s Assistance in the Intercalibration Exercise of Biological Elements of Maltese Coastal Waters
# MEPA T03/2014

## Intercalibration Report

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# 1 INTRODUCTION

The European Union's (EU) 'Water Framework Directive' (hereafter 'WFD' or 'Directive') is Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000, which establishes a framework for Community action in the field of water policy. The WFD establishes a framework for the protection of all water resources, comprising groundwater, inland surface waters, transitional waters (transitional between freshwater and coastal water) and coastal waters. The explicit and implicit aims of the WFD are (1) to prevent further deterioration of, and to protect and enhance the environmental status of aquatic systems; (2) to promote the sustainable use of water; (3) to reduce or eliminate discharges of hazardous substances and other negative impacts that compromise aquatic environments; (4) to contribute to the mitigation of the effects of floods and droughts; and (5) to provide national authorities with a legislative basis for the attainment of good water quality for all surface waters and for groundwater.

The overall objective of the WFD is for the EU Member States to achieve 'good status' for all water bodies by 2015 according to a defined timeframe. It is mandatory that such a status be achieved, or if it results that a water body has a lower status, to take action for the water body in question to achieve 'good status'. The WFD considers surface water status to have two components: 'chemical status' and 'ecological status'; both should satisfy the 'good status' criteria specified by the Directive before a water body can be said to have achieved a 'good status'.

The WFD was transposed into Maltese national legislation with Malta's accession to the European Union through the Water Policy Framework Regulations (2004), issued under the Environment Protection Act (2001), and the Malta Resources Authority Act (2000), and subsequently amended by the Water Policy Framework Regulations (2011) and the Environment and Development Planning Act (2010). The competent authorities responsible for local implementation of the WFD regulations are the Ministry for Energy and Health, and the Malta Environment and Planning Authority (MEPA), which falls under the auspices of the Ministry for Sustainable Development, the Environment and Climate Change. The former are responsible for inland waters (including groundwater) and the latter for coastal waters, and for surface waters located in protected areas or that support protected species.

The Maltese Islands (including a one nautical mile zone of coastal water around the islands) are considered to constitute one Water Catchment District; Maltese coastal water bodies have been classified into 4 different types based on their predominant physical characteristics (exposure, water depth and predominant currents; Figure 1):

- Deep waters that are very exposed;
- Waters of intermediate depth and exposure;
- Intermediate to deep waters that are exposed;
- Intermediate to deep exposed waters characterised by alternating currents.

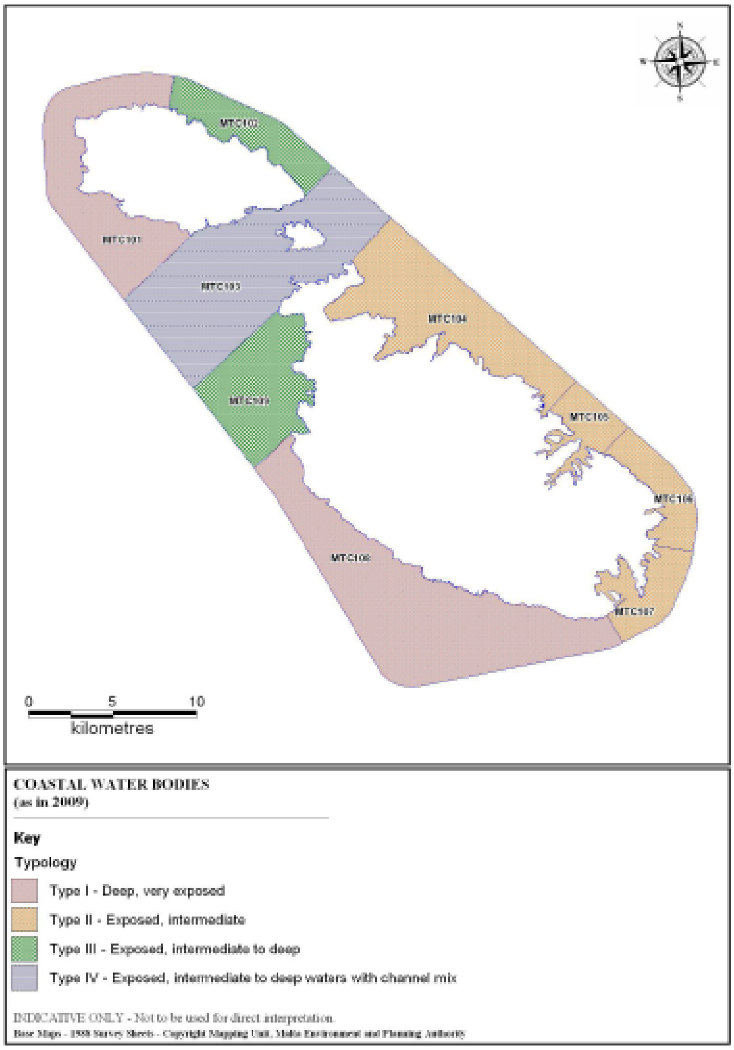


*Figure 1 Coastal water bodies of the Maltese Islands [Malta Water Catchment Management Plan]*[1]

[1] Malta Environment & Planning Authority. (2011). The Water Catchment Management Plan for the Maltese Islands. Accessed at: http://www.mepa.org.mt/topic-wcmp

The WFD requires assessment of the 'ecological status' of water bodies, which has to be based on use of a number of biological, and physico-chemical quality elements, of which the biological quality elements (BQEs) are given the greatest weighting. The WFD lists five major groups of BQEs: phytoplankton, aquatic flora (algae and angiosperms), benthic invertebrate fauna and fish fauna. The values obtained for BQEs of water bodies being assessed by Member States need to be compared to reference values for the same element found in a comparable water body that has suffered no or very minor disturbance due to anthropogenic activities. The 'ecological status' of a water body is then classified based on a combination of ratios of the observed values of each biological indicator to those found at the relevant reference site; this ratio is termed the 'Ecological Quality Ratio (EQR)'. On the basis of the obtained value of the EQR, water bodies are classified into one of five ecological status classes with a range from zero (bad ecological status) to one (high ecological status), and intermediate values corresponding to good, moderate, and poor status.

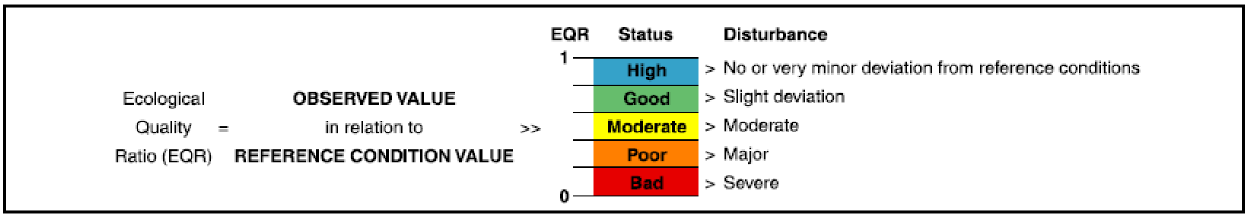


*Figure 2 The WFD classification of ecological status based on ecological quality ratios (Andersen et al. 2004, and references therein).*

The data on the basis of which the EQRs would be calculated, should be collected through a monitoring programme that is required by the WFD and which should have been initiated by Member States in 2007. This monitoring programme is sub-divided into three sub-programmes: (a) surveillance monitoring that is required to identify long-term trends, and to supplement the characterisation assessment; (b) operational monitoring that is required to address the pressures exerting an influence on the status of the water bodies; and (c) investigative monitoring that is required when the reasons for not meeting the objectives are unknown, or else in the case of accidental pollution. The monitoring systems applied need to take into consideration statistical factors such as the confidence in assigning water bodies to their true classes.

The Directive requires that the results of national classifications are compared between different Member States in the various ecoregions of the European Union to ensure that the established class boundaries and the reported monitoring results are consistent with the normative WFD definitions, and that they are comparable between Member States. In order to achieve this, an intercalibration exercise, as referred to in Annex V of the WFD, is required between Member States. This exercise is a key element in making this general environmental objective of 'good status' applicable in a harmonised way throughout the EU. Its objective is to establish a common understanding of 'good ecological status' in all Member States that is consistent with the definitions of the Directive. Intercalibration needs to take into account current scientific knowledge about the structure and functioning of aquatic ecosystems, and how human activities influence them and the results of the intercalibration exercise are expected to lead to appropriate boundary identification between the categories of high – good and good – moderate.

Ecoserv Ltd and UCV-IMEDMAR were commissioned by the MEPA (MEPA tender T03/2014) to provide scientific experts to render assistance in the intercalibration exercise of BQEs for Maltese coastal waters. As part of this assignment, the MEPA also requested assistance in the identification of reference sites for Maltese coastal water bodies; the criteria required by the WFD to set reference sites cannot be used in Malta since the requirements are too stringent.

The present submission reports on: (i) the results of the intercalibration exercise for the BQEs for which intercalibration was possible (*Posidonia oceanica* and macroalgae); (ii) data gaps and future monitoring needs for the BQEs which Intercalibration was not possible (benthic invertebrates and phytoplankton); (iii) methods used to determine reference sites in the Maltese Islands; (iv) approaches to intercalibration of heavily modified water bodies.

# 2 BQE INTERCALIBRATION

## *2.1 POSIDONIA OCEANICA*

### 2.1.1 Introduction

- Two Member States (Spain [Valencia] and Malta) compared and harmonised their national assessment systems.
- Intercalibration 'Option 2' was used; i.e. indirect comparison of assessment methods using a common metric.

- The comparative analyses show that national methods from all MS give a closely similar assessment (in agreement with comparability criteria defined in the IC Guidance), so no boundary adjustment was needed.
- The final results include the EQRs from the Spain-Valencia and Malta assessment systems.

### 2.1.2 Description of National Assessment Methods

*Table 1 Overview of the national assessment methods.*

| Member State | Method | Included in this IC exercise |
|---|---|---|
| Spain (Valencia) | Valencian-CS: Valencian Classification System | Yes |
| Malta | PREI: *Posidonia oceanica* Rapid Easy Index | Yes |

#### *2.1.2.1 Methods and Required BQE Parameters*

*Table 2 Overview of the metrics included in the national assessment methods.*

| Member State | Full BQE method | Abundance | Disturbance sensitive taxa | (Diversity) | Combination rule of metrics |
|---|---|---|---|---|---|
| Spain Valencian-CS | Yes | Shoot density, meadow cover, dead matte cover, shoot leaf surface area | 1 selected sensitive species, *Posidonia oceanica* + percent of plagiotropic rhizomes, rhizome baring/burial, percent of foliar necrosis, herbivore pressure, leaf epiphyte biomass | Not applicable only 1 species | Yes, See description of the national method below |
| Malta PREI | Yes | Shoot density, shoot leaf surface area, maximum depth of the meadow (lower limit), type of lower limit | 1 selected sensitive species, *Posidonia oceanica, +* ratio of epiphytic biomass to leaf biomass (E/L ratio) | Not applicabl e - only 1 species | Yes, See description of the national method below |

Most of the seagrass (angiosperm) meadows in the Mediterranean are monospecific, thus classification systems are based on variables related to that species. Moreover, Mediterranean seagrass experts agreed to develop classification systems on the basis of one selected species, *Posidonia oceanica*, due to its wide distribution, its sensitivity, the existing knowledge on this seagrass, and available data on its response to disturbance.

In addition, given the depth-dependence of most *Posidonia oceanica* descriptors, MS experts have agreed to collect data at a water depth of 15 ± 1 m.

**Valencian Classification System**

The metrics are combined using Principal Component Analysis (PCA). The EQR is calculated on the basis of the first component value using the following equation:

$EQR'_x = (CI_x - CI_{worst}) / (CI_{optimal} - CI_{worst})$

Where $EQR'_x$ is the ecological quality of the site *x*, $CI_x$ is the score of site *x* on the first component, $CI_{optimal}$ is the score of the 'optimal' site (reference site) on the first component, and $CI_{worst}$ is the score of the 'worst' site on the first component.

The Valencian classification system is based on nine metrics: shoot density, meadow cover, dead matte cover, percent of plagiotropic rhizomes, rhizome baring/burial, shoot leaf surface area, percent of foliar necrosis, herbivore pressure, and leaf epiphyte biomass (Fernandez-Torquemada et al. 2008, Table 2). All these metrics have been observed to respond to a number of anthropogenic impacts including an increase in nutrient and organic matter levels, direct mechanical impact (e.g. anchoring and fish trolling), industrial pollution, and fishing and fish farming activities, among others (Martinez-Crego *et al*. 2008).

**PREI**

The EQR is calculated according to the following equation:

$$EQR' = (N_{density} + N_{shoot\ leaf\ surface\ area} + N_{E/L}, + N_{lower\ limit})/3.5$$

Where $N_{density}$ = value measured/reference value; $N_{shoot\ leaf\ surface\ area}$ = value measured/ reference value; $N_{E/L}$ = [1-(E/L)]*0.5; $N_{Lower\ limit}$ = (N'-7)/(reference value –7).

The PREI is based on five metrics: shoot density, shoot leaf surface area, the ratio of epiphytic biomass and leaf biomass (E/L ratio), maximum depth of the meadow (lower limit), and type of lower limit (Gobert *et al*., 2009; Table 2). All these metrics have been observed to respond to a series of anthropogenic impacts including an increase in nutrient and organic matter levels, mechanical impact (e.g. anchoring and fish trolling), industrial pollution, and fishing and fish farming activities, among others (Martinez-Crego *et al*. 2008).

### *2.1.2.2 Sampling and Data Processing*

*Table 3 Overview of the sampling and data processing of the national assessment methods included in the IC exercise.*

| **Information provided in the online WISER project assessment method questionnaires** | |
|---|---|
| Sampling/survey device | All: SCUBA Diving transect, quadrats |
| How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? | Malta: annual sampling (August to September)<br>Spain Valencia: annual sampling (September-October) |
| Sampling/survey months | Malta: August to September<br>Spain Valencia: September -October |
| Which method is used to select the sampling /survey site or area? | Malta: expert judgement based on the presence of a meadow<br>Spain Valencia: Expert knowledge |
| How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? | Malta: e.g. station at 15 m: 20 replicates (shoots), 20 replicates (density), 1 replicate (visual census) for station lower limit<br><br>Spain-CS Valencia: 3 sites with 3-5 replicates per site at each locality |
| Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based | Malta: station at 15 m: estimate about 1000 square meters – station lower limit: along transect of 50 – 60 m Spain-CS Valencia: along a transect of 50 m x 10 m (500 m2) |

| | |
|---|---|
| Short description of field sampling/ survey procedure and processing (sub- sampling) | Malta: station at 15 m: sampling stations randomly positioned in an area of 1000 square meters distant one from each other by about 5 m<br><br>Spain- Valencian CS: Sampling was carried out by scuba divers at 17 locations within a depth range of 14-17 m. At each locality, three sampling sites separated by hundreds of metres were randomly selected to prevent spatial pseudo-replication. At each site, three 40 x 40 cm quadrates were randomly selected to measure shoot density, percentage of plagiotropic rhizomes, and rhizome baring. Living and dead *Posidonia* cover was estimated as the proportion of living and dead patches within three replicate 20 m transects. In addition, ten shoots were collected at random and transported to the laboratory for further analysis. |

### *2.1.2.3 National Reference Conditions*

*Table 4 Overview of the methodologies used to derive the reference conditions for national assessment methods included in the IC exercise.*

| Member State | Type and period of reference conditions | Number of reference sites | Location of reference sites | Reference criteria used for selection of reference sites |
|---|---|---|---|---|
| Spain (Valencia)- CS Valencia | Modelling considering the 3 best metrics in the region | No existing reference sites | No existing reference sites | Reference conditions were established in a manner identical to POMI.<br>Real reference sites were not available, so a virtual site was constructed, with the best values observed for all individual metrics (highest values for 'positive' metrics and lowest for the 'negative' ones) to serve as a reference. |
| Malta PREI | Modelling considering the 3 best metrics in the region | No existing reference sites | No existing reference sites | Because no truly unimpacted reference conditions exist in the area, a composite 'optimal' site was constructed based on PREI metrics with the assumption that this hypothetical site would have ecologically ideal conditions in relation to each of the metrics. |

All references are biogeographically corrected by the intercalibrated methods (PREI and CS-Valencia) itself. These 2 methods consider the reference conditions as the best of the 3 parameters found in the region where it is applied.

### 2.1.2.4 National Boundary Setting

*Table 5 Explanation for national boundary setting of the national methods included in the IC exercise.*

| Member State | Type of boundary setting: Expert judgment – statistical – ecological discontinuity – or mixed for different boundaries? | Specific approach for H/G boundary | Specific approach for G/M boundary | BSP: method tested against pressure |
|---|---|---|---|---|
| Spain-CS | The class boundaries are established by assuming that the system responds to all pressures in a linear way. While it is possible that this assumption may not always hold true, so far, no clear thresholds have been identified between element quality and the pressure gradient. Thus, following exactly the same boundary setting as PREI and POMI, the range from 0 to 0.099 was arbitrarily assigned to the bad ecological status and the other EQR boundaries were obtained by dividing the remaining scale (from 0.1 to 1) into four categories of equal amplitude (0.225 each). Therefore, where *P. oceanica* is present, the EQR is computed as follows: EQR = (EQR' + 0.11)/(1+0.10). | Equidistant division | Equidistant division | Yes quantitative |
| Malta PREI | The class boundaries are established by assuming that the system responds to all pressures in a linear way. While it is possible that this assumption may not always hold true, so far, no clear thresholds have been identified between the element quality and the pressure gradient. *P. oceanica* is very sensitive to anthropogenic disturbance and meadows disappearance has been reported in environmental conditions where macrofauna can still survive, hence the bad class has been defined as the ecological status in which *P. oceanica* cannot | Equidistant division | Equidistant division | Yes quantitative |

survive. In other words, wherever and whenever a *P. oceanica* bed is able to survive, albeit in a heavily degraded state, the ecological status is above bad. However, absence of the seagrass is not necessarily related to degradation, therefore the bad class can only be attributed to areas which show evidence of a recent die-off of the meadow (< 5years). Consequently, bad ecological status was arbitrarily assigned the range from 0 to 0.099. The other EQR boundaries were obtained by dividing the remaining scale (from 0.1 to 1) into four categories of equal amplitude (0.225 each). Therefore, when *P. oceanica* exists, the EQR is computed as follows: EQR = (EQR' + 0.11)/(1+0.10)

### *2.1.2.5 Results of WFD Compliance Checking*

*Table 6 List of the WFD compliance criteria and the WFD compliance checking process, and results of the national methods included in the IC exercise.*

| Compliance criteria | Compliance checking conclusions |
|---|---|
| 1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad). | Valencia and Malta have 4 classes (high, good, moderate and poor). Bad is only considered when an existing meadow is lost because *P. oceanica* is very sensitive to anthropogenic disturbances |
| 2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**) | Yes |
| • Scope of detected pressures | Integrated pressures |
| • Has the pressure-impact relationship of the assessment method been tested? | Yes |
| • Setting of ecological status boundaries: methodology and reasoning to derive and set boundaries | Yes:<br>equidistant division of the EQR gradient |
| • Boundary setting procedure in relation to the pressure:<br>Which amount of data/pressure indicators have been related to the method and what was the outcome of the relation? | The relationship of the integrated pressures using PREI (Malta Coast) and CS (with Valencian Coast) has been established with a regression. |
| • Reference and Good status community description:<br>Is a description of the communities of reference/ high – good – moderate status provided? Not only a formula or an EQR value, but the range of values for the different parameters included in the method that result in high- good- moderate status | Yes: the reference condition is a formula that includes the best different metrics |
| 3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into the BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole | Yes |

| Compliance criteria | Compliance checking conclusions |
|---|---|
| • Complete list of biological metric(s) used in assessment | Malta-PREI: shoot density, Leaf surface area, Lower depth limit, Typology of meadow at lower depth limit, Epiphyte biomass/leaf biomass.<br>Spain Valencia-CS: shoot density, meadow cover, dead matter cover, percent of plagiotropic rhizomes, rhizome baring/burial, shoot leaf surface area, percent of foliar necrosis, herbivore pressure, leaf epiphyte biomass |
| • Data basis for metric calculation | Malta-PREI: for intercalibration 26 sites with common data<br>Spain Valencia-CS: for intercalibration 15 sites common data |
| • Combination rule for multimetrics | Malta-PREI: average of the metrics<br>Spain Valencia-CS: multivariate analysis (PCA) |
| 4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT | Yes |
| • Is the assessment method applied to water bodies in the whole country? | Malta-PREI: yes<br>Valencia-CS: no- 1 region Valencia (Spain) |
| • Specify common intercalibration types | Only one type by now for all the Mediterranean |
| • Does the selection of metrics differ between types of water bodies? | No |
| 5. The water body is assessed against **type-specific near-natural reference conditions** | The water body is assessed in all methods against the best conditions of each individual parameter |
| • Scope of reference conditions | See section on National reference conditions: Site specific Least Disturbed Conditions, Modelling (extrapolating model results), Expert knowledge |
| • Key source(s) to derive reference conditions | Average of the best values of descriptor |
| • Number of sites, location and geographical coverage of sites used to derive reference conditions | Malta: 26 sampling sites<br>Spain: Valencian CS: 15 Localities in Valencian region |

| | |
|---|---|
| • Time period (months + years) of data of sites used to derive reference conditions | Malta: 2012-2013<br>Valencia: 2006-2009 |

| Compliance criteria | Compliance checking conclusions |
|---|---|
| • Reference site characterisation: criteria to select them | Reference site derived from the best values of a descriptor |
| • Is a true reference used for the definition of High status or an alternative benchmark estimation? | No, an alternative benchmark has been used |
| 6. Assessment results are expressed as **EQRs:**<br>• Are the assessment results expressed as Ecological Quality Ratios (EQR)? | Yes |
| 7. Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**<br>See info from WISER Questionnaires: | Yes |
| • Has the uncertainty of the method been quantified, and is it regarded in the assessment? | No, to be done<br>*Specification of uncertainty consideration:*<br>a) Choosing three sampling sites, separated by a distance of around from each other, and applying the entire protocol at each site, in eight of the meadows of the monitoring network, in two different sampling periods and in two deeps b) by performing a sensitivity test of the POMI (adding random variation to the descriptors) |
| 8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure** | Yes |
| 9. Selected taxonomic level achieves adequate **confidence and precision** in classification | No taxonomy is needed for these methods |
| • Minimum size of organisms sampled and processed | *Posidonia* shoot |
| • Record of biological data: level of taxonomical identification – what groups to which level | Not necessary |

**General conclusion of the compliance checking:** Compliance criteria are met

## 2.1.3 Results IC Feasibility Checking

### *2.1.3.1 Typology*

The Intercalibration is feasible irrespective of typology. In fact, typology is not relevant for BQE *Posidonia oceanica* in Mediterranean coastal waters. Common IC type: only one type; entire Mediterranean Sea, no subdivision.

*Table 7 Description of common Intercalibration water body types and the MS sharing each type*

| Method | Appropriate for IC types/subtypes | Remarks |
|---|---|---|
| Valencian CS and PREI | Used in entire Mediterranean Sea | |

### *2.1.3.2 Pressures Addressed*

*Table 8 Pressures addressed by the national methods and overview of the relationship between national methods and pressures.*

| Member State | Method/Metrics tested | Pressure | Pressure indicators | Amount of data | Strength of relationship |
|---|---|---|---|---|---|
| Spain | Valencian CS, | Eutrophication, organic matter and direct impact (direct habitat degradation) | Quantitative test: Estimates of six anthropogenic pressures that were considered most relevant to *Posidonia* meadows were correlated: coastal construction (length in km of artificial coastline per km of coastline by water body), beach regeneration (volume of sand in $m^3$ added per km of coastline), urban sewage (kg COD $d^{-1}$ $km^{-1}$ coast), industrial sewage (kg COD $d^{-1}$ $km^{-1}$ coast), pollution from rivers and channels (kg BOD5 $d^{-1}$ $km^{-1}$ coast), and pollution from agricultural soil use (ha mm precipitation $year^{-1}$). All variables were normalized and then incorporated in one index. | 35 sites | Linear regression $R^2 = 0.651$ $p<0.05$ |
| Malta | PREI | Eutrophication, organic matter and direct impact (direct habitat degradation) | A version of the LUSI index (MA-LUSIpo)[2] that takes into consideration indirect (land based: urban, commercial and industrial, agriculture) and direct (sea based: mariculture, sewage outfall) anthropogenic pressures along with confinement. | 26 sites | Linear regression $R^2=0.529$, $p<0.05$ |

Relationships between the method and pressures are shown in Figures 3 and 4 below.

[2] See ANNEX I.

**Malta-PREI**

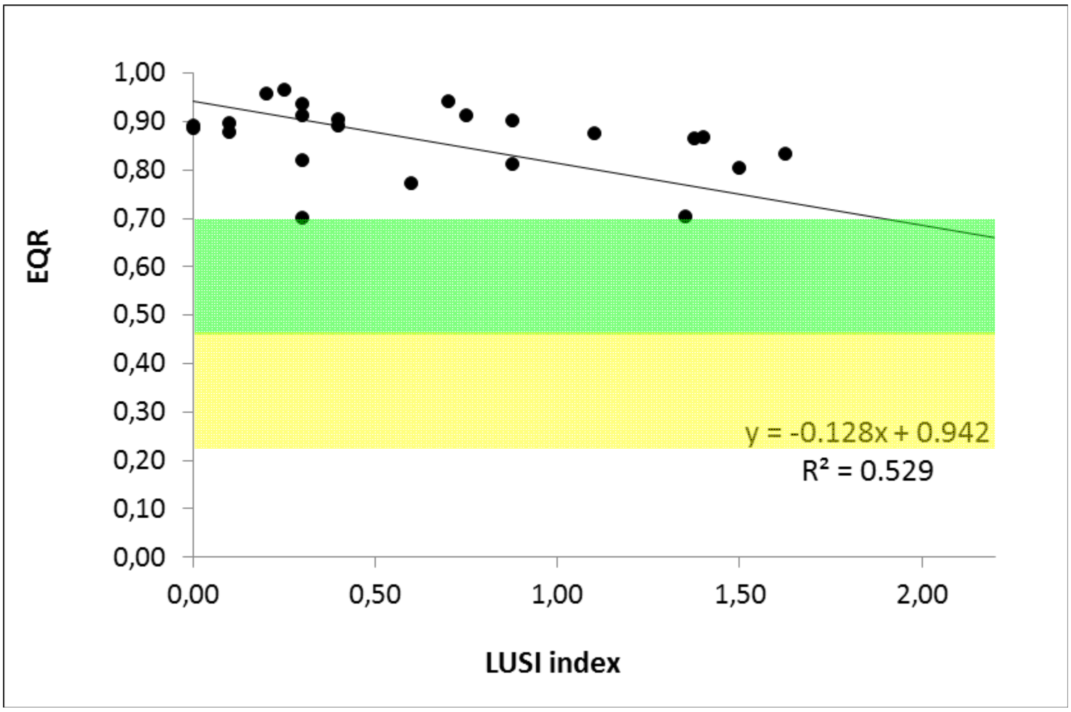


*Figure 3 Relationship between the PREI used in Malta and anthropogenic pressures, for which the combined pressure gradient is composed of the sum of significant pressures (LUSI[2] Index). The good and moderate ecological classes are indicated by green and yellow respectively.*

**Spain Valencian CS**

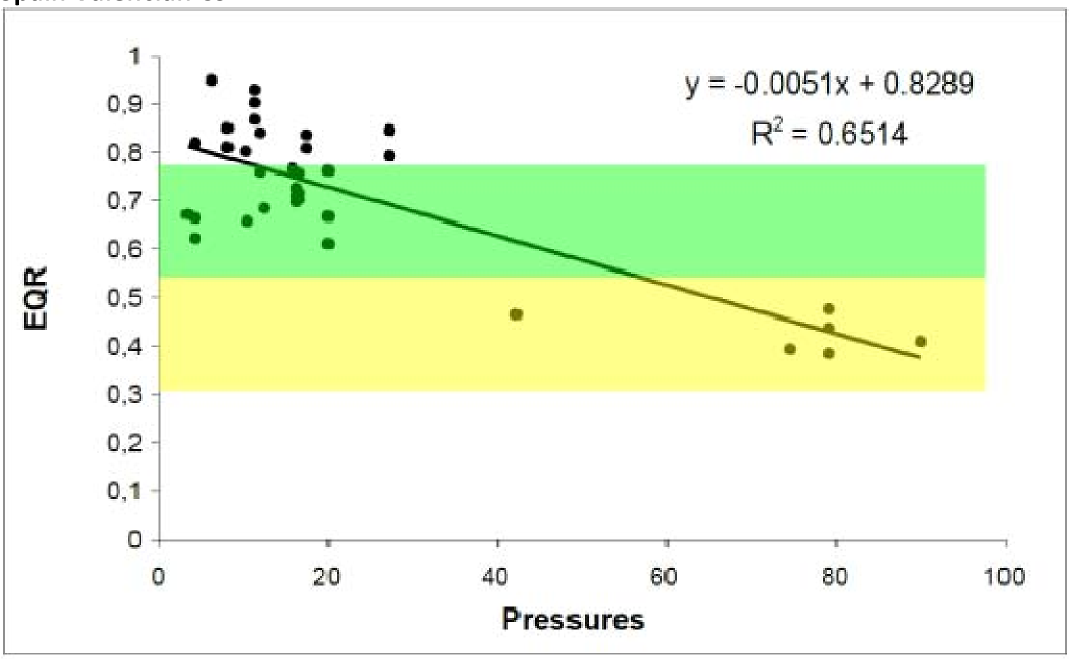


*Figure 4 Relationship between the Valencian CS and pressures. The pressures are calculated according to the procedure defined in the Catalan article 5 report, using the indicators coastal construction, beach regeneration, urban and industrial sewage, rivers and channels discharges, agricultural land use (Agencia Catalana del Aigua, 2006; Fernandez-Torquemada et al. 2008). The Valencian Classification system is presented as Component 1, before it is normalised on the EQR scale. The good and moderate ecological classes are indicated by green and yellow respectively.*

**Conclusion**

The Intercalibration exercise is feasible in terms of pressures addressed. The combination of the pressures is almost the same for all methods; all methods have a very similar combination of the pressures that include in large terms eutrophication, organic matter and direct impacts to the meadows (i.e. direct extraction)

*Table 9 Pressures considered in intercalibration*

| Method | Pressure |
|---|---|
| PREI (Malta) | Combination of pressures that include: fish farming, industrial development, agriculture, tourism, fishing and urbanization |
| Valencian CS (Spain-Valencia) | Coastal construction, beach regeneration, urban sewage, industrial sewage, pollution from rivers and channels, and pollution from agricultural soil use |

#### *2.1.3.3 Assessment Concept*

*Table 10 Intercalibration assessment concept*

| Method | Assessment concept |
|---|---|
| Malta PREI & VALENCIAN- CS | Based on a set of metrics related to structural and functional attributes of the system |

The Intercalibration exercise is feasible in terms of assessment concept. The two methods follow a very similar philosophy in which a set of metrics is combined and which includes structural and functional attributes of the ecosystem.

### 2.1.4 Collection of IC Dataset and Benchmarking

#### *2.1.4.1 Dataset Description*

*Table 11 Description of the data collection within the GIG.*

| Parameter | Description |
|---|---|
| Size of common dataset | 61 sites |
| Total number of sites | 61 sites used in the comparison calculation |
| Number of Member States | 2 |

| | |
|---|---|
| Repackage/disaggregation samples/WB results? | No, EQRs were used at the usual integrated level of the site of |
| Gradient of ecological quality | The upper 3 (in the analysis) or 4 classes are covered |
| Coverage per ecological quality class | High, good and moderate status in the analysis. |

*Table 12 Overview of the data set*

| Member State | Number of sites or samples or data values | | |
|---|---|---|---|
| | Biological data | Physico-chemical data | Pressure data |
| Malta – PREI - method | 26 sites | Not available, because data was of insufficient quality. | 26 sites |
| Spain - CS- method | 35 sites | Available | 35 sites |

The data set used in the Intercalibration exercise includes variables that are 'depth-independent'. *Posidonia* is very susceptible to depth and any data that includes shoot density or depth limit will be very difficult to be intercalibrated unless reference conditions are locally corrected (see previous explanation about reference conditions). To avoid this problem the variables chosen for the intercalibration where 'depth-independent' variables: typology of meadow at deep limit and shoot biomass.

The two regions, Malta and Valencia-Spain (which adopted different methods; PREI and CS respectively), considered these two common metrics (typology of meadow at deep limit and shoot biomass), hence these same 2 metrics are the ones used in the Option 2 (common metric) intercalibration procedure.

### *2.1.4.2 Data Acceptance Criteria*

*Table 13 List of data acceptance criteria used for the data quality control and description of the data acceptance checking process and results*

| Data acceptance criteria | Data acceptance checking |
|---|---|
| Data requirements (obligatory and optional) | All methods have been developed for *Posidonia oceanica* habitat (no other seagrasses have been included in this intercalibration exercise), in the same season (generally after summer) and at a fixed depth that in general is around 15 meters. |
| **Data acceptance criteria** | **Data acceptance checking** |

| | |
|---|---|
| Sampling and analytical methodology | All the adopted methodologies include diving on the *Posidonia oceanica* meadow where several attributes are measured (shoot density, cover, deep limit, etc.); additionally some shoot samples are also required. All sampling methods include at least 3 samples (up to 20 depending on the parameter) that are randomly collected within the meadow. |
| Level of taxonomic precision required and taxa lists with codes | Taxonomy is not required in any method. |
| The minimum number of sites / samples per intercalibration type | The minimum number of sites is 15 |
| Sufficient coverage of all relevant quality classes per type | All quality classes are covered except for the bad class (red) that is only included if a previous existing meadow of *P. oceanica* disappears. This is based on the fact that *P. oceanica* is very sensitive to anthropogenic pressures and cannot live under high levels of disturbance. |

#### *2.1.4.3 Common Benchmark*

The MEDGIG Group has defined an alternative benchmark for conditions of high status. This is defined as a location that has low impact (see below). The two methods use an alternative benchmark presenting very low pressures that responds to the following selected criteria:

- Low population density: no settlement within the next 3 km (or less than 10 habitats/ha within that area)
- Mooring density lower than 2 mooring ha$^{-1}$
- No harbour or mooring facility within 3 km
- No beach regeneration within 10 km
- No trawling is carried out in the area
- No industries within the 3 km
- No fish farms are present
- No desalination plants are present
- No evidence of meadow degradation due to other unconsidered impacts.

**Benchmark sites for CS-Spain and proposal benchmark sites for Malta - PREI:**

<u>CS-Spain</u>

Cap Santa Pola (38º 12' 44.7’ N; 000º 28' 52.8’ W)

Tabarca (38º 09' 36.1’ N; 000º 28' 38.7’ W)

PREI-Malta

Ramla l-Ħamra Bay (Gozo)[3] (36 º 04.269’ N ; 14 º 17.060’E)

Comino Channel (between Aħrax point and Comino) (36 º 00.334’ N; 14º 21.710 ‘E)

**Validation of the selection of the alternative benchmark with biological data:** In each benchmark the EQR was established.

**Description of boundary setting procedure set for the common IC type:** For each method and Member State, the boundaries were set up according to an equidistant division of a continuum as no evident discontinuities were detected. Consequently, the division of the ecological gradient in ecological quality classes with the class boundaries is very similar for the different methods/Member States. Since every Member State has selected its own reference sites to derive the reference conditions for the different parameters included in the method, the comparability of the high status class in relation to the pressures is checked through benchmarking.

#### *2.1.4.4 Benchmark Standardisation*

The EQRs of the unimpacted benchmark sites were very similar; no further adjustment of the common metric EQRs was needed as no geographical differences could be detected in the biological EQR values of the common metric in the same pressure environment. For the normalisation the subtraction option was used as the pressures behave in a parallel way.

### 2.1.5 Comparison of Methods and Boundaries

#### *2.1.5.1 IC Option and Common Metrics*

The IC option used is Option 2, as different metrics and different integration methods are used, and there are differences in data acquisition.

[3] This location was chosen instead of the alternative Dwejra (Gozo) (station CN01-1) based on expert knowledge on the size of the meadow, possible future impacts and resilience of the marine biocoenosis.

The IC common metric used is a biological common metric: the type of lower limit of the meadows and leaf surface area per shoot (Lopez y Royo et al, 2010).

### *2.1.5.2 Results of the Regression Comparison*

Overviews of the results of regression comparison are shown in Table 14, Figure 5, and Figure 6. All methods present a good correlation with the IC common metrics; therefore all of them are included in the IC exercise. The slope of the regression fulfils the requirement that the slope should lie between 0.5 and 1.5.

*Table 14 Results of regression analysis (National EQRs vs ICM)*

| Member State/Method | $R^2$ | slope |
|---|---|---|
| PREI | 0.812 | 0.935 |
| Valencian-CS | 0.897 | 1.097 |

**Checking the comparability of methods:** besides the regression analysis no statistical tests have been performed.

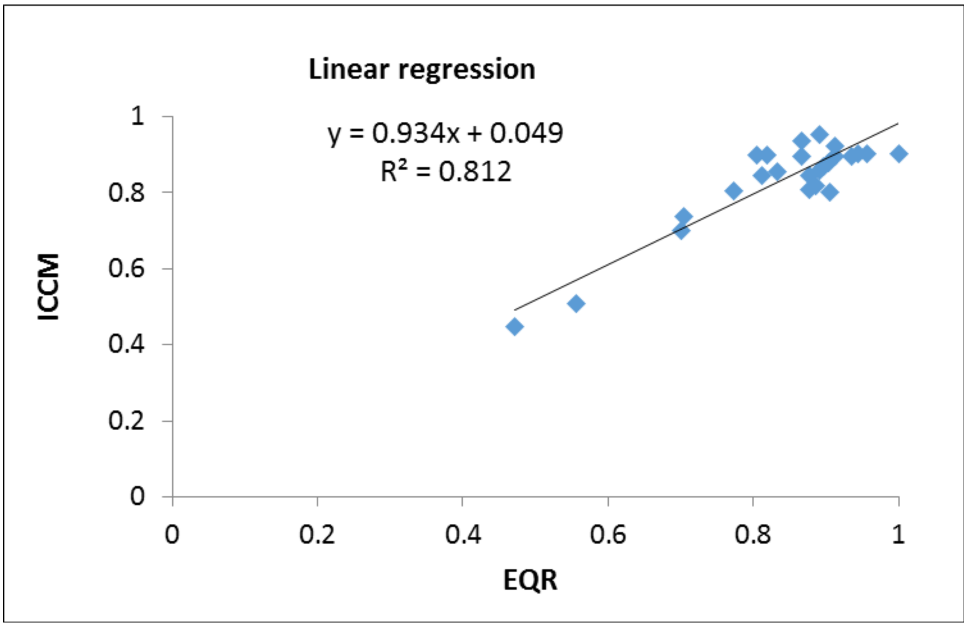


*Figure 5 Malta: PREI EQR on X-axis versus ICM EQR on Y-axis*

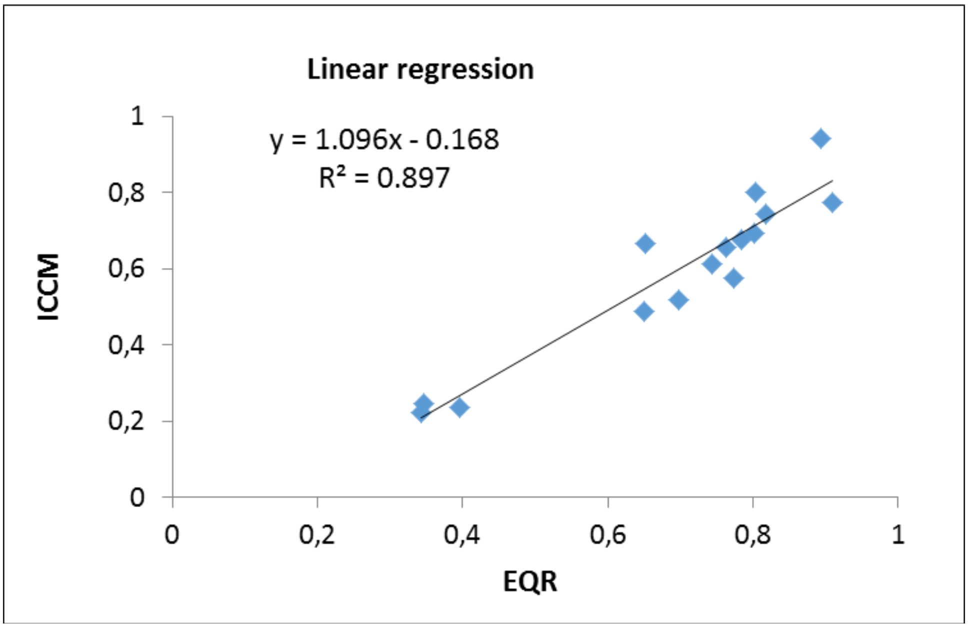


*Figure 6 Spain: CS-Valencian on X-axis versus ICM EQR on Y-axis*

### *2.1.5.3 Comparability Criteria*

**Assessing level of boundary bias**

No adjustment is needed. Class boundaries of each national classification system are 'translated' onto the ICCM, using the regression equations (Table 15).

*Table 15 Comparison of the methods: HG and GM boundary biases (HG- High-Good class boundary and Good-Moderate boundary).*

| Boundary | Malta | | Valencia | |
|---|---|---|---|---|
| | PREI | ICCM | Val | ICCM |
| **H/G** | 0.775 | 0.774 | 0.775 | 0.682 |
| **G/M** | 0.550 | 0.563 | 0.550 | 0.435 |

**Class Agreement:** No class agreement could be checked in the IC Option 2.

### 2.1.6 Final Results to be Included in the EC

#### *2.1.6.1 Table with EQRs*

*Table 16 Overview of the IC results for the national methods.*

| Biological Quality Element | | Seagrasses | |
|---|---|---|---|
| Results coastal waters: Ecological quality ratios of national classification systems | | | |
| Country | National classification systems intercalibrated | Ecological Quality Ratios | |
| | | High-Good boundary | Good-Moderate boundary |
| Malta | PREI - *Posidonia oceanica* Rapid Easy Index | 0.775 | 0.55 |
| Spain (Valencia) | Valencian-CS | 0.775 | 0.55 |

## 2.2 MACROALGAE

### 2.2.1 Description of National Assessment Methods

#### *2.2.1.1 Comparison between 2008 CARLIT and 2013 Square Sampling*

For macroalgae, data from the Final Coastal Water Monitoring Report of the Lot 3 ERDF project[4], were used. It transpires from this report that the algae were sampled using quadrats. This sampling procedure is considered inappropriate for the intercalibration procedures. The MEPA also provided data from the 2008 CARLIT survey. However, use of data from the 2008 CARLIT survey requires the assumption that the algal communities have not changed significantly since 2008. To assess such potential change, data from the 2013 surveys were used to validate the 2008 CARLIT data.

Comparison between the 2013 data and the 2008 CARLIT data required conversion of these two data sets to similar units: i.e. percent cover. For the quadrats from the 2013 stations, the

[4] MEPA tender reference CT3024/2011; Lot 3.

% cover values were estimated as the mean of the 5 quadrats sampled. For the 2008 CARLIT data, the % cover of macroalgae present in a coastal area some 1000 meters long was estimated. This coastal stretch was chosen on the basis of its proximity to the respective station from the 2013 survey (i.e. 500 m at each side of the station). The methodology applied is presented in Annex II.

The results of the comparison are presented in Table 17. Most (22) of the stations show acceptable similarities in % cover between both methods (black lines). Two stations CN04-1 and CP06 show differences between methods (red line) and three stations, CP04-2, CP06-2 and CN09, show different % cover values but similar ecological characteristics, since the communities from the different surveys are indicators of eutrophic and impacted areas (orange line). The differences between stations CN04-1 and CP06 and the corresponding CARLIT data are more probably due to sampling biases than to changes in the algal community between 2008 and 2013, since the direction of change is towards improving the quality status of the community, with a higher % cover of *Cystoreira*. Thus, the observed differences are more probably due to quadrat sampling being more biased than the CARLIT method. As a result, it was concluded that the CARLIT data can be validated using the quadrat data from the 2013 survey.

*Table 17 Comparison between the quadrat and CARLIT methods for 27 stations sampled in 2013 (% cover). Community codes are: Co= Corallinacea, Cys = Cystoseria spp, DD: Dendropoma spp. and L. bissoides structures, Lau= Laurencia spp, Photo= Photophilic algae and Ulva: Ulvacea (green algae).*

| W. BODY | SAMPLES | Method | Cys | Lau | Photo | Co | Ulva | DD |
|---|---|---|---|---|---|---|---|---|
| **1** | **CN01-1** | Quadrats | 85.00 | 0.00 | 2.00 | 13.00 | 0.00 | 0.00 |
| | | CARLIT | 86.96 | 0.00 | 0.00 | 10.38 | 0.00 | 2.66 |
| | **CN01-2** | Quadrats | 45.00 | 0.00 | 32.00 | 3.00 | 0.00 | 20.00 |
| | | CARLIT | 55.39 | 0.00 | 0.00 | 37.38 | 0.00 | 7.23 |
| | **CS01** | Quadrats | 34.00 | 0.00 | 20.00 | 40.00 | 0.00 | 6.00 |
| | | CARLIT | 56.10 | 0.00 | 0.00 | 37.80 | 0.00 | 6.09 |
| **2** | **CS02** | Quadrats | 96.00 | 0.00 | 0.00 | 4.00 | 0.00 | 0.00 |
| | | CARLIT | 83.09 | 0.00 | 0.00 | 16.91 | 0.00 | 0.00 |
| **3** | **CN03-1** | Quadrats | 82.00 | 10.00 | 2.00 | 6.00 | 0.00 | 0.00 |
| | | CARLIT | 66.87 | 0.00 | 0.00 | 29.11 | 4.02 | 0.00 |
| | **CN03-2** | Quadrats | 49.00 | 0.00 | 19.00 | 32.00 | 0.00 | 0.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | CARLIT | 54.25 | 0.00 | 0.00 | 45.75 | 0.00 | 0.00 |
| | **CN03-3** | Quadrats | 84.00 | 0.00 | 16.00 | 0.00 | 0.00 | 0.00 |
| | | CARLIT | 64.69 | 0.00 | 0.00 | 26.45 | 8.86 | 0.00 |
| | **CN03-4** | Quadrats | 85.00 | 0.00 | 2.00 | 13.00 | 0.00 | 0.00 |
| | | CARLIT | 72.08 | 0.00 | 0.00 | 27.92 | 0.00 | 0.00 |
| | **CN03-5** | Quadrats | 74.00 | 0.00 | 18.00 | 8.00 | 0.00 | 0.00 |
| | | CARLIT | 79.72 | 0.00 | 0.00 | 20.28 | 0.00 | 0.00 |
| | **CN03-6** | Quadrats | 50.00 | 0.00 | 48.00 | 2.00 | 0.00 | 0.00 |
| | | CARLIT | 100.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | **CS03** | Quadrats | 78.00 | 0.00 | 2.00 | 20.00 | 0.00 | 0.00 |
| | | CARLIT | 80.83 | 0.00 | 0.00 | 14.15 | 5.02 | 0.00 |
| **4** | **CN04-1** | Quadrats | 16.00 | 0.00 | 53.00 | 31.00 | 0.00 | 0.00 |
| | | CARLIT | 96.00 | 0.00 | 0.00 | 4.00 | 0.00 | 0.00 |
| | **CN04-4** | Quadrats | 8.00 | 0.00 | 41.00 | 51.00 | 0.00 | 0.00 |
| | | CARLIT | 39.41 | 0.00 | 0.00 | 60.59 | 0.00 | 0.00 |
| | **CN04-6** | Quadrats | 80.00 | 0.00 | 20.00 | 0.00 | 0.00 | 0.00 |
| | | CARLIT | 71.05 | 0.00 | 0.00 | 28.95 | 0.00 | 0.00 |
| | **CP04-1** | Quadrats | 90.00 | 10.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | CARLIT | 96.13 | 0.00 | 0.00 | 1.83 | 2.04 | 0.00 |
| | **CP04-2** | Quadrats | 40.00 | 52.00 | 1.00 | 7.00 | 0.00 | 0.00 |
| | * | CARLIT | 90.51 | 0.00 | 0.00 | 9.49 | 0.00 | 0.00 |
| **5** | **CN05** | Quadrats | 35.00 | 0.00 | 5.00 | 60.00 | 0.00 | 0.00 |
| | | CARLIT | 20.51 | 0.00 | 0.00 | 65.66 | 13.83 | 0.00 |
| | **CP05** | Quadrats | 14.00 | 0.00 | 10.00 | 76.00 | 0.00 | 0.00 |
| | | CARLIT | 17.82 | 0.00 | 0.00 | 80.35 | 1.83 | 0.00 |
| **6** | **CN06** | Quadrats | 73.00 | 10.00 | 7.00 | 10.00 | 0.00 | 0.00 |
| | | CARLIT | 92.92 | 0.00 | 0.00 | 7.08 | 0.00 | 0.00 |
| | **CP06-1** | Quadrats | 0.00 | 0.00 | 28.00 | 72.00 | 0.00 | 0.00 |
| | | CARLIT | 80.72 | 0.00 | 0.00 | 16.28 | 2.99 | 0.00 |
| | **CP06-2** | Quadrats | 18.00 | 0.00 | 45.00 | 37.00 | 0.00 | 0.00 |
| | ** | CARLIT | 78.04 | 0.00 | 0.00 | 21.96 | 0.00 | 0.00 |
| **7** | **CN07-1** | Quadrats | 95.00 | 0.00 | 0.00 | 5.00 | 0.00 | 0.00 |
| | | CARLIT | 87.19 | 0.00 | 0.00 | 0.00 | 12.81 | 0.00 |
| | **CP07** | Quadrats | 95.00 | 0.00 | 2.00 | 3.00 | 0.00 | 0.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *** | CARLIT | 100.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **8** | **CN08** | Quadrats | 72.00 | 0.00 | 20.00 | 8.00 | 0.00 | 0.00 |
| | | CARLIT | 71.37 | 0.00 | 0.00 | 26.66 | 0.00 | 1.97 |
| | **CS08** | Quadrats | 60.00 | 0.00 | 20.00 | 0.00 | 0.00 | 20.00 |
| | | CARLIT | 86.69 | 0.00 | 0.00 | 9.79 | 3.52 | 0.00 |
| **9** | **CN09** | Quadrats | 0.00 | 0.00 | 0.00 | 100.00 | 0.00 | 0.00 |
| | **** | CARLIT | 0.00 | 0.00 | 0.00 | 43.62 | 56.38 | 0.00 |
| | **CS09** | Quadrats | 86.00 | 0.00 | 5.00 | 9.00 | 0.00 | 0.00 |
| | | CARLIT | 60.20 | 0.00 | 0.00 | 25.95 | 13.85 | 0.00 |

Notes:

* Communities of medium-high density *Cystoseira* are often mixed with *Laurencia*, and represent similar water quality conditions. As CARLIT is focused on categories of *Cystoseira* density, sometimes a medium-density *Cystoseira* could, in fact, be a community dominated by *Laurencia* spp.
** Same as above: communities of medium-low density *Cystoseria* are often dominated by photophilic algae.
*** CN07-2 and CN07-3 represent sites that are located in a highly transformed water body, which are excluded from CARLIT analysis.
**** Two species of the same community showing anthropic impacts. Both species often co-exist in the same coastal area, and are therefore deemed equivalent.

*Table 18 Overview of the national assessment methods*

| Member State | Method | Included in this IC exercise |
|---|---|---|
| Spain | CARLIT = Cartography of Littoral and upper-sublittoral rocky-shore communities | Yes |
| Malta | CARLIT = Cartography of Littoral and upper-sublittoral rocky-shore communities | Yes |

### *2.2.1.2 Methods and Required BQE Parameters*

*Table 19 Overview of the metrics included in the national assessment methods*

| Member State | Full BQE method | Abundance | Disturbance sensitive taxa | (Diversity)* | Combination rule of metrics |
|---|---|---|---|---|---|
| Spain | Yes | Cover (length of coast in meters) of 9 pre-classified sensitivity classes | Communities sorted into 9 sensitivity classes | No | See below description of the method |
| Malta | Yes | Cover (length of coast in meters) of 9 pre-classified sensitivity classes | Communities sorted into 9 sensitivity classes | No | See below the description of the method |

*The optional non-obligatory parameter diversity is in brackets.

**CARLIT method**

The EQR is estimated using the following equation:

$$\mathrm{EQR} = \frac{\sum \frac{\mathrm{EQ}_{\mathrm{ssi}} * l_i}{\mathrm{EQ}_{\mathrm{rsi}}}}{\sum l_i}$$

Where

i = situation (geomorphologically relevant situation)

$EQ_{ssi}$ = EQ in the study site for situation i

$EQ_{rsi}$ = EQ in the reference sites for situation i

$l_i$ = coastal length in the study coast for situation i

### *2.2.1.3 Sampling and Data Processing*

*Table 20 Overview of sampling and data processing used in the national assessment methods included in the IC exercise.*

| **Information provided in the online WISER project assessment method questionnaires** | |
|---|---|
| Sampling/survey device | CARLIT: Visual sampling |
| How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? | CARLIT: Once a year |
| Sampling/survey months | Sampling time for CARLIT is May to June |
| Which method is used to select the sampling /survey site or area? | For CARLIT, the whole length of rocky coast is surveyed, except highly impacted areas (e.g. harbours), which are excluded |
| How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? | For CARLIT none if the whole length of rocky coast is surveyed. |
| Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of survey site or area is based | CARLIT: Around 400 km of study coast length and 250 km of reference zone coast length in Spain.<br><br>Around 200 km of coast length in Malta. |

| **Information provided in the online WISER project assessment method questionnaires** |
|---|
| Short description of field sampling/survey procedure and processing (sub-sampling):<br>The CARLIT method has been developed for littoral rocky coasts and for use at a fixed depth (0-0.5 m). Visual mapping is done, sorting the samples into 9 communities having different sensitivity levels.<br>A sampling unit is a sector of coast, at least 50 meters long, supporting a homogeneous community category (corresponding to a single community or combination of communities). The sectors are depicted on a graphical display. Rocky coasts are sampled by visual census of the dominant macroalgal community; this is carried out using small boats to enable surveying as close as possible (3-4 m) to the shoreline. The observed linear development of each macroalgal community is noted on a basemap (e.g. one obtained from aerial photography having a scale of 1:5000) and including details of the geomorphological features of the coastline being surveyed. The minimum length of the sampling unit is 50m. For some species (*Cystoseira* spp.) the % cover of the macroalgal belt is recorded, and macroalgae thalli samples are collected for the taxonomic identification, if necessary. |

### *2.2.1.4 National Reference Conditions*

*Table 21 Overview of the methodologies used to derive the reference conditions for the national assessment methods included in the IC exercise*

| **Member State** | **Type and period of reference conditions** | **Number of reference sites** | **Location of reference sites** | **Reference criteria used for selection of reference sites** |
|---|---|---|---|---|
| Spain | Expert knowledge, historical data in the Catalan coast before 1980's and in the adjacent Albères coast, least disturbed conditions from spring 2002 | 250 km of coast | Only 3 reference zones in natural parks from Corsica and Balearic Islands are considered representative for the entire Mediterranean Sea: Facade maritime du Parc Naturel Regional de Corse (France), Parc Natural de Ses Salines (Balearic Islands, Spain) and Reserva Marina del Nord de Menorca (Balearic Islands, Spain). | Undisturbed (or with only very minor disturbances) sites that cover a wide range of coastal geomorphologies, from different geological origins (volcanic, granite, calcareous, metamorphic) to different wave exposures and coastal morphologies. The reference zones present no or very low pressures as appear in the benchmark definition. |
| Malta | Expert knowledge | Near 14 km of coast | North coast of the Maltese Islands | Undisturbed (or with only very minor disturbances) sites that cover a wide range of coastal geomorphologies. The reference zones present no or very low pressures as per the benchmark definition. |

All the Member States have agreed on identification of reference sites according to the low pressures and impacts in accordance with Annex V of WFD.

Criteria used[5]:

- population density: no settlement, with more than 1000 individuals/km$^2$ in the next 15 km and/or more than 100 individuals/km$^2$ in the next 3 km within that area (winter population)
- no more than 10% of artificial coastline
- no harbour (more than 100 boats) within 3 km
- no beach regeneration within 1 km
- no industries within 3 km
- no fish farms within 1 km
- no desalination plants within 1 km
- no evidence of *Cystoseira* forest regression due to other unconsidered impacts; if there is evidence of *Cystoseira* regression (for example due to overgrazing), the quality element macroalgae may not be applied, depending on the method used).

Spain CARLIT: in 2001, with the first application of CARLIT, several water masses in NW Mediterranean Sea were selected (MPA of Menorca, Formentera, Corsica). More than 250 km of coastline were investigated using the cartographic approach. The selected sites presented no or very few human pressures (low inhabitant density, no industrial plants within the water basin, marine protected areas present). At present Spain, France and Italy are using the reference values proposed by Spain (Ballesteros *et al.*, 2007).

In the case of Malta, preliminary values using the full Mediterranean reference conditions show values higher than 1 EQR, hence, the selected Maltese reference zone is the northern parts of Gozo (WB MTC101)

[5] For Malta the experts have tried to satisfy of as many of the WFD criteria for reference sites as possible.

### *2.2.1.5 National Boundary Setting*

*Table 22 Explanations for national boundary setting of the national methods included in the IC exercise*

| Member State | Type of boundary setting: Expert judgment – statistical – ecological discontinuity – or mixed for different boundaries? | Specific approach for H/G boundary | Specific approach for G/M boundary | BSP: method tested against pressure |
|---|---|---|---|---|
| Spain - Malta | Boundaries are set according to biotic index and/or combined with the results of multivariate analysis. No statistical analysis exclusively to set boundaries. Calibrated against pre-classified sampling sites. Mixed. Continuum of possibilities with gradual disappearance/ appearance of different indicator species. Good-Moderate boundary indicated by the absence of *Cystoseira spp.* | Equidistant division: expert judgment of 25% deviation from the reference = EQR 0.75 | Equidistant division and modification: expert judgment of 40% deviation from the reference = EQR 0.6 | Yes, quantitative |

The boundaries were set up according to an equidistant division approach of a continuum as no evident discontinuities were detected.

### *2.2.1.6 Results of WFD Compliance Checking*

*Table 23 List of the WFD compliance criteria and the WFD compliance checking process and results of the national methods included in the IC exercise*

| Compliance criteria | Compliance checking conclusions |
|---|---|
| 1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad). | CARLIT: yes |
| 2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**) | CARLIT: yes |
| • Scope of detected pressures | See section on pressures addressed |
| • Has the pressure-impact relationship of the assessment method been tested? | Yes, see section on pressures addressed |
| • Setting of ecological status boundaries: methodology and reasoning to derive and set boundaries | See section on national boundary setting |
| • Boundary setting procedure in relation to the pressure:<br>• Which amount of data/pressure indicators have been related to the method and what was the outcome of the relation? | See section on pressures addressed |
| • Reference and Good status community description:<br>• Is a description of the communities of reference/high – good – moderate status provided? Not only a formula or an EQR value, but the range of values for the different parameters included in the method that result in high – good – moderate status | See section on ecological characteristics |
| 3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole. | CARLIT: yes |

| | |
|---|---|
| • Complete list of biological metric(s) used in assessment | See section on required BQE parameters |
| • Data basis for metric calculation | |
| • Combination rule for multimetrics | See section on required BQE parameters |
| 4. Assessment is adapted to intercalibration common types that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT | CARLIT: yes |
| • Is the assessment method applied to water bodies in the whole country? | Yes |
| • Specify common intercalibration types | See section on typology |
| • Does the selection of metrics differ between types of water bodies? | No |
| 5. The water body is assessed against type-specific near-natural reference conditions | CARLIT: yes |
| • Scope of reference conditions | CARLIT: Habitat-specific |
| • Key source(s) to derive reference conditions | CARLIT: Expert knowledge, historical data, least disturbed conditions; EEI: |
| • Number of sites, location and geographical coverage of sites used to derive reference conditions | CARLIT: 250 km of coast, only reference zones in natural parks from Corsica and Balearic Islands considered representative for the entire Mediterranean Sea (France & Spain). Very low-disturbed water body in the case of Malta. |
| • Time period (months + years) of data of sites used to derive reference conditions | CARLIT: Springtime 2002 (Spain), spring/summer 2008 (Malta) |
| • Reference site characterisation: criteria to select them | See section on National reference conditions |
| 6. Is a true reference used for the definition of High status or an | See section on National |

| | |
|---|---|
| alternative benchmark estimation? | reference conditions |
| 7. Sampling procedure allows for representative information about water body quality/ecological status in space and time | CARLIT: yes |
| • Has the uncertainty of the method been quantified and is it regarded in the assessment? | Yes, within the works carried out in the European WISER project |
| • Specify how the uncertainty has been quantified and regarded | To be done |
| 8. All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure | CARLIT: yes |
| 9. Selected taxonomic level achieves adequate confidence and precision in classification | CARLIT: yes |
| • Minimum size of organisms sampled and processed | CARLIT: Macroalgae (>1cm) |
| • Record of biological data: level of taxonomical identification – what groups to which level: | |
| CARLIT: Genus, Species/species groups. Algal communities (or combination of communities), the main algal species and the mussel *Mytilus galloprovincialis* or other dominant macroinvertebrates. | |

## 2.2.2 Results IC Feasibility Checking

### *2.2.2.1 Typology*

*Table 24 Description of common intercalibration water body types and the MS sharing each type*

| Method | Appropriate for IC types/subtypes |
|---|---|
| CARLIT | Rocky shore |

The Intercalibration is feasible in terms of typology. In fact, typology is not relevant for the BQE macroalgae in Mediterranean coastal waters. Common IC type: only one type - the entire Mediterranean Sea; there is no subdivision since the entire rocky shore of all Member States is considered.

During the early stages of the CIS the Mediterranean working group agreed to use only 2 parameters to distinguish different water types, namely substratum type and depth. Most of

the other geomorphological parameters, described in Directive Annex II (1.2.4), were not relevant (i.e. tidal regime) to distinguish different water types in relation to their ecological 'significance' for the Mediterranean Sea.
For intercalibration of macroalgae, the methods used are applied to macroalgal communities (species composition and abundance) of the upper infralittoral zone (3.5 to 0.2 m depth) on rocky coasts, with no distinction between types.

### *2.2.2.2 Pressures Addressed*

The relationship between pressures and EQR values has been calculated using the new MA-LUSI index[6]. This has been developed as an improvement of the LUSI index (Flo *et al*., 2011); specifically for shallow water macroalgal communities. The new MA-LUSI[6] index includes not only pressures from the original LUSI Index but also some other pressures that affect macroalgae communities, such as mariculture, sewage outfalls, harbour activities and irregular fresh water inputs. In some countries, the background trophic status has also been included as a local pressure. The MA-LUSI[5] values were calculated using a 1 km buffer zone around the sampling site on pressure maps. For the CARLIT method, the site has been defined as 1 km of coastline.

*Table 25 Pressures addressed by the national methods and overview of the relationship between national methods and the pressures. (Pressures: EU = eutrophication, GD = general degradation*

| Member State | Method/ Metrics tested | Pressure | Pressure indicators | Amount of data | Strength of relationship |
|---|---|---|---|---|---|
| Spain | CARLIT | EU, GD | MA-LUSI[6] index<br>Following pressures are included:<br>a) Indirect pressures from Corine database (urban, commercial & industrial, agriculture)<br>b) Direct pressures (sewage | 40 sites | Linear regression ($p<0.05$) |

[6] See ANNEX III.

| | | | | | |
|---|---|---|---|---|---|
| | | | outfall, mariculture, sediment nutrient release, irregular freshwater inputs, harbour activites)<br>c) Confinement | | |
| Malta | CARLIT | EU, GD | MA-LUSI[5] index<br>Following pressures are included:<br>a) Indirect pressures extracted from the MEPA pressure maps (urban, commercial & industrial, agriculture, harbour activities)<br>b) Direct pressures (Sewage outfall, aquaculture, bunkering zones, reverse osmosis installations)<br>c) Confinement | 9 sites | Linear regression ($p<0.05$) |

The regression of each method in relation to pressures is presented below (Figure 7 and 8):

**Spain-CARLIT**

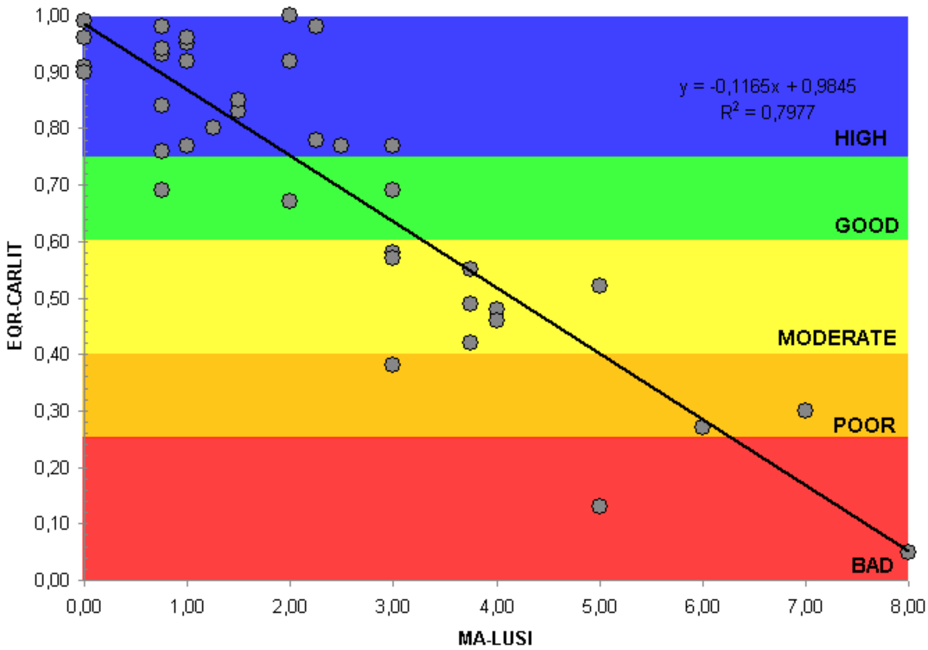


*Figure 7 Relation between CARLIT and MA-LUSI[5] in Spanish coastal waters*

**MALTA – CARLIT**

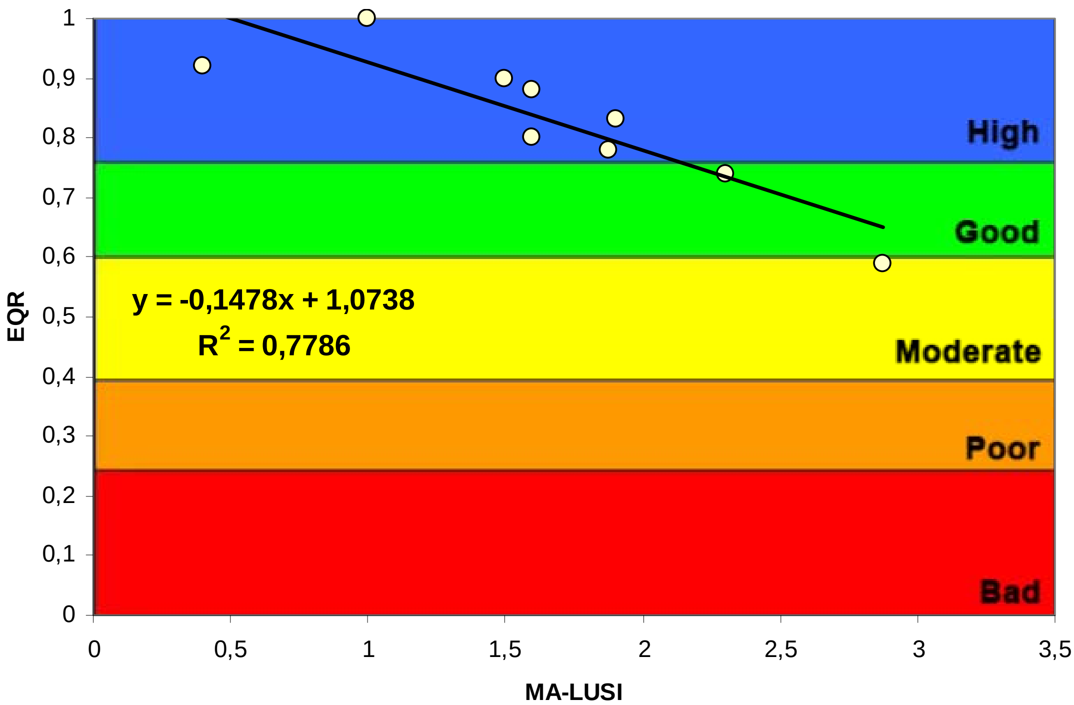


*Figure 8 Relation between EQR and pressure data (expressed as MA-LUSI[5] index) in Maltese coastal waters*

*Table 26 Pressures considered in intercalibration*

| Method | Pressure |
|---|---|
| CARLIT | Eutrophication, aquatic habitat destruction, general degradation, pollution by organic matter |

**Conclusion**

According to the available data and the results shown, the IC is feasible in terms of pressures. The MS present a good correlation between MA-LUSI[5] and EQR values.

## 2.2.3 Collection of IC Dataset and Benchmarking

### *2.2.3.1 Dataset Description*

*Table 27 Description of data collection within the GIG*

| | |
|---|---|
| Size of common dataset: total number of sites | 49 sites, 40 sites for Spain, 9 for Malta |
| Number of Member States | 2 |
| Repackage/disaggregation of samples/ WB results? | No |
| Gradient of ecological quality | Fully covered (only for Spain 5 classes) |
| Coverage per ecological quality class | For Spain the 5 ecological quality classes are covered, for Malta 3 classes are covered (no bad or poor status) |

*Table 28 Overview of the number of sites/samples/data values*

| Member State | | Number of sites or samples or data values | |
|---|---|---|---|
| Spain | CARLIT: Cartography of Catalonia, Balearic Island and Valencia coast. More than 100 sample sites* in the entire Spanish Mediterranean coast | In Catalonia yes, from the same WBs, but not collected in the monitoring sites | X according to MA-LUSI[6]) |
| Malta | CARLIT: Cartography of full Malta coastline. | Yes, from the monitoring sites | X (according to MA-LUSI[6]) |

* For the CARLIT method a site is defined as 1km of coastline.

### 2.2.3.2 Data Acceptance Criteria

*Table 29 Overview of the data acceptance criteria used for the data quality control*

| Data acceptance criteria | Data acceptance checking |
|---|---|
| Data requirements (obligatory and optional) | The CARLIT method is developed for littoral rocky coasts and at a fixed depth (0-3 m). Sampling time for CARLIT is scheduled from May to June |
| The sampling and analytical methodology | Visual mapping for CARLIT |
| Level of taxonomic precision required and taxa lists with codes | The samples are assigned to 9 communities that have a different sensitivity for CARLIT |
| The minimum number of sites / samples per intercalibration type | - |
| Sufficient covering of all relevant quality classes per type | All quality classes are covered sufficiently. |

### 2.2.3.3 Common Benchmark

In the case of macroalgae, no standardization in needed. The CARLIT method is fully tested and accepted as a valid method of the EQR calculation. Using this methodology to calculate the EQR avoids problems with intercalibration and common metrics, since the same method is used, and direct comparison is feasible.

*Table 30 List of reference sites Macroalgae*

| MS | Number of sites | EQR ± SD | Name of sites | Coordinates (WGS84) | Date of sampling |
|---|---|---|---|---|---|
| Spain | 6 sites | 0.94±0.08 | Catalan coast: 5, 17, 21,47, 54, 72 | X Y<br>525625 4684715<br>523308 4678208<br>519449 4645757<br>513752 4694591<br>514911 4635747<br>495111 4618869 | Spring 2009 |
| Malta | 1 site | 1 | Malta WB MTC101[7] | 436503 3990903 | Spring 2008 |

[7] WB MTC101 is chosen due to its extent and variety of habitats and communities, combined with a low pressure (MA-LUSIma Index). Although WB MTC108 has a lower Ma-LUSIma index (a priori lower impacted zone), it supports lower habitat complexity, which produces a lower EQR value. So, based on expert opinion, WB MTC101 fits more appropriately in the definition of a reference zone than WB MTC108.

Screening of the biological data for impacts caused by pressures not regarded in the reference criteria was ensured so that true reference sites are selected; however, some large-scale pressures cannot be avoided (for example climate change). The biological assemblages were not screened in order to select reference sites, because the present authors feel that the choice of reference sites has to be independent of the biological assemblages.

A description of setting reference conditions (summary statistics used) is the following: The reference conditions for the infralittoral fringe macroalgal assemblages (in the Mediterranean Sea) are generally considered mostly to be the *Cystoseira* spp. assemblages (but not exclusively, for example *Lithophyllum byssoides* may also be used). No statistical analyses were carried out for the present BQE, given that this is largely described in the scientific literature (both old and recent).

### 2.2.4 Final Results to be included in the EC

#### *2.2.4.1 Table with EQRs*

*Table 31 Overview of the IC results for the national methods*

| **Biological Quality Element** | **Macroalgae** | | |
|---|---|---|---|
| Results for coastal waters: Ecological quality ratios of national classification systems<br>The following results apply to the upper infralittoral zone (3.5 – 0.2 m depth) on rocky coasts: | | | |
| **Country** | **National classification systems intercalibrated** | **Ecological Quality Ratios** | |
| | | **High-Good boundary** | **Good-Moderate boundary** |
| Spain | CARLIT - Cartography of Littoral and upper-sublittoral rocky-shore communities | 0.75 | 0.60 |
| Malta | CARLIT - Cartography of Littoral and upper-sublittoral rocky-shore communities | 0.75 | 0.60 |

#### *2.2.4.2 Correspondence Common Types versus National Types*

It is not necessary to transform the common intercalibration types and common boundaries into the national typologies/assessment systems. The results are directly applicable to the national types that belong to the common type.

#### *2.2.4.3 Gaps of the Current Intercalibration*

The major gap in the current intercalibration is the lack of temporal replication since only data from the 2008 CARLIT survey and the 2013 quadrat survey (a different method) were available.

### 2.2.5 Ecological Characteristics

#### *2.2.5.1 Description of Reference or Alternative Benchmark Communities*

The common views for reference conditions can be summarized as follows:

1. Macroalgal communities of high diversity (more than 20 species (Eastern) and 40 species (Western) both at reference and benchmark sites) should be dominated by brown algae mainly of the order Fucales in high irradiance sites and red algae of the order Corallinales (or other sciaphilic species) on vertical cliffs.
2. Dense well-developed macroalgal communities thriving in the upper infralittoral zone with most characteristic species belonging to the genera *Cystoseira, Sargassum, Lithophyllum, Peyssonnelia, Corallina* and *Padina*. Other common species belong to the genera *Halopteris, Stypocaulon, Dictyota, Dictyopteris, Laurencia, Cladophora* and *Jania*.
3. In the shadow zones (exposed steep vertical cliffs) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir). In marine caves with scarce light conditions a sciaphilic community of red and green algae is dominant.
4. Spatio-temporal variability of the community's composition and abundance is affected by the availability of hard substratum, intense and frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intense, and by limiting factors such as nutrient levels.

CARLIT:

Rocky shore sites exposed to high irradiance levels and characterized by dense communities of several *Cystoseira* species: *C. mediterranea/amentacea var*. *stricta*, C. crinita, C. *brachyparpa var. balearica*, *C. foeniculacea*/*barbata*/*spinosa* var. *tenui*or/*compressa var*. *pustulata*. Alternatively, in shadow zones (steep vertical cliffs, high hydrodynamic conditions) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir).

### *2.2.5.2 Description of Good Status Communities*

A description of IC type-specific biological communities representing the 'borderline' conditions between good and moderate ecological status, considering possible biogeographical differences (as much as possible based on the common dataset and common metrics) is as follows:

Species of the genus *Cystoseira* (Fucales, Cystoseiraceae) dominate the Mediterranean upper-infralittoral communities (Feldmann, 1937; Boudouresque, 1971) and are particularly sensitive to any natural (Gros, 1978; Verlaque, 1987) or anthropogenic stress (Bellan-Santini 1966; Ballesteros *et al*., 1984; Hoffmann *et al*., 1988; Soltan *et al.*, 2001) and, therefore, have experienced profound changes and decline over extensive areas (Thibaut *et al.*, 2005). The highly structured and productive *Cystoseira mediterranea/stricta/crinita* communites are observed in hydrodynamic environments and non-polluted waters along the northwestern Mediterranean coasts (Boudouresque, 1969; Ballesteros, 1988). Increasing levels of organic matter and nutrients results in *Cystoseira*-dominated communities being replaced by the red alga *Corallina elongata* (Bellan-Santini, 1965, 1968; Ballesteros *et al*., 1984; Giaccone, 1993) and the mussel *Mytilus galloprovincialis* (Bellan-Santini, 1965, 1968; Bellan and Bellan-Santini, 1972). Green ephemeral algae begin to dominate in highly disturbed environments and near freshwater discharges: Ulva (Golubic, 1970; Bellan and Bellan-Santini, 1972; Rodriguez-Prieto and Polo, 1996), *Cladophora* (Belsher, 1977) or *Enteromorpha* (Ballesteros *et al.*, 1984; Kadari- Meziane, 1994). Finally, the dominance of blue-green algae (*Oscillatoria, Lyngbya, Phormidium*) indicates very degraded environments (Golubic, 1970; Littler and Murray, 1975; Murray and Littler, 1978).

Based on these ecological changes, along pollution gradients, the boundary between High and Good conditions is defined when the *Cystoseira* communities occur in patches and do not comprise extensive-continuous assemblages, and the *Lithophyllum byssoides* belt displays symptoms of degradation. Samples of *Cystoseira* assemblages indicate a lower biomass of *Cystoseira* spp. and the substitution of sciaphilic species inhabiting the underlayer dense *Cystoseira* assemblages by *Corallina elongata* or *Mytilus galloprovincialis*. The disappearance of these sensitive species and replacement by stress tolerant species such as *C. elongata* and *M. galloprovincialis* defines the boundary between Good and Moderate situations.

Nevertheless, the presence and abundance of littoral and sublittoral communities respond not only to water quality but also to other anthropogenic disturbances and variability of natural geomorphological and physical factors in coastal areas. In situations defined as high water quality conditions, the sensitive species can be replaced by stress-tolerant ones, where other stress factors (e.g. low irradiance or sand abrasion) are affecting them.

Despite the communities representing the borderlines are the same all around the Mediterranean coast, there are some important differences that should be considered. *Cystoseira compressa* is a plastic species that can live either in pristine or in moderately degraded coastal waters. This phenomenon is a bit more pronounced in Aegean Sea and in the Gulf of Trieste (North Adriatic), which are in comparison to other Mediterranean parts, very shallow. In a recent paper (Orfanidis *et al.* 2011) SIMPER analysis was used to quantify the contribution of *C. compressa* to the *C. crinita* community in Aegean pristine sites; this turned out to be up to 7.17 (%) (Mean coverage 15.5). Therefore, the abundance of *Cystoseira compressa* in the eastern High and Good sites is considerably higher than in the western sites.

# 3 DATA GAPS AND FUTURE MONITORING NEEDS

## 3.1 BENTHIC INVERTEBRATES

The recommendations are aimed at 1) avoiding data collection problems in the future, 2) filling the data gaps identified in the Maltese data sets, 3) facilitating and simplifying sampling procedures, and 4) supporting the intercalibration process in future years.

### 3.1.1 Avoiding data collection problems in the future.

The following problems related to data collection were identified:

*Table 32 Extract from Table 1 of the interim report[8] with identified problems with respect to 14 and 16.*

| ID | Problem | Solution | Done/Solved |
|---|---|---|---|
| **14** | The benthic samples were not collected at the monitoring stations used for other parameters such as physico-chemical parameters, while the geographical coordinates of the actual sampling stations are not available. Consequently, essential data on sediment grain size are not available. | The maps of indicative substratum types for Gozo and Malta provided by MEPA cannot be used without an indication of the location where the samples were collected. Without an indication of sediment grain size, intercalibration cannot be carried out since data for benthic invertebrates collected from similar soft sediments are required for comparison between Member States. | NO/NO |
| **16** | Samples were collected from a water depth of 50 m. | Intercalibration of benthic invertebrates will remain incomplete. Basic calculations and procedures to mitigate for this will be provided and applied to the available data. | NO/NO |

[8] Ecoserv Ltd. And UCV-IMEDMAR (2014). Service Tender for the Provision of Scientific Expert/s Assistance in the Intercalibration Exercise of Biological Elements of Maltese Coastal Waters – Interim Report on Proposed Plan of Work [MEPA tender reference: T03/2014]. Mosta, Malta: Ecoserv Ltd; October 2014; 19 pp.

| | | | |
|---|---|---|---|
| | | Other countries have collected benthic samples from shallow sites, which means intercalibration with Maltese samples will not be possible. | |

The recommendations for these 2 problems are aimed at adoption of correct sampling procedures to study the marine benthos of sublittoral waters. Stations should always be correctly georeferenced, and it should be ensured that samples are collected in the geo-referenced site. Additionally, MS agreed to collect samples within the 8 - 12 m depth range to enable successful intercalibration. The MS have collected most samples within this depth range. It is highly recommended to adopt this depth range to facilitate future intercalibration and comparisons with other MS. In exceptional circumstances, such as when a sandy seabed is scarce, the water depth range could be extended to between 6 m and 15 m, and very exceptionally to 18 m, but providing justification as to why a different depth was used (see below). Assessing the granulometry of the soft sediments is strongly recommended, in order to correctly interpret the invertebrate community in the context of the substratum type where the community is present. This has a further advantage, as described in point 2).

If sandy sediment is not found in an area, the recommended procedure is the following:

i. If a sandy seabed is not present in an area within the 8 - 12 m depth range, because, for example, there is a dense seagrass meadow or a rocky bottom, the first approach is simply to not use the site and instead use an alternative station located within a few km around the original site.
ii. If still no sandy seabed is present within a few km of the original station, then he 6 m - 15 m depth range at the original site should be explored.
iii. If still no sandy sediment is found, then proceed to explore the limit between 6 m and 15 m in the area a few km around the original station.
iv. If no one of the previous solutions solves the problem, then explore the seabed in the general area to a maximum depth of 18 m. This would be an exceptional solution if all the previous procedures do not solve the problem and no sandy seabed is located off a considerable stretch of coast (e.g. 5 km long). Knowing details of the sediment granulometry becomes more important if sampling is carried out at this depth.

v. If no sandy seabed is present in the area within a few km around the station and within the 6 - 18 m depth range, sampling at the problematic station will not be carried out and the sampling team should proceed to the nearest station location where a soft sediment seabed is present within the 8 - 12 m depth range, and considering points i to iv above.

### 3.1.2 Filling the data gaps identified in the Maltese data sets

The most relevant data gap observed in the Maltese data sets is the lack of studies of granulometry and organic matter content of the sediments. As explained above, assessing these sediment attributes has many advantages and introduces important information that facilitates the intercalibration process. Knowing the sediment type and organic matter content may be used to better understand the invertebrate community associated with the soft sediment. It also facilitates comparison among stations because sediments with different characteristics usually support different invertebrate species.

Furthermore, knowing the sediment type and invertebrate community composition within the station itself helps one to understand the community dynamics. For example, in Valencia (Spain) some stations had a community composition that included species associated with sandy bottoms found in sediments enriched with mud, as determined by the granulometric assessment. This situation was interpreted as one in which the site concerned was being gradually silted and enabled i) the prediction of a future shift in the invertebrate community and ii) an early warning sign that some process was modifying the typical coastal dynamics at the site. Finally, collecting time series data on the sediment granulometric characteristics at the same stations over a number of years allows monitoring of potential changes in sediment type, and renders relevant indirect information on the occurrence of possible impacts that could change the properties of the sediments.

### 3.1.3 Facilitating and simplifying sampling procedures

Two main improvements may make the sampling procedure more reliable; these are summarized in problem 15 from Table 1 of the interim report:

*Table 33 Extract from Table 1 of the interim report with problem 15.*

| **ID** | Problem | Solution | Done/Solved |
|---|---|---|---|
| **15** | A 1 mm mesh size was used to process benthic samples, when it should have been 0.5 mm. | Since the grab size is large (0.1 m2) there is the possibility that the number of invertebrates collected could partly compensate for the loss of material that resulted from use of a large mesh size. Moreover, both Greece and Cyprus used a 1 mm mesh; although a 1 mm mesh size is not ideal for Malta, this should not be a serious problem. | YES/YES |

The samples may be collected with a $0.025m^2$ Van Been grab instead of using a much heavier $0.1m^2$ grab. The smaller grab, together with collection of samples within the 8 m - 12 m depth range enables easier handling of the grab by just two persons and avoiding use of a jib. This has the additional advantage of possibly using a smaller vessel, such as a small boat or rubber dingy. This, in turns, reduces considerably the sampling time during the surveys. Depending on the distance between stations, up to 8 stations, including two[9] replicates per station could be sampled per day.

The utilization of a 0.5 mm mesh is recommended to reduce loss of invertebrates and to improve the reliability of sampling.

Given the heterogeneous distribution of *Posidonia oceanica* and rocky bottom habitats around Malta, it is recommended to consider first carrying out a pilot survey that will be aimed at establishing the locations of stations on a soft sediment seabed and appropriate depth range. This could be easily done by a priori selection of an excess number of potential stations on a map, and then investigating them further using SCUBA diving and/or ROV techniques. In this way it will be possible to establish which stations are the most appropriate for undertaking the actual sampling of benthic invertebrates.

[9] The number of replicates depends mostly on the quantity of invertebrates present in the sediment. If low numbers are observed, more replicates per station could be necessary.

### 3.1.4 Supporting the intercalibration process in future years.

The most important feature to support the intercalibration in future years is temporal replication of the surveys. This is not only recommendable in order to complete the intercalibration, but also to obtain the necessary quantitative information for use in interpretation of the intensity of impacts, understanding of the dynamics of the benthic invertebrate community and plan mitigation strategies, if necessary. Considering the length of Malta coasts, we recommend sampling 40 stations, although the number may vary slightly up or down depending on the variability of the stations.

*Table 34 Extract from Table 1 of the interim report with problem 7.*

| ID | Problem | Solution | Done/Solved |
|---|---|---|---|
| **7** | Lack of temporal replication of surveys (all BQEs) | The WFD indicates that the sampling procedure should be representative, providing information about the quality of the water masses both in space and time; surveys should thus be replicated in time. No such temporal replicates are available for the Maltese Islands, and as a consequence the available data can only be considered to provide a preliminary indication of the status of Maltese coastal water bodies. Intercalibration will be carried out wherever possible as part of the present tender, but the results may nevertheless not be accepted by the EU's JRC due to the preliminary nature of the data and in particular the lack of such temporal replication. | NO |
| **17** | AMBI is not intercalibrated among countries. | BENTIX and BOPA would have been calculated, and the BENTIX, AMBI and BOPA could have been compared using correlation analysis. Such a comparative analysis is however not possible due to the lack of disaggregated data (see point 13 above). | NO/YES |

As explained in problem 17 shown in Table 3, we recommend calculating the BENTIX index, because it has been already intercalibrated among countries. This would facilitate the intercalibration process in future years.

It is also recommendable that samples be collected from impacted sites and adjacent areas. This will help establish the extent of the impact and possibly control its progression with time. Samples may be collected close to the impacted site and at two reference stations situated c. 0.5 to 1 km from the impacted site, depending on the type and intensity of the impact.

Regarding the invertebrate species to be identified, it is recommended to focus on the following: polychaetes, molluscs, decapods crustaceans and echinoderms. If other groups such as cummaceans, amphipods or tanaidaceans are abundant, then it would be recommendable to identify them as well.

## 3.2 PHYTOPLANKTON

The below recommendations are aimed at avoiding data collection problems in the future, and to facilitate the use of existing data on phytoplankton.

### 3.2.1 Avoiding data collection problems in the future.

The following problems related to phytoplankton data collection were noted (Table 35):

*Table 35 Extract from Table 1 of the interim report[10] with problems 9 and 12.*

| ID | Problem | Solution | Done/Solved |
|---|---|---|---|
| **9** | Phytoplankton samples were collected at a water depth of 5 m. Other Member States collected samples at several water depths, and also took | It will only be possible to use the Maltese phytoplankton dataset for intercalibration if it can be demonstrated that the data collected at 5 m depth are representative of the whole water column, i.e. that there is a | NO/NO |

[10] Ecoserv Ltd. and UCV-IMEDMAR (2014). Service Tender for the Provision of Scientific Expert/s Assistance in the Intercalibration Exercise of Biological Elements of Maltese Coastal Waters – Interim Report on Proposed Plan of Work [MEPA tender reference: T03/2014]; 19pp.

| | | | |
|---|---|---|---|
| | more than one sample by sampling throughout the whole water column. Additionally, other Member States have also included a surface water sample (e.g. Cyprus has three samples of water for the whole column, and one of them is collected at the surface). | completely mixed water column with regards to light, temperature, water transparency etc. The necessary data on the hydrodynamics of Maltase water columns are not available, making intercalibration of this BQE is impossible. | |
| **10** | Two different methods are used to measure chlorophyll (SPFT & SPFL) and the method which is not normally used in WFD monitoring (SPFL) is then chosen. | The spectrofluorometrically obtained data set will be analysed in detail. The SPFT method is the most widely used because the equipment is available in most laboratories; the fluorimeter has to be calibrated with chlorophyll *a* standards, which are very susceptible to degradation. SPFL is more sensitive than the SPFT (so a smaller sample can be used), but to achieve acceptable results it is necessary to calibrate the fluorometer spectrophotometrically with a sample from the same data source. | NO/YES |
| **11** | Not all plankton sampling stations are located in the nearshore zone (i.e., located 500 - 1500 m from the shore) as required for WFD monitoring | A common decision taken by the phytoplankton group during intercalibration is that only coastal nearshore data (500 m - 1500 m from the shore) is used for comparing the ecological status between Member States[11]. All reference conditions and boundaries proposed in the intercalibration exercise are thus referring | NO/NO |

[11] JRC Scientific and Technical Reports Water Framework Directive intercalibration technical report. Part 3: Coastal and Transitional waters. Edited by Alessandro Carletti and Anna-Stiina Heiskanen. EUR 23838 EN/3 – 2009. ©

| | | | |
|---|---|---|---|
| | | to this zone. Not all the samples collected in Malta were collected within a distance of 500 m - 1500 m from the coast, since the majority of stations were located inshore (7 of a total of 12 phytoplankton sampling stations). A relationship between inshore/nearshore/offshore samples would thus have to be established in order estimate the nearshore equivalent of inshore sites. In Valencia (Spain) such a conversion was carried out using data collected from several transects laid perpendicular to the coastline. However, such transects are not available for Malta and as a consequence it is not possible to estimate the nearshore equivalent of samples collected inshore. | |
| **12** | In the MSFD initial assessment for the water column habitats report it is stressed that there are two spikes of chlorophyll *a*, one in late summer and the other in winter. However, no survey in winter is included in the data provided. | Chlorophyll *a* data collected during winter is required in order to establish the correct ecological status of the water bodies, which is an important prerequisite for the intercalibration process. No such data are available for the Maltese Islands. | NO/NO |

The following recommendations to address the problems listed in Table 35 are related to the application of the recommended sampling procedures for intercalibration of the phytoplankton BQE. A common decision taken by the phytoplankton group during intercalibration is that only coastal nearshore data (500 m - 1500 m from the shore) should be used to compare the status between

Member States[12]. All the reference conditions and boundaries proposed in the intercalibration exercise thus refer to this zone.

Chlorophyll needs to be monitored at least quarterly – monitoring periods are also vital and the actual months should be stipulated. However, in order to identify chlorophyll peaks, a time series of precise and complete data is required, which is not yet available for the Maltese Islands. As an example, to find when the peaks occurred in Valencia (Spain) the Spanish plankton experts collected samples at 110 stations on a monthly basis during 5 years. With the information obtained from this extensive five year survey, it was possible to reduce the number of sampling stations to 70, and the temporal scale to 'seasonally' (i.e. four surveys a year, taking place in spring, summer, autumn, and winter, instead of the former twelve surveys a year). By analysing a time series of detailed surveys it was possible to select and specify the best month to collect samples during every season.

Consequently it is recommended to collect monthly samples during at least one year at a suitable number of sampling stations[13]. Sampling stations should always be geo-referenced, in order to ensure that samples are always collected at the same site, and at the same distance from the shore (nearshore if possible). Samples should be collected at superficial depth (0 m). It is highly recommended to collect samples at this distance and depth to facilitate future intercalibration and comparison with other MS. The data from such a detailed survey should then be analysed in order to identify the most suitable sampling stations and sampling months to monitor seasonal phytoplankton variations.

### 3.2.2 Re-utilisation of existing data

During the Lot 3 ERDF baseline study phytoplankton samples were collected at a water depth of 5 m at each of 12 stations located around the Maltese Islands. Chlorophyll *a* levels were determined spectrophotometrically (SPFT) and spectrofluorometrically (SPFL), and the main taxa found in the samples identified microscopically. Stations were located either inshore (up to 500 m from the coast; N= 7 see Annex II) or nearshore (500 m – 1500 m distance from shore; N= 5). However, only data

[12] JRC Scientific and Technical Reports Water Framework Directive intercalibration technical report. Part 3: Coastal and Transitional waters. Edited by Alessandro Carletti and Anna-Stiina Heiskanen. EUR 23838 EN/3 – 2009. ©

[13] Malta coast length is approximately half of that of *Comunidad Valenciana* (Spain), so we recommend sampling c. 55 stations in the monthly surveys of the first year. These stations would be reduced in the following years eliminating those found to produce redundant data.

from nearshore stations can be used for the purpose of the intercalibration exercise; hence it will be necessary to attempt establishing a relationship between the inshore and nearshore stations in order to use the entire dataset. Such a relationship can however only be established using data on phytoplankton from coastal transects, which are currently not available for the Maltese Islands.

Since samples were only collected at a water depth of 5 m, this is a further major shortcoming of the available dataset. Other Member States have collected samples at several water depths, and several samples were collected in order to characterise the vertical profile of the water column. Moreover, the surface water sample is always collected. It would only have been possible to use the Maltese phytoplankton dataset for intercalibration by demonstrating that the data collected at 5 m depth are representative of the whole water column, i.e. that there is a completely mixed water column with regards to light, temperature, water transparency etc. Detailed data on the hydrodynamics of Maltase water columns would have been required to achieve this, which do not appear to be available.

Therefore, to reuse the data already collected it is necessary to select 4-5 stations and study vertical and horizontal transects to:

1. Check whether the water column is more or less homogeneous horizontally (i.e. with distance to the shore) and vertically. If so, the old data are automatically usable, or
2. Calculate a correction factor to apply to the previous data to make it usable. This correction factor is calculated from the vertical and horizontal profiles.

Depending on the previous, if 1) is achieved, and the data can be reused, then it would be possible to continue working in the same manner as for the ERDF Lot 3 baseline study (i.e. using the same sampling stations and the same depths), and including  the missed seasons. However if objective 1) is not achieved, then samples should be collected at the water surface at all stations. It should at all times be clearly defined whether samples are taken at inshore or nearshore sites. If objective 1 cannot be achieved, the existing phytoplankton dataset cannot be used.

Re-utilisation of the existing data would provide further information to ensure the selection of representative sampling sites when designing a phytoplankton monitoring survey as described in section 3.2.1 above.

# 4 DETERMINATION OF REFERENCE SITES

To establish reference sites, four criteria are used:

1- Lowest values of MA-LUSI indices;

2- Satisfaction of as many of the WFD criteria for reference sites as possible;

3- Highest EQR values;

4-Expert knowledge on Maltese marine biocoenosis.

Pressure values for the calculation of the LUSI index were assigned based on expert judgement, using the available data from GIS layers and pressure maps provided by MEPA. The stations or WB with lower LUSI index values were then checked for compliance with as many of the WFD criteria as possible, as well as for high EQR values. For example, for the *Posidonia* BQE the reference points selected were those corresponding to stations CS03 and CSC2. Ramla l-Ħamra Bay (Gozo) (Station CS02) was selected instead of Dwejra (Gozo) (Station CN01-1) because expert knowledge of conditions in the area indicate that in Dwejra (Gozo) the meadow is relatively small. Furthermore, there could be impacts from run-off of the nearby valley, which, together with the size if the meadow in this site, could affect future EQR values, rendering the reference point obsolete. Instead, the presence of larger and healthier seagrass meadows at Ramla l-Ħamra Bay (Gozo) justify the choice of this location as a seagrass reference point for the Maltese Islands, despite that slightly higher value of MA-LUSIpo were recorded at this location during the Lot 3 ERDF baseline surveys than at Dwejra (Gozo).

*Table 36 Stations selected as possible reference sites for Posidonia BQE.*

| Sampling Station | LUSI[6] | EQR | Station Name | Lat (°N) | Long (°E) |
|---|---|---|---|---|---|
| CN01-1 | 0.00 | 0.89 | Dwejra (Gozo) | 36 03.181 | 14 11.171 |
| CS03* | 0.00 | 0.89 | Comino Channel (between Aħrax point and Comino) | 36 00.334 | 14 21.710 |
| CS02* | 0.10 | 1.01 | Ramla l-Ħamra Bay (Gozo) | 36 04.269 | 14 17.060 |
| CS09 | 0.40 | 0.91 | Gnejna (Malta) | 35 55.665 | 14 19.882 |

*Stations retained as reference sites in the Maltese Islands.

# 5 INTERCALIBRATION OF HEAVILY MODIFIED WATER BODIES

No criteria regarding intercalibration of heavily modified WB have been established by the European Commission. The recommendation, and working strategy used by MS has been to remove these stations from the intercalibration. Once the intercalibration exercise is completed with inclusion of the rest of data, the quality of stations sampled in these WB can be extrapolated from the EQR values. Many countries retain sampling of stations in the heavily modified WB in order to have time series data of the evolution of the impacts in these areas.

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# ANNEX I – APPLICATION OF LUSI TO POSIDONIA BQE

**Malta Land Uses Simplified Index used for *Posidonia* BQE (MA-LUSIpo)**

Land Uses Simplified Index (LUSI) is calculated from a specific combination of pressures that influence a Water Body. This index was developed after Flo *et al*. (2011), adapting it to regional level. The aim of the index is giving a single value to the combination of all human pressures that may produce impacts on coastal areas, and whose relationship (pressure-impact) is due to a well-known mechanism. Each pressure receives a score and the Index is made up of the different pressures that affect the Biological Quality Indicator ('*Posidonia*' in this case).

To adapt this Index to Malta waters in order to identify anthropogenic pressures related to *Posidonia* impacts on coastal areas, the following parameters were considered:

- **Land uses**
  Urban fabric,
  Most of the land is covered by buildings; roads and artificially surfaced area cover almost all the ground.
  Agriculture,
  Areas principally occupied by agriculture, interspersed with significant natural areas
  Green urban areas,
  Areas with vegetation within urban fabric. Includes parks and cemeteries with vegetation
  Port areas,
  Industrial or commercial units,
  Artificially surfaced areas (with concrete, asphalt, tarmacadam, or stabilised, e.g. beaten earth) devoid of vegetation, occupy most of the area in question, which also contains buildings and/or vegetated areas.
  Complex cultivation patterns,
  Juxtaposition of small parcels of diverse annual crops, pasture and/or permanent crops.

- **Other Significant Pressures**[14]
  Aquaculture,
  Bunkering area,
  WB Sewage treatment plant,
  Industrial pollution,
  Shipyard,
  Trawling
  Hydromorphological pressures,
  Population.

14 Other Significant pressures not considered in the above are also considered e.g. indirect pressures that may suffer water bodies due to its own activities in the body of water or adjacent water bodies

The assessment of pressures considered in the index has been developed as follows. First of all, the existence of pressures has been identified for each sampling point in each water body (pressures maps provided by MEPA) and second, values have been assigned to each of them. The values assigned to the identified pressures are:

*Table 1. Values assigned to the identified pressures*

| | Pressure | Assigned value*[15] |
|---|---|---|
| **Land Uses** | Urban fabric | 0.7 |
| | Agriculture | 0.1 |
| | Green urban areas | 0.2 |
| | Port areas | 1 |
| | Industrial or commercial units | 1 |
| | Complex cultivation patterns | 0.5 |
| **Others** | Aquaculture | 0.2 |
| | Bunkering area | 0.1 |
| | Sewage treatment plant | 0.5 |
| | Industrial pollution | 0.3 |
| | Shipyard | 0.1 |
| | Trawling | 0.2 |
| | Hydromorphological pressure | 0.7 |
| | Population density | 0.2 / 0.4 |

* Values assigned according to expert knowledge

Afterwards, a correction factor is applied to the sum of the whole pressures in order to take into account the degree of confinement that could emphasize or diminish the effect of these pressures on the water body. Depending on the shape of the coastal line the sum is multiplied by the correction number (see Table 2).

*Table 2. Correction factor depending on the coastal line*

| Confinement | Correction number |
|---|---|
| **Sheltered** | 1.25 |
| **Open** | 1.00 |

Finally MA-LUSIpo index is calculated as follows:

**LUSI = (Land Uses + Other Significant Pressures) * Correction factor** Ec (1)

**Land Uses** =Urban fabric + Agriculture + Green urban areas + Port areas + Industrial or commercial units + Complex cultivation patterns Ec (2)

**Other Significant Pressures** = Aquaculture + Bunkering area + WB Sewage treatment plant + Industrial pollution + Shipyard + Trawling + Hydromorphological pressures + Population Ec (3)

**LUSI Index: Determination**

[15] The MA-LUSIpo index has been calculated from expert judgement using the available data from GIS layers and pressure MAPS. The experts inform that to be able to assign pressure values with higher precision for each station more data would have been necessary. The recommendation is to gather data about precise quantitative value of the impact. For example, how much volume of water discharged from sewage treatment plants, estimated number of moorings in a water body, statistics of bunkering areas, etc.

The LUSI Index is determined with the available data. It is calculated at each sampling point. The average values for each WB will be used for Posidonia BQE.

a) Land Uses Pressure determination

In those sampling points where pressure is identified due to a particular land use, the value assigned to such pressure is introduced as indicated in the previous section (Table 1). Finally, applying Ec (2) the value of Land use is determined for each sampling point.

*Table 3. Land use values for each sampling point*

| | | LAND USES | | | | | |
|---|---|---|---|---|---|---|---|
| Water Body | Sampling point | Urban fabric | Agriculture | Green urban areas | Port areas | Industrial or commercial units | Complex cultivation patterns |
| MTC101 | CN01-1 | | | | | | |
| MTC101 | CN01-2 | 0.7 | | | | | |
| MTC101 | CS01 | | | 0.2 | | | |
| MTC102 | CN02-1 | 0.7 | | | | | |
| MTC102 | CS02 | | 0.1 | | | | |
| MTC103 | CN03-1 | | 0.1 | | | | |
| MTC103 | CN03-2 | 0.7 | 0.1 | | | | |
| MTC103 | CN03-3 | | 0.1 | | | | |
| MTC103 | CN03-4 | | 0.1 | | | | |
| MTC103 | CN03-6 | | | | | | |
| MTC103 | CS03 | | | | | | |
| MTC104 | CN04-1 | | | | | | |
| MTC104 | CN04-3 | | | | | | |
| MTC104 | CN04-4 | 0.7 | | | | | |
| MTC104 | CN04-5 | 0.7 | | | | | |
| MTC104 | CN04-6 | 0.7 | | | | | |
| MTC104 | CP04-1 | | | | | | |
| MTC104 | CP04-2 | 0.7 | | | | | |
| MTC105 | CN05-1 | 0.7 | | 0.2 | 1 | | |
| MTC105 | CP05 | 0.7 | | | | 1 | |
| MTC106 | CN06-1 | 0.7 | | | | | |
| MTC106 | CP06-1 | | | | | | |
| MTC106 | CP06-2 | 0.7 | | | | | |
| MTC107 | CN07-1 | | | | | | |
| MTC107 | CN07-2 | | | | | | 0.5 |
| MTC107 | CN07-3 | | | | | | 0.5 |
| MTC107 | CP07 | | | | | | |
| MTC108 | CN08-1 | | | | | | |
| MTC108 | CS08 | | | | | | |
| MTC109 | CN09-1 | | 0.1 | | | | |
| MTC109 | CS09 | | 0.1 | | | | |

b) Other Significant Pressures determination.

Other Significant Pressures for those water bodies receiving some anthropic pressure defined in Table 1 are also assigned. The sum of all identified pressures (Ec (3)) determines the final value for each station.

*Table 4. Other Significant Pressure values for each sampling point*

| | | | | | OTHER | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Water Body** | **Sampling point** | **Aquaculture** | **Bunkering area** | **WB Sewage treatment plant** | **Industrial pollution** | **Shipyard** | **Trawling** | **Hydro morphological pressure** | **Population** |
| MTC101 | CN01-1 | | | | | | | | |
| MTC101 | CN01-2 | | | | | | | | |
| MTC101 | CS01 | | | | | | | | |
| MTC102 | CN02-1 | | | | | | | | |
| MTC102 | CS02 | | | | | | | | |
| MTC103 | CN03-1 | | | | | | 0.2 | | |
| MTC103 | CN03-2 | | | | | | | | |
| MTC103 | CN03-3 | | | | | | 0.2 | | |
| MTC103 | CN03-4 | | | | | | 0.2 | | |
| MTC103 | CN03-6 | | 0.1 | | | | | | |
| MTC103 | CS03 | | | | | | | | |
| MTC104 | CN04-1 | 0.2 | | | | | | | 0.4 |
| MTC104 | CN04-3 | | 0.1 | | | | | | |
| MTC104 | CN04-4 | 0.2 | | | | | | | 0.4 |
| MTC104 | CN04-5 | | | | | | | | |
| MTC104 | CN04-6 | | | | | | | | 0.4 |
| MTC104 | CP04-1 | | | | | | | | 0.4 |
| MTC104 | CP04-2 | | | | | | | | 0.4 |
| MTC105 | CN05-1 | | | | 0.3 | 0.1 | | 0.7 | |
| MTC105 | CP05 | | | | 0.3 | 0.1 | | 0.7 | |
| MTC106 | CN06-1 | | | 0.5 | | | | | |
| MTC106 | CP06-1 | | 0.1 | 0.5 | | | | | |
| MTC106 | CP06-2 | | 0.1 | 0.5 | | | 0.2 | | |
| MTC107 | CN07-1 | 0.2 | | | | | | | |
| MTC107 | CN07-2 | | | | 0.3 | | | 0.7 | 0.4 |
| MTC107 | CN07-3 | | | | 0.3 | | | 0.7 | 0.4 |
| MTC107 | CP07 | | | | 0.3 | | | 0.7 | 0.4 |
| MTC108 | CN08-1 | | | | | | 0.2 | | |
| MTC108 | CS08 | | | | | | 0.2 | | 0.2 |
| MTC109 | CN09-1 | | | | | | 0.2 | | |
| MTC109 | CS09 | | 0.1 | | | | 0.2 | | |

c) Correction factor determination

The following table shows for each sampling point if it is Inshore or Nearshore type, as reported by MEPA. The confinement of the point is also shown:

*Table 5. Type and confinement for each sampling point*

| WATER BODY | SAMPLING POINT | CONFINEMENT |
|---|---|---|
| CN01-1 | MTC101 | Open |
| CN01-2 | MTC101 | Sheltered |
| CS01 | MTC101 | Open |
| CN02-1 | MTC102 | Open |
| CS02 | MTC102 | Open |
| CN03-1 | MTC103 | Open |
| CN03-2 | MTC103 | Sheltered |

| | | |
|---|---|---|
| CN03-3 | MTC103 | Open |
| CN03-4 | MTC103 | Open |
| CN03-6 | MTC103 | Open |
| CS03 | MTC103 | Open |
| CN04-1 | MTC104 | Sheltered |
| CN04-3 | MTC104 | Open |
| CN04-4 | MTC104 | Sheltered |
| CN04-5 | MTC104 | Sheltered |
| CN04-6 | MTC104 | Sheltered |
| CP04-1 | MTC104 | Open |
| CP04-2 | MTC104 | Open |
| CN05-1 | MTC105 | Sheltered |
| CP05 | MTC105 | Open |
| CN06-1 | MTC106 | Sheltered |
| CP06-1 | MTC106 | Open |
| CP06-2 | MTC106 | Open |
| CN07-1 | MTC107 | Sheltered |
| CN07-2 | MTC107 | Sheltered |
| CN07-3 | MTC107 | Sheltered |
| CP07 | MTC107 | Open |
| CN08-1 | MTC108 | Open |
| CS08 | MTC108 | Open |
| CN09-1 | MTC109 | Open |
| CS09 | MTC109 | Open |

d) Calculation of the LUSI index by sampling points:

Finally, LUSI index is determined by applying the Ec (1). The final results are shown in the following table:

*Table 6. LUSI index value for each sampling point*

| *WATER BODY* | *SAMPLING POINT* | *LAND USES SUM* | *OTHERS SUM* | *SHELTER/OPEN* | *LUSI* |
|---|---|---|---|---|---|
| CN01-1 | MTC101 | 0 | 0 | 1 | 0.0 |
| CN01-2 | MTC101 | 0.7 | 0 | 1.25 | 0.9 |
| CS01 | MTC101 | 0.2 | 0 | 1 | 0.2 |
| CN02-1 | MTC102 | 0.7 | 0 | 1 | 0.7 |
| CS02 | MTC102 | 0.1 | 0 | 1 | 0.1 |
| CN03-1 | MTC103 | 0.1 | 0.2 | 1 | 0.3 |
| CN03-2 | MTC103 | 0.8 | 0 | 1.25 | 1.0 |
| CN03-3 | MTC103 | 0.1 | 0.2 | 1 | 0.3 |
| CN03-4 | MTC103 | 0.1 | 0.2 | 1 | 0.3 |
| CN03-6 | MTC103 | 0 | 0.1 | 1 | 0.1 |
| CS03 | MTC103 | 0 | 0 | 1 | 0.0 |
| CN04-1 | MTC104 | 0 | 0.6 | 1.25 | 0.8 |
| CN04-3 | MTC104 | 0 | 0.1 | 1 | 0.1 |
| CN04-4 | MTC104 | 0.7 | 0.6 | 1.25 | 1.6 |
| CN04-5 | MTC104 | 0.7 | 0 | 1.25 | 0.9 |
| CN04-6 | MTC104 | 0.7 | 0.4 | 1.25 | 1.4 |
| CP04-1 | MTC104 | 0 | 0.4 | 1 | 0.4 |

| | | | | | |
|---|---|---|---|---|---|
| CP04-2 | MTC104 | 0.7 | 0.4 | 1 | 1.1 |
| CN05-1 | MTC105 | 1.9 | 1.1 | 1.25 | 3.8 |
| CP05 | MTC105 | 1.7 | 1.1 | 1 | 2.8 |
| CN06-1 | MTC106 | 0.7 | 0.5 | 1.25 | 1.5 |
| CP06-1 | MTC106 | 0 | 0.6 | 1 | 0.6 |
| CP06-2 | MTC106 | 0.7 | 0.8 | 1 | 1.5 |
| CN07-1 | MTC107 | 0 | 0.2 | 1.25 | 0.3 |
| CN07-2 | MTC107 | 0.5 | 1.4 | 1.25 | 2.4 |
| CN07-3 | MTC107 | 0.5 | 1.4 | 1.25 | 2.4 |
| CP07 | MTC107 | 0 | 1.4 | 1 | 1.4 |
| CN08-1 | MTC108 | 0 | 0.2 | 1 | 0.2 |
| CS08 | MTC108 | 0 | 0.4 | 1 | 0.4 |
| CN09-1 | MTC109 | 0.1 | 0.2 | 1 | 0.3 |
| CS09 | MTC109 | 0.1 | 0.3 | 1 | 0.4 |

# ANNEX II – DATA EVALUATION FOR MACROALGAE BQE

**Detailed example of CARLIT 2008 data validation**

To validate 2008 CARLIT data, we compare the algal communities from the 2008 CARLIT with the data of quadrats sampled in 2013. Below we detail the comparison of three stations (CN 101, CP 05 and CS 03) sampled in May 2013 with the corresponding CARLIT data. We used the 2013 data sheets provided by MEPA.

**Station CN 101 Baseline II**

**Step 1**. First we calculate the % of presence of the different categories of macroalgae in the sampled quadrats (in this case *Cystoseira spp* (Cys), Photophilic algae (Photo), *Corallinacea* (Co) and *Litophyllum byssoides* (LiBy)) (Table 1).

*Table 1. Example of % of presence of the different categories of macroalgae in the sampled quadrats in station CN101.*

| Quadrats | Cys | Photo | Co | LiBy | TOTAL |
|---|---|---|---|---|---|
| **Surface** | 425 | 10 | 65 | 0 | 500 |
| **%** | 85 | 2 | 13 | 0 | 100 |

**Step 2**. Then we situate the sampling point in the CARLIT ArcGIS layer using the site coordinates: latitude 3990134.58, longitude 426700.89. After doing this we measure 500 meters on each side of the sampling point in the CARLIT and re-calculate with CARLIT the % of each category of macroalgae (Cys, Photo, Co and LiBy). It is necessary to group the different categories of abundance of *Cystoseira spp.* into one single category (Table 2).

*Table 2. Example of re-calculation with CARLIT of the % of each category of macroalgae in station CN101.*

| Carlit | Cys | CO | Photo | LiBy | Total |
|---|---|---|---|---|---|
| **Meters** | 930 | 111 | 0 | 28 | 1069 |
| **%** | 87 | 10 | 0 | 3 | 100 |

**Step 3**. Finally, we compare the % of each community using both, quadrates and CARLIT method, in order to validate the CARLIT 2008 (Table 3).

*Table 3. Example of comparison between quadrat sampling and CARLIT method. Names of columns as in previous tables.*

| CN101 | Cys | CO | Photo | LiBy | Total |
|---|---|---|---|---|---|
| **% Quadrats** | 85 | 2 | 13 | 0 | 100 |
| **% CARLIT** | 87 | 10 | 0 | 3 | 100 |

In the case of CN101, values of the main algal communities (*Cystoseira spp.*) are significantly similar so we can validate the methodology. Anyway, a little variation in the values between the two methods is expected because different measurement methods were used.

**Station CP 05 Baseline II**

**Step 1**. We calculate the % of presence of the different categories of macroalgae in the sampled quadrats (in this case *Cystoseira spp* (Cys), Photophilic algae (Photo), *Corallinacea* (Co) and Ulvacea (Ulva)).

*Table 4. Example of % of presence of the different categories of macroalgae in the sampled quadrats in station CP 05.*

| Quadrats | Cys | Photo | Co | Ulva | TOTAL |
|---|---|---|---|---|---|
| **Surface** | 70 | 50 | 380 | 0 | 500 |
| **%** | 14 | 10 | 76 | 0 | 100 |

- We situate the sampling point in the CARLIT ArcGIS layer with the coordinate data of the site: latitude 3973265.31 longitude 457155.02

**Step 2**. - As above.

*Table 5. Example of re-calculation with CARLIT of the % of each category of macroalgae (Cys, Photo, Co and LiBy) in station CP05.*

| Carlit | Cys | Photo | Co | Ulva | TOTAL |
|---|---|---|---|---|---|
| Meters | 174 | 0.0 | 784 | 18 | 976 |
| % | 18 | 0.0 | 80 | 2 | 100 |

**Step 3**. As above.

*Table 6. Example of comparison between quadrat sampling and CARLIT method. Names of columns as in previous tables.*

| CP05 | Cys | Photo | Co | Ulva | TOTAL |
|---|---|---|---|---|---|
| **% Quadrats** | 14 | 10 | 76 | 0 | 100 |
| **% CARLIT** | 18 | 0 | 80 | 2 | 100 |

In the case of CP05, the values of the main algal communities (*Corallinacea*) are quite similar; the second community (*Cystoseira spp)* shows also similar values.

**Station CS 03 Baseline II**

**Step 1**. We calculate the % of presence of the different categories of macroalgae in the sampled quadrats (in this case *Cystoseira spp* (Cys), Photophilic algae (Photo), *Corallinacea* (Co) and Ulvacea (Ulva)).

*Table 7. Example of % of presence of the different categories of macroalgae in the sampled quadrats in station CS03.*

| Quadrats | Cys | Photo | Co | Ulva | TOTAL |
|---|---|---|---|---|---|
| **Surface** | 390 | 10 | 100 | 0 | 500 |
| **%** | 78 | 2 | 20 | 0 | 100 |

- We situate the sampling point in the CARLIT ArcGIS layer with the coordinate data of the site: latitude 3984754.66, longitude 442487.93

**Step 2**. As above.

*Table 8. Example of re-calculation with CARLIT of the % of each category of macroalgae in station CS03.*

| Carlit | Cys | Photo | Co | Ulva | TOTAL |
|---|---|---|---|---|---|
| **Meters** | 1098 | 0 | 192 | 68 | 1358 |
| **%** | 81 | 0 | 14 | 5 | 100 |

**Step 3**. As above.

*Table 9. Example of comparison between quadrat sampling and CARLIT method. Names of columns as in previous tables*

| CS03 | Cys | Photo | Co | Ulva | TOTAL |
|---|---|---|---|---|---|
| **% Quadrats** | 78 | 2 | 20 | 0 | 100 |
| **% CARLIT** | 81 | 0 | 14 | 5 | 100 |

Again, the values of the main algal communities (Cys) are quite similar; the second community (Co*)* shows also similar values.

The rest of stations show similar results, indicating that we can proceed with the intercalibration using the data from CARLIT 2008.

# ANNEX III – APPLICATION OF LUSI TO MACROALGAE BQE

**Malta Land Uses Simplified Index used for Macroalgae BQE (MA-LUSIma)**

Land Uses Simplified Index (LUSI) is calculated from a specific combination of pressures that influence a Water Body. This index was developed after Flo et al. (2011), adapting it to regional level. The aim of the index is giving a single value to the combination of all human pressures that may produce impacts on coastal areas, and whose relationship (pressure-impact) is due to a well-known mechanism. Each pressure receives a score and the Index is made up of the different pressures that affect the Biological Quality Indicator ('Macroalgae' in this case).

To adapt this Index to Malta waters in order to identify anthropogenic pressures related to Macroalgae impacts on shallow water coastal areas, we consider the following parameters:

- **Land uses**
  Urban fabric,
  Most of the land is covered by buildings; roads and artificially surfaced area cover almost all the ground.
  Agriculture,
  Areas principally occupied by agriculture, interspersed with significant natural areas
  Port areas,
  Industrial or commercial units,
  Artificially surfaced areas (with concrete, asphalt, tarmacadam, or stabilised, e.g. beaten earth) devoid of vegetation, occupy most of the area in question, which also contains buildings and/or vegetated areas.

- **Other Significant Pressures**[16]
  Aquaculture,
  Bunkering area,
  WB Sewage treatment plant,
  Reverse osmosis installations

The assessment of pressures considered in the index has been developed as follows. First of all, the existence of pressures has been identified for each water body using pressures maps provided by MEPA, assigning values to the identified pressures as follows:

[16] Other Significant pressures not considered in the above are also considered e.g. indirect pressures that may suffer water bodies due to its own activities in the body of water or adjacent water bodies

*Table 1: Values assigned to indirect and direct impacts*

| | Pressure | Assigned value*[17] |
|---|---|---|
| **Land uses** | Urban uses | 0.5 |
| | Industrial and commercial uses | 0.5 |
| | Agriculture uses | 0.1 |
| | Harbour facilities | 0.5 |
| **Other significant pressures** | Aquaculture installations | 0.1 |
| | Bunkering uses | 0.2 |
| | Sewage outfalls and installations | 1 |
| | Reverse osmosis installations | 0.2 |

*Values assigned according to expert knowledge

Afterwards, a correction factor is applied to the sum of the whole pressures in order to take into account the degree of confinement that could emphasize or diminish the effect of these pressures on the water body. Depending on the shape of the coastal line the sum is multiplied by the correction number (see Table 2).

*Table 2. Correction factor depending on the coastal line*

| Coast morphology | Correction factor |
|---|---|
| Sheltered | 1.25 |
| Open | 1.00 |

Finally, MA- LUSIma index is calculated as follows:

**LUSI = (Land Uses + Other Significant Pressures) * Correction factor** Ec(1)

**Land Uses** =Urban uses + Industrial and commercial uses + Agriculture uses + Harbour uses Ec(2)

[17] The MA-LUSIma index has been calculated from expert judgement using the available data from GIS layers and pressure MAPS. The experts inform that to be able to assign pressure values with higher precision for each station more data would have been necessary. The recommendation is to gather data about precise quantitative value of the impact. For example, how much volume of water discharged from sewage treatment plants, estimated number of moorings in a water body, statistics of bunkering areas, etc.

**Other significant pressures** = Aquaculture installations + bunkering uses + sewage outfalls and installations + reverse osmosis installations. Ec(3)

**2. –LUSI Index: Determination**

The LUSI Index is determined with the available data, and it is calculated for each WB, using it for macroalgae BQE

a) Land Uses Pressure determination

In those water bodies where pressure is identified due to a particular land use, the value assigned to such pressure is introduced as indicated in the previous section (Table 1). Finally, applying Ec (2) the value of Land use is determined for each water body.

Pressure values for each water body has been calculate as follows:

*Table 3. Land use values for each sampling point*

| Water Body | Urban | Industrial | Agriculture | Harbour |
|---|---|---|---|---|
| MTC101 | | | | |
| MTC102 | 0.5 | | 0.1 | |
| MTC103 | 0.5 | | 0.1 | |
| MTC104 | 0.5 | 0.5 | | |
| MTC105 | 0.5 | 0.5 | | 0.5 |
| MTC106 | 0.5 | 0.5 | | |
| MTC107 | 0.5 | 0.5 | | 0.5 |
| MTC108 | | | 0.1 | |
| MTC109 | 0.5 | 0.5 | 0.1 | |

b) Other Significant Pressures determination.

Other Significant Pressures for those water bodies receiving some anthropic pressure defined in Table 1 are also assigned. The sum of all identified pressures (Ec (3)) determines the final value for each station.

*Table 4. Other Significant Pressures values for each sampling point*

| WB | Aquacult. | Bunkering | Sewage | Osmosis |
|---|---|---|---|---|
| MTC101 | | | 1 | |
| MTC102 | | | 1 | |
| MTC103 | 0.1 | | 1 | 0.2 |
| MTC104 | 0.1 | 0.2 | | 0.2 |

| | | | | |
|---|---|---|---|---|
| MTC105 | | | | |
| MTC106 | 0.1 | 0.2 | 1 | |
| MTC107 | 0.1 | | | |
| MTC108 | 0.1 | | | 0.2 |
| MTC109 | | 0.2 | 1 | |

c) Correction factor determination

The following table shows for each sampling point if it is Inshore or Nearshore type, as reported by MEPA. The confinement of the point is also shown:

*Table 5. Type and confinement for each sampling point*

| WB | Coastal line | Correction |
|---|---|---|
| MTC101 | Open | 1 |
| MTC102 | Open | 1 |
| MTC103 | Open | 1 |
| MTC104 | Open | 1 |
| MTC105 | Sheltered | 1.25 |
| MTC106 | Open | 1 |
| MTC107 | Open | 1 |
| MTC108 | Open | 1 |
| MTC109* | Sheltered | 1.25 |

* Note: Water Body number 9 has a theoretically Open coastline,

but we considered Sheltered because of great number of inlets.

d) Calculation of the LUSI index by water bodies:

Finally, LUSI index is determined by applying the Ec (1). The final results are shown in the following table:

*Table 6. LUSI index value for each sampling point*

| WB | Land Uses Sum | Others Sum | Shelter/ Open | LUSI |
|---|---|---|---|---|
| MTC101 | 0 | 1 | 1 | 1 |
| MTC102 | 0.6 | 1 | 1 | 1.6 |
| MTC103 | 0.6 | 1.3 | 1 | 1.9 |
| MTC104 | 1 | 0.5 | 1 | 1.5 |
| MTC105 | 1.5 | 0 | 1.25 | 1.875 |
| MTC106 | 1 | 1.3 | 1 | 2.3 |
| MTC107 | 1.5 | 0.1 | 1 | 1.6 |

| | | | | |
|---|---|---|---|---|
| MTC108 | 0.1 | 0.3 | 1 | 0.4 |
| MTC109* | 1.1 | 1.2 | 1.25 | 2.875 |

# ANNEX IV – APPLICATION OF LUSI TO PHYTOPLANKTON BQE

**Malta Land Uses Simplified Index used for Phytoplankton BQE (MA-LUSIphy)**

Land Uses Simplified Index (LUSI) is calculated from a specific combination of pressures that influence a Water Body. This index was developed after Flo et al. (2011), adapting it to regional level. The aim of the index is giving a single value to the combination of all human pressures that may produce impacts on coastal areas, and whose relationship (pressure-impact) is due to a well-known mechanism. Each pressure receives a score and the Index is made up of the different pressures that affect the Biological Quality Indicator ('phytoplankton' in this case).

To adapt this Index to Malta waters in order to identify anthropogenic pressures related to phytoplankton impacts on coastal areas, we consider the following parameters:

- **Land uses**

<u>Urban fabric,</u>

Most of the land is covered by buildings; roads and artificially surfaced area cover almost all the ground.

<u>Agriculture,</u>

Areas principally occupied by agriculture, interspersed with significant natural areas

<u>Green urban areas,</u>

Areas with vegetation within urban fabric. Includes parks and cemeteries with vegetation

<u>Port areas,</u>

<u>Industrial or commercial units,</u>

Artificially surfaced areas (with concrete, asphalt, tarmacadam, or stabilised, e.g. beaten earth) devoid of vegetation, occupy most of the area in question, which also contains buildings and/or vegetated areas.

<u>Complex cultivation patterns,</u>

Juxtaposition of small parcels of diverse annual crops, pasture and/or permanent crops.

- **Other Significant Pressures**[18]

<u>Aquaculture,</u>

<u>Bunkering area,</u>

[18] Other Significant pressures not considered in the above are also considered e.g. indirect pressures that may suffer water bodies due to its own activities in the body of water or adjacent water bodies

WB Sewage treatment plant,

Industrial pollution,

Shipyard,

Hydromorphological pressures.

The assessment of pressures considered in the index has been developed as follows. First of all, the existence of pressures has been identified for each sampling point in each water body (pressures maps provided by MEPA) and second, values have been assigned to each of them. The values assigned to the identified pressures are:

*Table 1. Values assigned to the identified pressures*

| | Pressure | Assigned value*[19] |
|---|---|---|
| **Land Uses** | Urban fabric | 0.7 |
| | Agriculture | 0.1 |
| | Green urban areas | 0.2 |
| | Port areas | 1 |
| | Industrial or commercial units | 1 |
| | Complex cultivation patterns | 0.5 |
| **Others** | Aquaculture | 0.2 |
| | Bunkering area | 0.1 |
| | Sewage treatment plant | 0.5 |
| | Industrial pollution | 0.3 |
| | Shipyard | 0.1 |
| | Hydromorphological pressure | 0.7 |
| | Population density | 0.2 / 0.4 |

* Values assigned according to expert knowledge

[19] The MA-LUSI index has been calculated with the available data from GIS and pressure MAPS. The experts inform that to be able to assign pressure values with high precision for each station more data would have been necessary. The recommendation is to gather data about precise quantitative value of the impact. For example, how much volume of water discharged from sewage treatment plants, estimated number of moorings in a water body, statistics of bunkering areas, etc.

Afterwards, a correction factor is applied to the sum of the whole pressures in order to take into account the degree of confinement that could emphasize or diminish the effect of these pressures on the water body. Depending on the shape of the coastal line the sum is multiplied by the correction number (see Table 2).

*Table 2. Correction factor depending on the coastal line*

| Confinement | Correction number |
|---|---|
| **Sheltered** | 1.25 |
| **Open** | 1.00 |

Finally MA-LUSI index is calculated as follows:

**LUSI = (Land Uses + Other Significant Pressures) * Correction factor** Ec (1)

**Land Uses =**Urban fabric + Agriculture + Green urban areas + Port areas + Industrial or commercial units + Complex cultivation patterns Ec (2)

**Other Significant Pressures =** Aquaculture + Bunkering area + WB Sewage treatment plant + Industrial pollution + Shipyard + Hydromorphological pressures Ec (3)

**2. –LUSI Index: Determination**

The LUSI Index is determined with the available data. It is calculated at each sampling point. The average values for each WB will be used for Posidonia BQE

a) Land Uses Pressure determination

In those sampling points where pressure is identified due to a particular land use, the value assigned to such pressure is introduced as indicated in the previous section (Table 1). Finally, applying Ec (2) the value of Land use is determined for each sampling point.

*Table 3. Land use values for each sampling point*

| | | LAND USES | | | | | |
|---|---|---|---|---|---|---|---|
| **Water Body** | **Sampling point** | **Urban fabric** | **Agriculture** | **Green urban areas** | **Port areas** | **Industrial or commercial units** | **Complex cultivation patterns** |
| MTC101 | CN01-1 | | | | | | |
| MTC101 | CN01-2 | 0.7 | | | | | |
| MTC101 | CS01 | | | 0.2 | | | |
| MTC102 | CN02-1 | 0.7 | | | | | |
| MTC102 | CS02 | | 0.1 | | | | |
| MTC103 | CN03-1 | | 0.1 | | | | |
| MTC103 | CN03-2 | 0.7 | 0.1 | | | | |
| MTC103 | CS03 | | | | | | |
| MTC104 | CN04-1 | | | | | | |
| MTC104 | CN04-4 | 0.7 | | | | | |
| MTC104 | CN04-6 | 0.7 | | | | | |
| MTC104 | CP04-1 | | | | | | |
| MTC104 | CP04-2 | 0.7 | | | | | |
| MTC105 | CN05-1 | 0.7 | | 0.2 | 1 | | |
| MTC105 | CP05 | 0.7 | | | | 1 | |
| MTC106 | CN06-1 | 0.7 | | | | | |
| MTC106 | CP06-1 | | | | | | |
| MTC107 | CN07-2 | | | | | | 0.5 |
| MTC107 | CN07-3 | | | | | | 0.5 |
| MTC107 | CP07 | | | | | | |
| MTC108 | CS08 | | | | | | |
| MTC109 | CN09-1 | | 0.1 | | | | |
| MTC109 | CS09 | | 0.1 | | | | |

b) Other Significant Pressures determination.

Other Significant Pressures for those water bodies receiving some anthropic pressure defined in Table 1 are also assigned. The sum of all identified pressures (Ec (3)) determines the final value for each station.

*Table 4. Other Significant Pressures values for each sampling point*

| | | OTHER | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Water Body** | **Sampling point** | **Aquacult.** | **Bunkering area** | **WB Sewage treat. plant** | **Industrial pollution** | **Shipyard** | **Hydro morphological pressure** | **Population** |
| MTC101 | CN01-1 | | | | | | | |
| MTC101 | CN01-2 | | | | | | | |
| MTC101 | CS01 | | | | | | | |
| MTC102 | CN02-1 | | | | | | | |
| MTC102 | CS02 | | | | | | | |
| MTC103 | CN03-1 | | | | | | | |
| MTC103 | CN03-2 | | | | | | | |
| MTC103 | CS03 | | | | | | | |
| MTC104 | CN04-1 | 0.2 | | | | | | 0.4 |
| MTC104 | CN04-4 | 0.2 | | | | | | 0.4 |
| MTC104 | CN04-6 | | | | | | | 0.4 |
| MTC104 | CP04-1 | | | | | | | 0.4 |
| MTC104 | CP04-2 | | | | | | | 0.4 |
| MTC105 | CN05-1 | | | | 0.3 | 0.1 | 0.7 | |
| MTC105 | CP05 | | | | 0.3 | 0.1 | 0.7 | |
| MTC106 | CN06-1 | | | 0.5 | | | | |
| MTC106 | CP06-1 | | 0.1 | 0.5 | | | | |
| MTC107 | CN07-2 | | | | 0.3 | | 0.7 | 0.4 |
| MTC107 | CN07-3 | | | | 0.3 | | 0.7 | 0.4 |
| MTC107 | CP07 | | | | 0.3 | | 0.7 | 0.4 |
| MTC108 | CS08 | | | | | | | 0.2 |
| MTC109 | CN09-1 | | | | | | | |
| MTC109 | CS09 | | 0.1 | | | | | |

c) Correction factor determination

The following table shows for each sampling point if it is Inshore or Nearshore type, as reported by MEPA. The confinement of the point is also shown:

*Table 5. Type and confinement for each sampling point*

| WATER BODY | SAMPLING POINT | CONFINEMENT |
|---|---|---|
| MTC101 | CN01-1 | Open |
| MTC101 | CN01-2 | Sheltered |
| MTC101 | CS01 | Open |
| MTC102 | CN02-1 | Open |
| MTC102 | CS02 | Open |
| MTC103 | CN03-1 | Open |
| MTC103 | CN03-2 | Sheltered |
| MTC103 | CS03 | Open |
| MTC104 | CN04-1 | Sheltered |
| MTC104 | CN04-4 | Sheltered |
| MTC104 | CN04-6 | Sheltered |
| MTC104 | CP04-1 | Open |
| MTC104 | CP04-2 | Open |
| MTC105 | CN05-1 | Sheltered |
| MTC105 | CP05 | Open |
| MTC106 | CN06-1 | Sheltered |
| MTC106 | CP06-1 | Open |
| MTC107 | CN07-2 | Sheltered |
| MTC107 | CN07-3 | Sheltered |
| MTC107 | CP07 | Open |
| MTC108 | CS08 | Open |
| MTC109 | CN09-1 | Open |
| MTC109 | CS09 | Open |

d) Calculation of the LUSI index by sampling points:

Finally, LUSI index is determined by applying the Ec (1). The final results are shown in the following table:

*Table 6. LUSI index value for each sampling point*

| WATER BODY | SAMPLING POINT | LAND USES SUM | OTHERS SUM | SHELTER/OPEN | LUSI |
|---|---|---|---|---|---|
| MTC101 | CN01-1 | 0 | 0 | 1.00 | 0.0 |
| MTC101 | CN01-2 | 0.7 | 0 | 1.25 | 0.9 |
| MTC101 | CS01 | 0.2 | 0 | 1.00 | 0.2 |
| MTC102 | CN02-1 | 0.7 | 0 | 1.00 | 0.7 |
| MTC102 | CS02 | 0.1 | 0 | 1.00 | 0.1 |
| MTC103 | CN03-1 | 0.1 | 0 | 1.00 | 0.1 |
| MTC103 | CN03-2 | 0.8 | 0 | 1.25 | 1.0 |
| MTC103 | CS03 | 0 | 0 | 1.00 | 0.0 |
| MTC104 | CN04-1 | 0 | 0.6 | 1.25 | 0.8 |
| MTC104 | CN04-4 | 0.7 | 0.6 | 1.25 | 1.6 |
| MTC104 | CN04-6 | 0.7 | 0.4 | 1.25 | 1.4 |
| MTC104 | CP04-1 | 0 | 0.4 | 1.00 | 0.4 |
| MTC104 | CP04-2 | 0.7 | 0.4 | 1.00 | 1.1 |
| MTC105 | CN05-1 | 1.9 | 1.1 | 1.25 | 3.8 |
| MTC105 | CP05 | 1.7 | 1.1 | 1.00 | 2.8 |
| MTC106 | CN06-1 | 0.7 | 0.5 | 1.25 | 1.5 |
| MTC106 | CP06-1 | 0 | 0.6 | 1.00 | 0.6 |
| MTC107 | CN07-2 | 0.5 | 1.4 | 1.25 | 2.4 |
| MTC107 | CN07-3 | 0.5 | 1.4 | 1.25 | 2.4 |
| MTC107 | CP07 | 0 | 1.4 | 1.00 | 1.4 |
| MTC108 | CS08 | 0 | 0.2 | 1.00 | 0.2 |
| MTC109 | CN09-1 | 0.1 | 0 | 1.00 | 0.1 |
| MTC109 | CS09 | 0.1 | 0.1 | 1.00 | 0.2 |

Once the LUSI index is applied the lowest values (0.0) are those corresponding to CS03 and CN01-1. The CS03 is one is recommended to be selected as the first reference site. Then WFD criteria should be checked to accomplish for as many as possible. The other two selected points by MEPA as reference sites are CS02 and CS09, revealing a low LUSI value too (0.1 and 0.2 respectively). Moreover, it is also noticed that those sample points that belong to harbours, in heavily modified WB, exhibit the higher LUSI values, CN05-1 (3.8); CP05 (2.8); CN07-2 (2.4); CN07-3 (2.4).

**3 –LUSI index *vs* Chlorophyll *a* correlation**

Once LUSI index value is determined for each sampling point it is correlated to chlorophyll *a* mean value (Figures 1 to 3). In table 7 LUSI index values and Chlorophyll *a* mean value for the whole 23 data set are shown.

*Table 7. LUSI index values and Chlorophyll a mean value for each sampling point*

| TYPE | WATER BODY | SAMPLING POINT | LUSI | Chl *a* mean |
|---|---|---|---|---|
| | MTC101 | CN01-1 | 0.00 | 0.118 |
| Inshore | MTC101 | CN01-2 | 0.88 | 0.202 |
| | MTC101 | CS01 | 0.20 | 0.138 |
| | MTC102 | CN02-1 | 0.70 | 0.188 |
| Nearshore | MTC102 | CS02 | 0.10 | 0.111 |
| Inshore | MTC103 | CN03-1 | 0.10 | 0.142 |
| | MTC103 | CN03-2 | 1.00 | 0.335 |
| Nearshore | MTC103 | CS03 | 0.00 | 0.138 |
| Nearshore | MTC104 | CN04-1 | 0.75 | 0.250 |
| | MTC104 | CN04-4 | 1.63 | 0.185 |
| Inshore | MTC104 | CN04-6 | 1.38 | 0.212 |
| Nearshore | MTC104 | CP04-1 | 0.40 | 0.148 |
| | MTC104 | CP04-2 | 1.10 | 0.190 |
| Inshore | MTC105 | CN05-1 | 3.75 | 0.574 |
| | MTC105 | CP05 | 2.80 | 0.470 |
| | MTC106 | CN06-1 | 1.50 | 0.335 |
| Inshore | MTC106 | CP06-1 | 0.60 | 0.396 |
| | MTC107 | CN07-2 | 2.38 | 0.325 |
| Inshore | MTC107 | CN07-3 | 2.38 | 0.242 |
| Inshore | MTC107 | CP07 | 1.40 | 0.184 |
| | MTC108 | CS08 | 0.20 | 0.168 |
| | MTC109 | CN09-1 | 0.10 | 0.140 |
| Nearshore | MTC109 | CS09 | 0.20 | 0.096 |

Figure 1 shows the relationship between the chlorophyll a values of these 23 sampling points (Nearshore / Inshore) and the corresponding LUSI value. A good correlation between pressure (LUSI) and impact (chlorophyll a value) is achieved ($R^2$=0.6564), with a p-value<0.01.

Figure 2 shows the correlation between pressure (LUSI) and impact (chlorophyll a value) only for Nearshore stations, which are the most suitable data to conduct an intercalibration. In this case, the correlation obtained is better ($R^2$=0.7385), but it is non-significant (p-value>0.05), probably because the number of stations is too low (only 5). Moreover, these samples show low LUSI values, except the CN04-1 point with a value of 0.75. In fact, three of these 5 stations are considered reference sites in this example (CS02, CS03 y CS09). Two of them (CS03 y CS09) were also considered reference sites by MEPA.

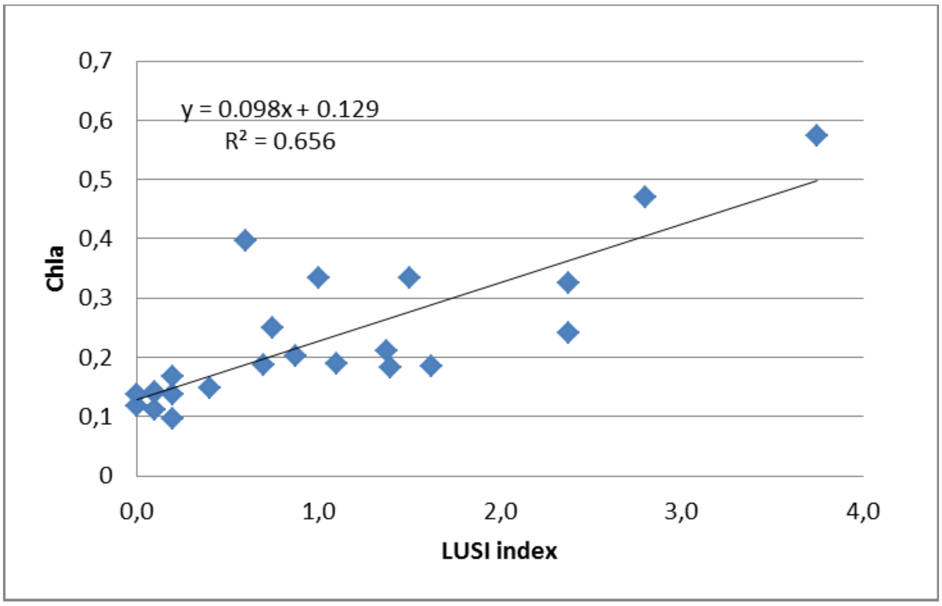


*Figure 1. Relationship between pressure (LUSI index) and impact (Chlorophyll a)*

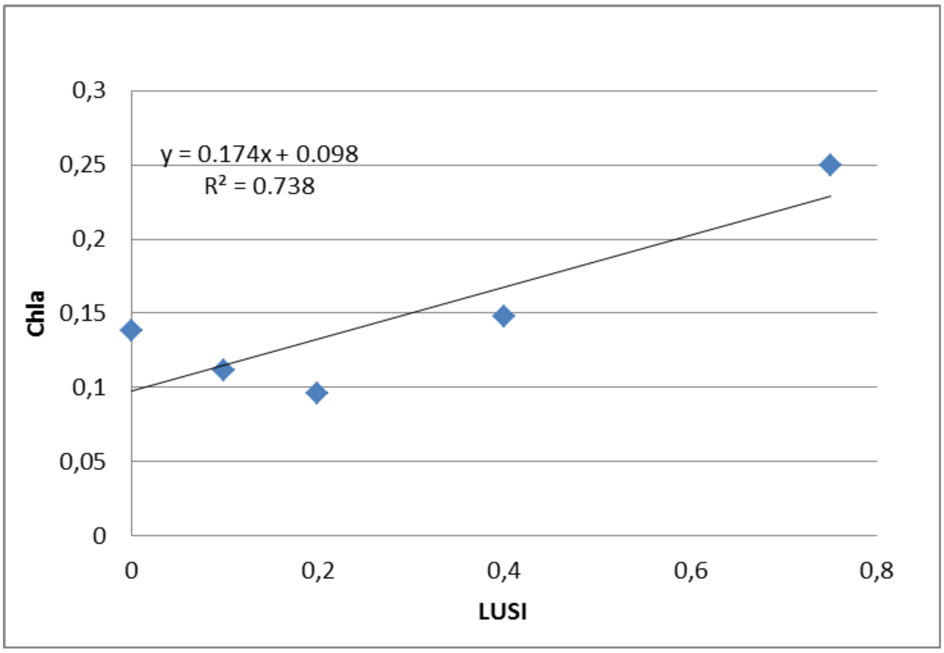


*Figure 2. Relationship between pressure (LUSI index) and impact (Chlorophyll a) of Nearshore sampling sites.*

Figure 3 shows the correlation between pressure (LUSI) and impact (chlorophyll a value) for inshore stations. The correlation coefficient is much lower ($R^2$=0.4859, and it is non-significant, p-value > 0.05). This might be due to the fact that LUSI values are higher since three of the stations belong to harbour zones (CN05-1, CN07-3, and CP07). Station CN05-1 shows the highest LUSI value (3.75).

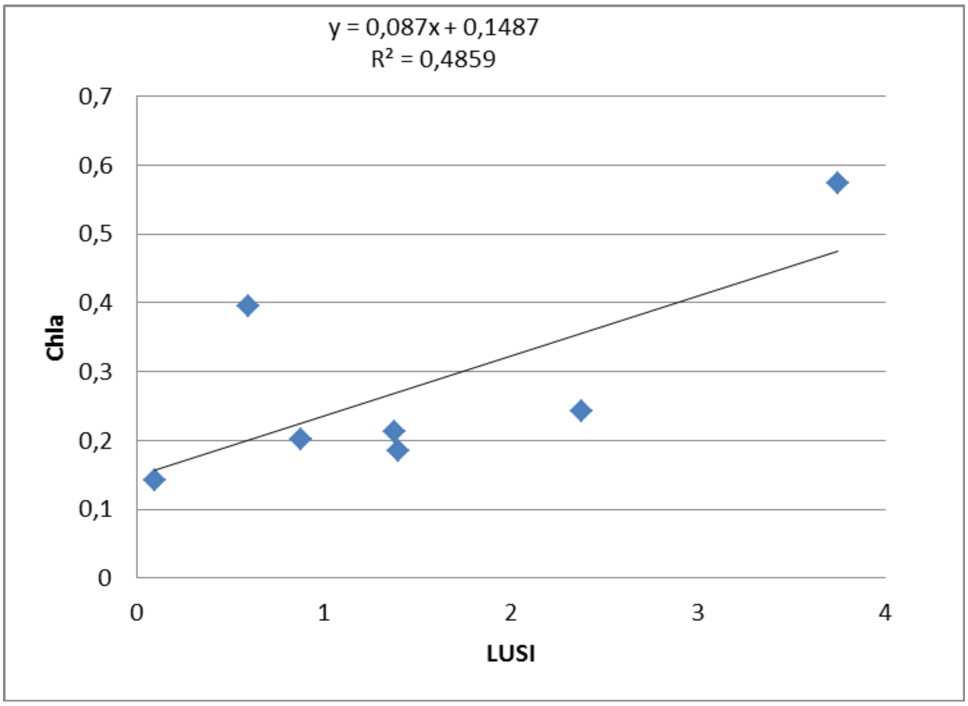


*Figure 3 Relationship between pressure (LUSI index) and impact (Chlorophyll a) of Inshore sampling site.*

**4- Ecological status assessment (Nearshore Sampling site) with 90th percentile Chlorophyll *a* (µg/L)**

In order to establish the Ecological Status for water bodies for Malta using the BQE phytoplankton, the 90th percentile of Chlorophyll *a* values is used, as it is said in the COMMISSION DECISION of 20 September 2013.

*Table 8. Commission Decision boundaries*

| Type III E | Values Chl a 90th percentile (µg/L) | |
|---|---|---|
| | High-Good | Good-Moderate |
| **Cyprus** | 0.10 | 0.40 |
| **Greece** | 0.10 | 0.40 |

Although the WFD establishes a 5-6 year period when 90th percentile are used or one year in the case of geomean, a first approach with the available data from Malta coastal water bodies has been developed. Only Nearshore sampling points have been used (n=5). The results obtained are shown in Table 9.

*Table 9. Ecological status for sampling points*

| Water Body | Sampling Point | Chl a 90th percentile (µg/L) | Ecological Status |
|---|---|---|---|
| MTC101 | CN01-1 | 0.19 | Moderate |
| | CN01-2 | 0.34 | |
| MTC102 | CN02-1 | 0.33 | |
| MTC103 | CN03-1 | 0.26 | |
| | CN03-2 | 0.63 | |
| MTC104 | CN04-1 | 0.56 | |
| | CN04-4 | 0.28 | |
| | CN04-6 | 0.30 | |
| MTC105 | CN05-1 | 0.93 | |
| MTC106 | CN06-1 | 0.58 | |
| MTC107 | CN07-2 | 0.38 | |
| | CN07-3 | 0.31 | |

| | | | |
|---|---|---|---|
| MTC109 | CN09-1 | 0.22 | |
| MTC104 | CP04-1 | 0.24 | Good |
| | CP04-2 | 0.21 | |
| MTC105 | CP05 | 0.71 | |
| MTC106 | CP06-1 | 0.81 | |
| MTC107 | CP07 | 0.27 | |
| MTC101 | CS01 | 0.23 | |
| MTC102 | CS02 | 0.15 | Good |
| MTC103 | CS03 | 0.26 | Good |
| MTC108 | CS08 | 0.31 | |
| MTC109 | CS09 | 0.12 | Good |

This approach reveals four stations in a Good and one Moderate Ecological Status.