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# Using seaweed assemblages on intertidal rocky shores to assess the ecological status of Andalusian coasts. Biogeographical implications.

## Utilización de comunidades de macroalgas en intermareales rocosos para la evaluación del estado ecológico en las costas de Andalucía. Implicaciones biogeográficas

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presentada por


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para la obtención del título de doctor por la Universidad de Cádiz

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INFORMAN:

Que la presente memoria titulada *"Utilización de comunidades de macroalgas en intermareales rocosos para la evaluación del estado ecológico en las costas de Andalucía. Implicaciones biogeográficas"*, presentada por **Ricardo Bermejo Lacida**, ha sido realizada bajo nuestra dirección y autorizan su presentación y defensa para optar al Grado de Doctor por la Universidad de Cádiz.

Puerto Real, a 15 de enero de 2014

Prof. Dr. Ignacio Hernández Carrero　　　Prof. Dr. Juan José Vergara Oñate

Para la realización de esta tesis doctoral, Ricardo Bermejo Lacida ha disfrutado de una beca de posgrado del Programa Nacional de Formación de Profesorado Universitario (FPU) del Ministerio de Ciencia e Innovación (AP2008-01209. Su actividad investigadora se ha realizado como miembro del grupo de investigación "Estructura y Dinámica de Ecosistemas Acuáticos" (PAIDI RNM-214) en el marco del Campus de Excelencia Internacional del Mar. Además, los resultados de esta memoria de Tesis han sido financiados por los siguientes proyectos:

- Contrato OT2010/102 *"Desarrollo y aplicación de indicadores biológicos basados en macroalgas y angiospermas marinas en aguas de transición y costeras de Andalucía en cumplimiento de la Directiva Marco de Agua"* con la Consejería de Medio Ambiente de la Junta de Andalucía.
- Proyecto SeaLive - *"Retroalimentaciones y trade-offs en praderas de fanerógamas marinas: el coste de vivir en ecosistemas acuáticos"* del Ministerio de Ciencia e Innovación (CTM2011-24482).
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Parte de los resultados presentados en esta memoria de tesis doctoral han sido publicados en revistas internacionales indexadas (Ecological Indicators, Marine Pollution Bulletin y Journal of Applied Phycology), están siendo evaluados para su publicación (PlosOne) o se encuentran en preparación para su publicación. Otros resultados no presentados pero derivados de este trabajo han sido también publicados en revistas no indexadas de ámbito nacional (ALGAS, Ambientalia, Migres y Acta Botanica Malacitana). Finalmente, los resultados de las investigaciones se han llevado como contribuciones a congresos nacionales e internacionales (ICES annual science conference 2010, Nantes; 5th European Phycological Congress, Rodas 2011; III Simposio Internacional de Ciencias del Mar, Cádiz 2012; I Congreso Iberoamericano de GIAL, Cádiz 2012, XVII Simpósio Ibérico de Estudios de Biología Marina, San Sebastían 2012).

## Glossary of common terms used in Water Framework Directive.

**Artificial water body**: body of surface water created by human activity.

**Biological Quality Element:** group of organisms (e.g. phytoplankton, macroalgae, angiosperms, benthic invertebrate fauna, and fish) that can be used to assess the ecological status of a water body.

**Coastal water:** surface water on the landward side of a line, every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured, extending where appropriate up to the outer limit of transitional waters.

**Ecological Status:** expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters. It is categorised on five classes: high, good, moderate, poor or bad.

**Ecological Quality Ratio:** numerical expression of the ecological status. It should be quantified into a single numerical value between 0 and 1, which represents the ratio between the current and the reference (i.e. pristine or near-pristine) condition.

**Ecoregion:** relatively large areas of land or water, which contain characteristic, geographically distinct assemblages of natural communities and species.

**Geographical Intercalibration Group:** group of Member States that share ecological types of rivers, lakes and coastal/transitional waters within an ecoregion, and can thus compare monitoring results between themselves.

**Heavily modified water body:** body of surface water, which as a result of physical alterations by human activity is substantially changed in character.

**Reference condition:** biological, chemical and morphological conditions associated with no or very low anthropogenic pressure. It can be inferred from historical datasets, spatial comparisons, modelling or may be derived using combination of these methods.

**Surface water:** discrete and significant element of surface water such as a lake, a reservoir, a stream, river or canal, part of a stream, river or canal, a transitional water or a stretch of coastal water.

**Transitional waters:** bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows.

**Water body:** discrete and significant element of surface or ground water.

## List of abbreviations.

| | |
|---|---|
| **AACD** | Absolute Average Class Difference |
| **AMOVA** | Analysis of MOlecular VAriance |
| **ANOSIM** | ANalysis Of SIMilarities |
| **ANOVA** | ANalysis Of VAriance |
| **BQE** | Biological Quality Element |
| **CARLIT** | CARtography of LITtoral rocky-shore communities |
| **CCO** | Cover, Characteristic species, Opportunistic species on rocky bottoms |
| **CFR** | quality of rocky bottoms / Calidad Fondos Rocosos |
| **CF** | Correction Factor |
| **DB** | Decimetric Blocks |
| **dNTP** | deoxynucleotide |
| **EAG** | Eastern Alboran Gyre |
| **EEI** | Ecological Evaluation Index |
| **EEIc** | Ecological Evaluation Index continuous formula |
| **EOF** | Empirical Orthogonal Function |
| **EQR** | Ecological Quality Ratio |
| **ES** | Ecological Status |
| **ESG** | Ecological Status Group |
| **GIG** | Geographic Intercalibration Group |
| **GIS** | Geographic Information Systems |
| **GPS** | Geographical Positioning System |
| **GRS** | Geomorphological Relevant Situation |
| **HC** | High Coast |
| **LUSI** | Land Use Simplified Index |
| **LC** | Low Coast |
| **QW** | Quality Water value |
| **MarMAT** | Marine Macroalgae Assessment Tool |
| **MB** | Metric Blocks |
| **MCI** | Metal Content Index |
| **MED** | Mediterranean |
| **MDS** | Non-metric multiDimensional Scaling |
| **MPA** | Marine Protected Areas |
| **MS** | Member States |
| **MSW** | Mediterranean Surface Waters |
| **mt 23S** | mitochondrial 23S |
| **NEA** | North East Atlantic |
| **NJ** | Neighbour Joining |
| **PA** | Pressure Assessment |

| | |
|---|---|
| **PCA** | Principal Component Analysis |
| **PCR** | Polymerase Chain Reaction |
| **RA** | Relative species Abundance |
| **RICQI** | Rocky Intertidal Community Quality Index |
| **RSL** | Reduced Species List |
| **SD** | Standar Deviation |
| **SIMPER** | SIMilarity PERcentage analysis |
| **SIMPROF** | SIMilarity PROFile analysis |
| **SL** | Sensitivity Level |
| **SS** | Suspended Solids |
| **SST** | Sea Surface Temperature |
| **WAG** | Western Alboran Gyre |
| **WB** | Water Body |
| **WFD** | Water Framework Directive |
| **WPP** | Water Policy Plan |

# CONTENTS

# Abstract

## Resumen

*"If I were called in*
*to construct a religion*
*I should make use of water"*

Philip Arthur Larkin
(Collected Poems of Philip Larkin, 1989)

*Cystoseira elegans* and *Sargassum vulgare* in Cabo de Gata. Julio 2011. Photograph by Antonio Bermejo Lacida

## Abstract

According to the Water Framework Directive (WFD), the ecological status of European coastal waters must be assessed using different biological quality elements (BQE). One of the four proposed BQE is based on the composition and abundance of the marine macroalgae. Because of the biogegraphical differences along the European coasts, six ecoregions have been considered for biological indices development (Atlantic, Baltic, North Sea, Barents Sea, Norway Sea and Mediterranean Sea). The geographical position of Andalusia (Southern Spain), as a transition zone between the Atlantic and the Mediterranean Sea implies some technical and theoretical difficulties. Coastal waters of Andalusia belong to two different ecoregions, and their evaluation can be carried out with up to seven different macroalgal based indices. Moreover, the existence of a natural gradient along this coast interferes in the final value of the indices.

The main objectives of this thesis were: i) the adaptation and comparison of indices based on macroalgae for the assessment of the ecological status in coastal waters of Andalusia; and ii) the provision of useful information for management about the ecology and the biogeography of littoral communities in southern Iberian Peninsula.

The first objective is addressed in three chapters. In chapters 1 and 2, the Reduced Species List (RSL) and CARtography of LITtoral communities (CARLIT) indices were adapted to the particularities of Andalusian coasts. Afterwards, both indices were compared in the Strait of Gibraltar and the western Alboran Sea (chapter 3). The results showed that these indices were suitable to assess the ecological status in Andalusian coastal waters, and they yielded similar results. Overall, the ecological status of Andalusian water bodies (WBs) was good or high, excepting some highly modified WBs.

The second block is focused on the ecology and biogeography of macroalgal communities in southern Iberian Peninsula. In chapter 4 the biogeographical patterns of the Alboran Sea were studied based on the landscape and the species composition of littoral and upper-sublitoral communities, and compared to regional oceanographic patterns. The results pointed out the influence of regional oceanographic patterns in the littoral communities, and the existence of three different subregions: western, central and eastern Alboran. In chapter 5, considering the ecological importance of *Cystoseira mediterranea, C. amentacea* and *C. tamariscifolia*, a genetic approach based on microsatellites was developed to assess the taxonomic identity and the genetic structure of these populations along the southern Iberian Peninsula. The preliminary results suggest that only a genetic entity, probably *C. tamariscifolia*, is present in the Alboran Sea. Furthermore, these populations showed a moderate differentiation among them, being the most genetically diverse populations those in western and central Alboran. The knowledge of these ecological and biogeographic patterns will be essential for a proper management (e.g. design a network of marine protected areas) and to interpret the results yielded by indices based on macroalgae.

## Resumen

De acuerdo con la Directiva Marco del Agua (DMA), el estado ecológico de las masas de agua costeras de Europa se tiene que evaluar mediante diferentes elementos de calidad biológicos. Uno de los cuatro elementos de calidad propuestos se basa en la composición y abundancia de las comunidades de macroalgas. Debido a las diferencias biogeográficas existentes a lo largo de las costas europeas, se han considerado seis grandes ecorregiones para el desarrollo y aplicación de estos índices (Atlántico, Báltico, Mar del Norte, Mar de Barents, Mar de Noruega y Mediterráneo). La situación de Andalucía como zona de transición entre el Atlántico y el Mediterráneo supone una serie de dificultades tanto técnicas como conceptuales a la hora de abordar su estudio, ya que, al pertenecer sus aguas a dos ecorregiones, su evaluación puede llevarse a cabo hasta con siete índices distintos. Además, la existencia de un gradiente natural a lo largo de estas costas interfiere en los valores de los indicadores.

Los objetivos generales de esta tesis fueron: i) la adaptación y comparación de índices basados en macroalgas para la estimación del estado ecológico de las costas andaluzas; y ii) la aportación de información útil para la gestión sobre la ecología y biogeografía de las comunidades litorales en estas costas.

El primer objetivo se trata en un primer bloque formado por tres capítulos. En los capítulos 1 y 2, los índices "Reduced Species List" (RSL) y "CARtography of LITtoral communities" (CARLIT) se adaptaron para la evaluación del estado ecológico en las aguas costeras andaluzas. En el capítulo 3, los índices se compararon en el estrecho de Gibraltar y oeste del mar de Alborán. Los resultados indicaron que estos índices fueron adecuados para la evaluación del estado ecológico y mostraron resultados comparables. En términos generales, el estado ecológico de los cuerpos de agua andaluces fue bueno o muy bueno exceptuando algunos cuerpos de agua altamente modificados.

En el segundo bloque se estudia la ecología y biogeografía de las comunidades de macroalgas del sur de la Península Ibérica. En el capítulo 4 se analiza el paisaje y la composición específica de las comunidades litorales de la costa norte del mar de Alborán y su relación con la oceanografía de la zona, identificándose tres subregiones: Alborán occidental, Alborán central y Alborán oriental. En el capítulo 5, dada la importancia ecológica y para la gestión de *Cystoseira mediterranea, C. amentacea* y *C. tamariscifolia*, se estudió la identidad taxonómica y la estructura genética en el sur de la península ibérica de las diferentes poblaciones de este grupo de especies utilizando microsatélites. Los resultados preliminares sugieren que solo una especie está presente en el mar de Alborán, probablemente *C. tamariscifolia*, en lugar de tres como actualmente se supone. Estas poblaciones mostraron una diferenciación moderada entre ellas, siendo las poblaciones del oeste y centro del mar de Alborán las que presentaron una mayor diversidad genética. Toda esta información ecológica y biogeográfica será clave para una adecuada gestión (por ejemplo, para el diseño de una red de áreas marinas protegidas) e interpretación de la información dada por los índices basados en macroalgas.

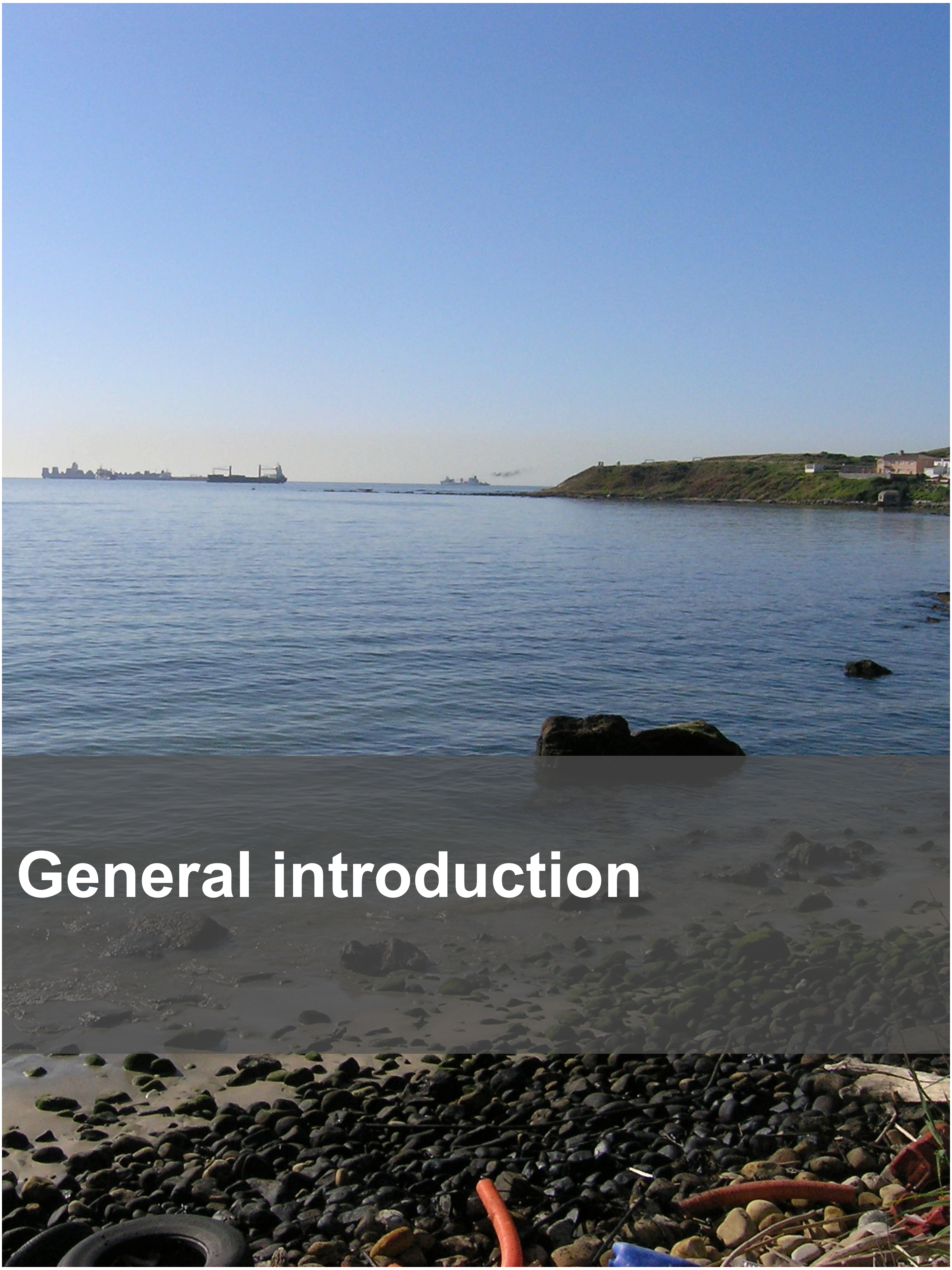
## Resumo

De acordo com a Directiva do Quadro da Água (DQA), o estado ecológico das massas de água costeira europeias avalia-se por meio de diferentes elementos de qualidade biológica. Um dos quatro elementos de qualidade proposto é baseado na composição e abundância das comunidades de macroalgas. Devido às diferenças biogeográficas existentes junto às costas europeias, definiram-se seis grandes ecorregiões (Atlântico, Báltico, Mar do Norte, Mar de Barents, Mar da Noruega e Mediterrâneo). A situação da Andaluzia como zona de transição entre o Atlântico e o Mediterrâneo, representa uma série de dificuldades, tanto técnicas como conceituais na abordagem do seu estudo. Devido às suas águas pertencerem a duas ecorregiões diferentes, a sua avaliação pode ser realizada com sete índices diferentes. Além disso, a existência de um gradiente natural ao longo destas costas interfere nos valores e nos indicadores.

Os objetivos gerais desta Tese de Doutoramento foram: i) adaptar e comparar os índices baseados em macroalgas destinados a estimar o estado ecológico das costas andaluzas; e ii) fornecer informações úteis para a gestão relativa à ecologia e biogeografia das comunidades litorais destas costas.

O primeiro objectivo é abordado préviamente e formado por três capítulos. Nos capítulos 1 e 2, os índices RSL e CARLIT foram adaptados para a avaliação do estado ecológico das águas costeiras andaluzas atlânticas e mediterrâneas. No capítulo 3, os índices foram comparados entre si no Estreito de Gibraltar e a oeste do Mar Alborán, dando resultados semelhantes. Os resultados indicaram que estes índices foram adequados para avaliar o estado ecológico e mostraram resultados comparáveis. Em termos gerais, o estado ecológico das massas de água da Andaluzia foi bom ou alto, com excepção de alguns corpos de água altamente modificados.

O segundo bloco fornece informações sobre a ecologia e biogeografia do sul da Península Ibérica. No capítulo 4 estuda-se a paisagem e a composição específica das comunidades litorais da costa norte do Mar de Alborán e a sua relação com a oceanografia da área, identificando-se três sub-regiões: Alboran ocidental, central e oriental. No capítulo 5, dada a importância ecológica e para a gestão de *Cystoseira mediterranea*, *C. amentacea* e *C. tamaricifolia*, estudou-se a identidade taxonómica e a estrutura genética, no sul da Península Ibérica de diferentes populações deste grupo de espécies utilizando microssatélites. Os resultados preliminares sugerem que uma única entidade genética está presente no Mar de Alborán, sendo provavelmente C*. tamariscifolia*. Além disso, estas populações mostraram uma diferenciação moderada entre elas, sendo as populações da zona a oeste e ao centro do Mar de Alborán as que apresentam uma maior diversidade genética. Toda a informação ecológica e biogeográfica obtida irá ser a chave para uma adequada gestão (por exemplo, no planeamento de uma rede de áreas marinhas protegidas) e interpretação através dos índices baseados em macroalgas.



# General introduction

*"Las cosas que comunalmente pertenecen a todas las criaturas que biuen en este mundo, son estas; el ayre, e las aguas de las lluvia, e el mar, e su ribera..."*

Alfonso X el sabio
(Ley de las Siete Partidas, S. XIII)

Algeciras Bay. June 2012. Photograph by Antonio Bermejo Lacida

## General introduction

The footprint of man is easily visible in the coastal zone, where human activities have been historically concentrated (Lotze et al., 2006) since the early years of the human civilization. More than the 40% of the world´s population is settled within 150 kilometres of the coast (UN, 2014). The Atlantic coast of Europe and the Mediterranean basin have been inhabited for millennia, being the alteration of environmental conditions and anthropogenic pressures stronger than in other coastal areas of the world (Airoldi and Beck, 2007; Coll et al., 2010); thus the European estuaries and coastal areas are among the most severely degraded coastal temperate systems worldwide (Lotze et al., 2006). To prevent further deterioration of the environment and to protect the natural heritage, the European Union has adopted different international conventions and has developed a wide legislative framework that directly or indirectly protect the coastal environment, its associated biodiversity and other natural resources (Table 1).

**Table 1-** Summary of main protection initiatives adopted by the European Union and State Members that directly or indirectly address issues related to the protection of Iberian marine coasts (modified from Airoldi and Beck, 2007).

| Initiative | Description |
|---|---|
| Ramsar Convention | Ramsar Convention on Wetlands, Ramsar (1971). Provides the framework for the conservation and wise use of wetlands of international importance especially as waterfowl habitat. Includes salt marshes and some lagoon systems and marine waters to a depth of 6 m. |
| Bonn Convention | Convention on the Conservation of Migratory Species of Wild Animals, Bonn (1979). Intergovernmental treaty, aiming to conserve terrestrial, marine and avian migratory species throughout their range. |
| Rio Convention | Convention on Biological Diversity, Rio de Janeiro (1992). Provides legal framework for biodiversity conservation and sustainable development. The Jakarta Mandate (1995) leads activity in marine biodiversity management and conservation. |
| Bern Convention | Convention on the Conservation of European Wildlife and Natural Habitats, Bern (1979). Aims at preserving wild flora and fauna and their natural habitats through national programmes using the co-operation between European States. |
| ICES Convention | Convention for the International Council for the Exploration of the Sea, Copenhagen (1964). Coordinates and promotes marine research in the North Atlantic, including the Baltic Sea and North Sea, and the Common Fisheries Policy on the protection of the marine environment and the regulation of fisheries. |
| OSPAR Convention | Convention for the Protection of the Marine Environment of the northeast Atlantic, Paris (1992). Merged the 1972 Oslo Convention on dumping waste at sea and the 1974 Paris Convention on land-based sources of marine pollution. It guides the protection of the marine environment of the northeast Atlantic and the identification of priority habitats and species. |
| Barcelona Convention | Amended in 1995 as the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean, Barcelona (1976). Provides legal framework to Mediterranean Action Plan (1975), under UNEP Regional Seas Programme. Aims to control human impacts and protect the marine and coastal Mediterranean environments. |
| Birds Directive (79/923/EEC) | Council Directive on the Conservation of Wild Birds. Identifies 194 endangered species and subspecies of birds for which the E.U. Member States are required to designate Special Protection Areas (SPAs). Over 4000 SPAs have been designated to date, covering 8% of E.U. territory. |
| Habitats Directive (92/43/EEC) | Council Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora. Aims to protect wildlife species and habitats, which have conservation that requires the designation of Special Areas of Conservation (SACs). These sites, together with the SPAs of the Birds Directive, make up the NATURA 2000 network, currently covering about 15% of E.U. coasts. Marine habitats broadly defined, and few marine species listed. |
| Shellfish Waters Directive (79/923/EEC) | Council Directive on the Quality Required of Shellfish Waters. Aims to ensure a suitable environment for shellfish harvest. Member States are required to designate coastal and brackish waters that need improvement to support shellfish fisheries. |
| Water Framework Directive (2000/60/EC) | Integrates and updates existing E.U. water legislations (e.g., Discharges of Dangerous Substances, Urban Waste Water Treatment, Nitrates Directive) and provides for water management. The Water Framework Directive requires surface and ground water bodies - such as lakes, streams, rivers, estuaries, and coastal waters - to be ecologically sound by 2015. |
| Marine Strategy Directive (2008/56/EC) | The proposed directive aims to define common objectives and principles at E.U. level to achieve good environmental status of the European marine environments by 2020, which is in line with the objetives of Water Framework Directive. It will establish European Marine Regions as management units for implementation. |

In this sense, the Water Framework Directive (WFD), which is inspired in the US Clean Water Act (Hering et al., 2010), supposed an important innovation for the assessment of the water quality in Europe, as this Directive represents a change in the scope of water management from the local scale to the basin one (Apitz et al., 2006). The WFD is mainly based on an ecological approximation rather than a traditional physico-chemical one, with ecosystems at the center of the management decision (Borja, 2005). The ultimate objective of this legislation is the achievement of a good ecological status (or good ecological potential in modified water bodies) for surface waters by 2015. Conversely, member states must develop necessary actions to solve this situation and reach a good ecological status of their waters. This ecological status is understood as an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, taking into account the physico-chemical nature of the water and sediments, the flow characteristics of the water and the physical structure of the water body.

The implementation of the WFD is a challenge for managers in general and ecologists in particular due to the complex requirements of the Directive concerning biological indicators (Hering et al., 2010): i) ecological status should be quantified into a single numerical value between 0 and 1, the Ecological Quality Ratio (EQR), which represents the ratio between the current and the reference (i.e. pristine or near-pristine) condition; ii) EQR should show a significant relationship with anthropogenic pressures and iii) classification should encompass five status classes (high, good, moderate, poor and bad). Furthermore, to ensure adequate comparability, the different indices developed within a determined geographical area (ecoregion) must be intercalibrated. These requirements have received criticism (Moss, 2007; Dufour and Piégay, 2009; Hering et al., 2010; Lopez y Royo et al., 2011) related to: the lost of information due to the integration of all ecological variables in an unique value; the impossibility to find pristine or near-pristine habitats in Europe; the difficulties to define anthropogenic pressures due to the diversity of human-induced disturbances (e.g. acidification, eutrophication, heavy metals, invasions by alien species, pollution by organic compounds and by organic matter and so on); and the concerns due to the intercalibration based on partial comparisons between methodologies for ecological assessment with different philosophies developed in different geographical areas. Nevertheless, this innovative point of view of the WFD has been welcomed and seen as an opportunity by many researchers, increasing knowledge of the biodiversity and the ecology of European surface waters. For instance, almost 4,000 peer-reviewed papers have resulted from research projects associated with the implementation of the Directive (query 'Water Framework Directive' in SCOPUS at 04/01/2014).

*Why this interest in WFD?*

Human-induced disturbances and their effects on the environment must be understood at a global and a local scale in order to comprehend natural processes and interactions with anthropogenic activities, and to target management actions effectively (Lopez-Royo et al., 2009). The widespread use of indicators as informative tools in environmental management fulfil the objective of a comparable and replicable system of information and evaluation (OECD, 2005). Nutrients, turbidity and heavy metals are usually used as variables to define the water quality in consequence of the visible effects of eutrophication and the chemical pollution in marine nearshore ecosystems at a local scale, the risk for human health and the negative impact in economic activities such as tourism or fishing. A considerable effort has been made by the international community to monitor the distribution of these variables in the sea and to determine their effects on marine ecosystems. However, analyses of water samples may give accurate, but local and transient information. Furthermore, this approach cannot determine the long-term effect of pollutants (in broad sense) on benthic communities (McCormick and Cairns, 1994; Licata et al., 2004). In contrast, biological indicators avoids drawbacks associated with a direct survey of pollutants in water samples, such as the need to repeat the samplings periodically or the fluctuation in pollution levels (Ostapczuk et al., 1997). Moreover, biological indicators may indicate the long-term effects of pollutants in benthic communities when these pollutants cannot be measured or have disappeared from the environment (Licata et al., 2004). The most important theoretical advantage is that bioindicators are a direct measurement of the pollution effects in organisms, which is often the main goal of the studies. Therefore, the use of bioindicators can yield a more integrated response than that provided by physico-chemical indicators.

*Why macrophyte communities?*

In the context of the WFD, one of the four biological quality elements (BQEs) proposed for coastal waters are macroalgae. The use of macrophytes as bioindicators to assess pollution in the marine environment has been proved successful in many ecological studies (e.g. Orfanidis et al., 2001; Leoni et al., 2006; Arévalo et al., 2007). The sedentary condition of attached macrophytes integrates the effects of long-term exposure to different anthropogenic pressures resulting in a decrease or even disappearance of the most sensitive species and their replacement by highly resistant, nitrophilic and/or opportunists species (e.g. Borowitzka, 1972; Diez et al., 1999). Very different stressors can produce directly or indirectly impacts in rocky shore communities dominated by macrophytes (Crowe et al., 2000): nutrient pollution (Arévalo et al., 2007), thermal pollution (Teixeira et al., 2012), heavy metals (Castilla, 1996; Sales et al., 2011), overfishing (Thibaut et al., 2005; Coll et al., 2010), harvesting (Ugarte, 2011), trampling (Milazzo et al., 2002; Rita et al., 2011), siltation/turbidity (Thibaut et al., 2005; Echavarri-Erasun et al., 2007) or alien species introduction (Montefalcone et al., 2010), among others.

Marine macrophytes are conspicuous elements for many temperate coasts (Lüning, 1990). They are an ecologically relevant group for littoral and upper sublittoral communities as are the primary structural link in the ecosystem food web (Norderhaug et al., 2007; Mangialajo et al., 2008; Vizzini, 2009). In addition, they form an irregular landscape of distinct habitats providing a complex set of interspersed physical and biological environments (fig. 1) that affect the development and maintenance of the associated fauna (Tuya et al., 2012). Consequently, changes in composition and abundance of littoral macrophyte communities will have significant effects in shore ecosystems (e.g. Dayton et al., 1992; Serio et al., 2006; Tuya et al., 2009).

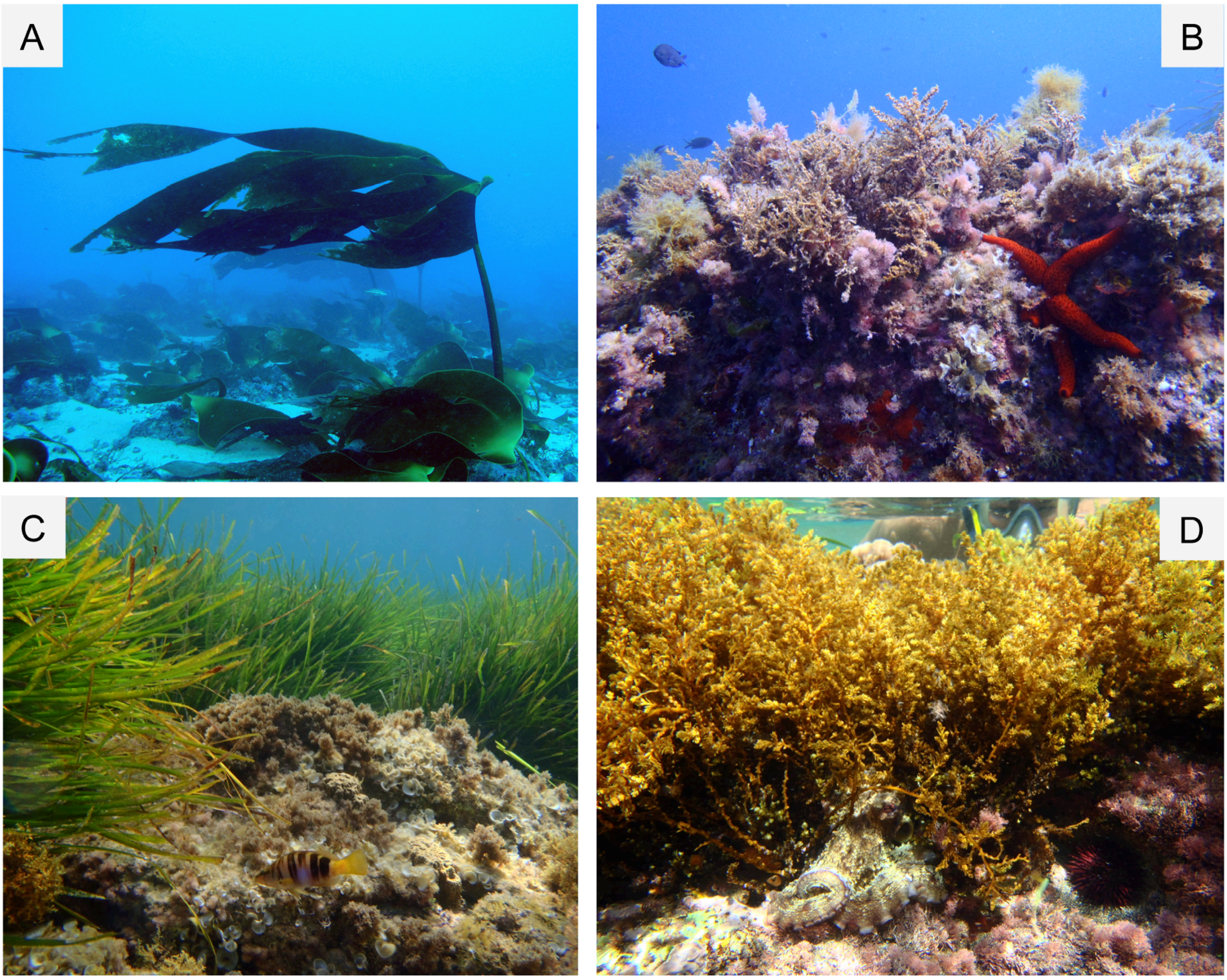



**Fig. 1.-** Different sublittoral marine systems dominated by structural complex macrophytic communities, A: subtidal kelp forest of *Laminaria ochroleuca* in Tarifa island (30 m of depth; Photography by G. Mourente), B: subtidal macroalgal assemblage dominated by *Cystoseira spinosa* in Cala del Toro (Cabo de Gata Natural Park; 10 m of depth); C: Shallow *Posidonia oceanica* meadow in Los Escullos (Cabo de Gata Natural Park; 2 m of depth); D: Upper-sublittoral meadow of *Cystoseira amentacea* var. *stricta* in Isleta del Moro (Cabo de Gata Natural Park; 0.20-0.30 m of depth).

It is remarkable that seagrasses and seaweeds of the orders Tilopteridales, Laminariales and Fucales, some of the most important bioengineering species in the Mediterranean and Atlantic phytal zone (Lüning, 1990; Giaccone et al., 1994; Figueroa et al., 2014; Pérez-lloréns et al., 2014), are suffering a general decline (Thibaut et al., 2005; Diaz-Almela et al., 2007; Fernández, 2011; Pérez-lloréns et al., 2014). This is producing a simplification in the structural complexity of shore communities and the landscape homogenization (i.e. habitat destruction or degradation). In this sense, habitat destruction or degradation is considered the most important threat to the diversity, structure, and functioning of marine coastal ecosystems and to the goods and services they provide (Lotze et al., 2006; Airoldi and Beck, 2007; Coll et al., 2010). The implementation of the WFD offers a good opportunity for the assessment of the intensity and extension of this phenomenon.

Thus, macrophyte communities have many desirable attributes as indicators of ecosystem integrity and environmental change, because they are: i) sensitive to different anthropogenic pressures, yielding an integrative response; ii) ecologically and socially relevant; iii) broadly extended along European coast; and iv) present in almost all ecological situations (from pristine to highly degraded environment). However, inherent limitations should be considered to assess properly the ecological status (see below).

*Some considerations about indices based on macroalgal communities*

Indices based on macroalgal communities can be used to assess the extent of biological changes from reference conditions. However, the causes of this change can be difficult to determine due to the integrated response of these communities and the cumulative impacts in natural conditions (McCormick and Cairns, 1994). Moreover, the non-linear response and the time of response of the community to some stressors (Knowlton, 2004) make difficult to propose specific management measures to avoid ecosystem degradation. For this reason, the WFD considers that the ecological status has to be evaluated using BQEs supported by hydromorphological and physico-chemical quality elements, as the information supplied by the BQEs is different and complementary to the physico-chemical assessment.

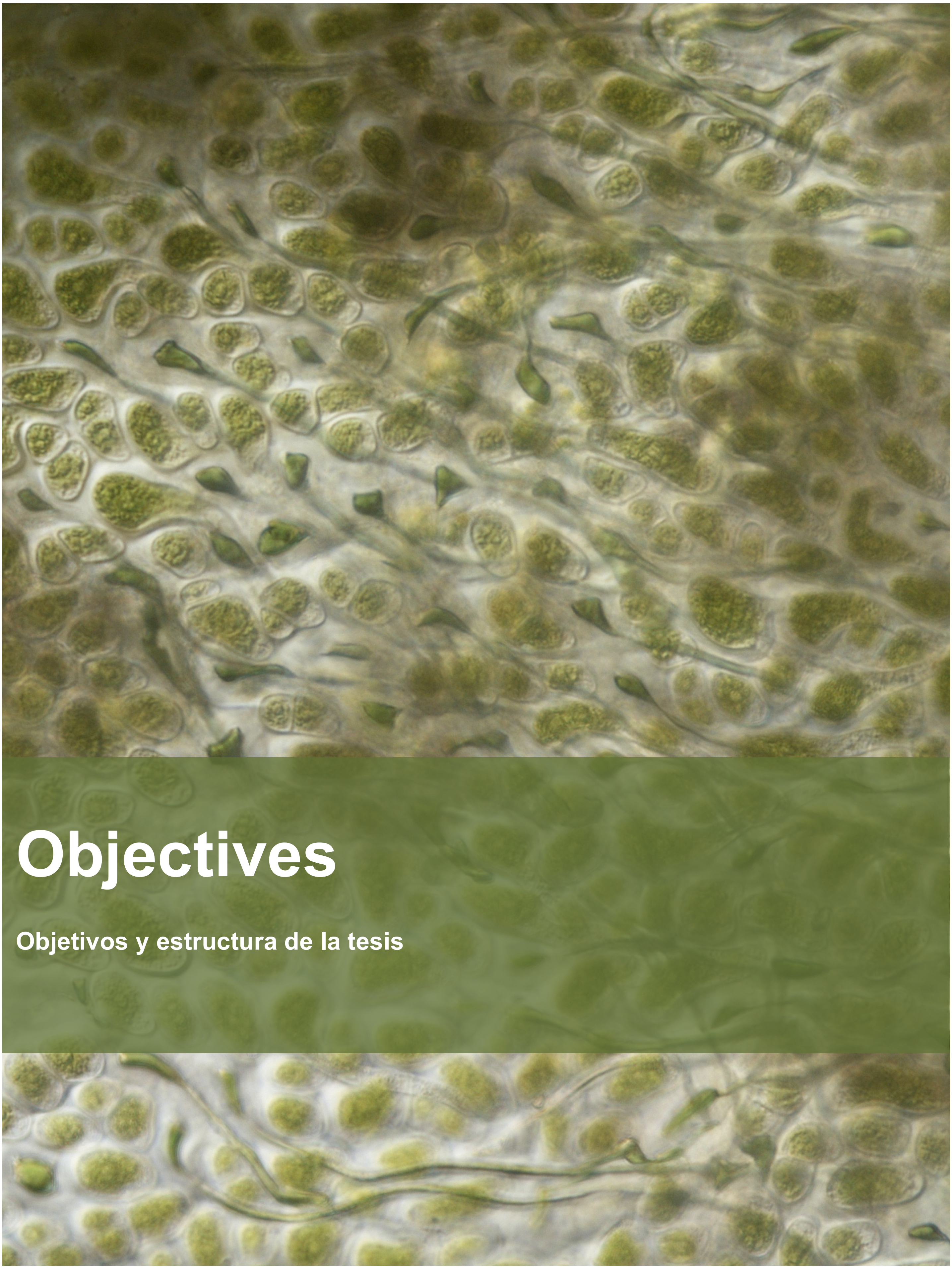
Macroalgal communities are sensitive to an array of anthropogenic pressures. These pressures are superimposed on those caused by natural environmental factors (Crowe et al., 2000). In this sense, quite often most of the community change is unexplained even when the whole environmental variables are included (e.g. nutrients, temperature, salinity, tidal range...). In fact, variability in environmental and biological factors, and biogeographic patterns are major drivers of community structure (Moss, 2007). Thus, the knowledge of ecological and biogeographic patterns of a studied region is necessary to define homogeneous sets and reference conditions (Karr, 1999), reducing the influence of natural variability in the final result of the biological indices.

To address the reduction of the influence of this biological variability in the ecological assessment, the WFD divided the European waters in relevant eco-regions (Mediterranean, Baltic, Black Sea and Atlantic). In these eco-regions, coastal waters have been classified according to environmental characteristics to delimit different types (IES, 2009). In spite of this practical classification, it is of little value to develop a single indicator, even based on the same BQE, to assess the ecological status of all coastal waters within the same eco-region. There are biogeographic differences, which may not be acknowledged by indices that are developed for a particular area. In this sense, Guinda et al. (2008) pointed out that, although the water framework directive considers the Northeast Atlantic as an entire ecoregion, the Iberian coastal marine ecosystem are clearly different from northern coastal areas (EEA, 2006). Furthermore, despite Alboran Sea is included in the Mediterranean ecoregion (IES, 2009), the biogeographic particularities of this area makes necessary to define new reference conditions and to propose methodological adjustments (Ballesteros et al., 2007). Accordingly, when a biological indicator designed in one sub-region is intended to be applied in others, it should be modified and reassessed (Ballesteros et al., 2007; Juanes et al., 2008). On the other hand, although Member States (MS) are allowed to use their own national classification systems, adequate comparability and consistence has been searched through the process of intercalibration, undertaken by the different MS within an eco-region (European Commission, 2000 – Annex V). However, in particular, border areas, as the Gibraltar Strait, the intercalibration between indices developed for different eco-regions is necessary to manage coastal waters and to avoid any bias in the ecological status. This fact is especially important considering legal implications when a good ecological status is not achieved (European Commission, 2000).

*To reach a good ecological status*

The ultimate aims of the WFD are the prevention of further deterioration of marine habitats and the restoration of degraded water bodies. Restoration can be understood in different ways, from treatment of symptoms to treatment of ultimate cause, being the latter the most suitable interpretation (Moss, 2007). Restoration actions should put their efforts into reduce direct interventions minimizing intrusive actions and favouring natural resilience and recovery of littoral ecosystems. Thus, a deep scientific knowledge about the biogeographic and ecological patterns of littoral and upper sublittoral communities is necessary for a proper assessment and management of coastal water bodies, identifying the ultimate cause of degradation and the potential of natural recovery of littoral ecosystems. For instance, the knowledge about the coupling between regional oceanography and littoral communities is a first step to forecast the possible effects of climate exchange in these communities (Boaventura et al., 2002; Blanchette et al., 2008). The study of genetic structure patterns of habitat forming species with limited dispersion, growth, and/or recolonization abilities as *Astroides calycularis* (Goffredo et al., 2010; Casado-Amezúa et al., 2012), *Cystoseira* spp. (Susini et al., 2007; Mangialajo et al., 2012) or *Posidonia oceanica* (Gobert et al., 2006; Alagna et al., 2013) is essential for the design of a net of Marine Protected Areas (MPA) to favour natural recolonization and to accomplish with protection needs of these species, or to identified proper donors populations for an hypothetical restoration (e.g. Sales et al., 2011; Gianni et al., 2013) avoiding problems as genetic contamination or homogenization.

In summary, the present study is intended to apply and to reassess two indices based on macroalgae in two biogeographical areas of the coast of southern Spain; and to gain an insight into the ecology and biogeography of littoral communities, and population genetics of *Cystoseira ericaefolia* in these areas, which is essential for a better interpretation of the indices and the management of coastal water bodies.



# Objectives

## Objetivos y estructura de la tesis

*"The Directive is a truly revolutionary document. If its spirit is respected, it can be an early step in the sort of changes that are ultimately necessary for the survival, in reasonable comfort, of a large human population."*

Brian Moss

(Shallow lakes, the water framework directive and life.

What should it all be about? Hydrobiologia (2007) 58 4: 381-394)

Microscopic surface view of *Gayralia oxysperma*. April 2010. Photograph by Ricardo Bermejo Lacida

## Objectives and structure of the thesis

The main goals of this PhD Thesis are:

1. Application and reassessment of the Reduced Species List (RSL) index, which is based on the species richness of seaweed assemblages on intertidal rocky seashores to assess the ecological status under the Water Framework Directive in the Atlantic coast of Southern Spain.
2. Application and reassessment of the Littoral and sublittoral Cartography (CARLIT) index, which is based on the cartography of littoral and upper sublittoral communities to assess the ecological status under the Water Framework Directive in the Mediterranean coast of Southern Spain.
3. Comparison between CARLIT and RSL indices based on seaweed assemblages in the boundary between two ecoregions (Gibraltar Strait and western Alboran).
4. Study of the effect of meso-scale oceanographic patterns on the biogeographical variability of seaweed assemblages on rocky seashores along the Alboran Sea.
5. Study of the genetic patterns and taxonomic identity of the habitat forming species of *Cystoseira ericaefolia*-group along southern Iberian Peninsula.

To address the objectives, the results of the thesis have been structured in five chapters:

Chapters 1 and 2 address the first and second of the main objectives proposed, respectively. In chapter 1, the reduced species list (RSL; Wells et al., 2007) index is applied and reassessed in the Atlantic coast of Andalusia (Bermejo et al. 2012). Similarly, in chapter 2 the cartography of littoral and upper sublittoral communities (CARLIT; Ballesteros et al., 2007) is adapted to the Mediterranean coast of Southern Spain (Bermejo et al. 2013).

Subsequently, in chapter 3 these indices are compared in Western Alboran Sea and the Gibraltar straight, where both indices can be applied (Bermejo et al. 2014). The principal aim of this chapter was to ensure comparability among methodologies and to guarantee that the two indices provide equivalent ecological quality assessment.

In chapter 4 is assessed the link between oceanographic patterns and littoral and upper sublittoral communities in the Alboran Sea (Bermejo et al., submitted).

Finally, in chapter 5, the habitat-forming populations belonging to the *Cystoseira ericaefolia*-group, constituted by three species (*C. amentacea, C.mediterranea* and *C. tamariscifolia*), are analysed based on preliminary results obtained using a genetic approach through microsatellite markers.

## Objetivos y estructura de la tesis

Los principales objetivos de esta tesis doctoral han sido:

1. El desarrollo y adaptación del índice RSL (Reduced Species List), el cual está basado en el estudio de la composición y riqueza específica de las comunidades intermareales rocosas dominadas por macroalgas para la estimación del estado ecológico de las aguas costeras del Atlántico andaluz, en el contexto de la Directiva Marco del Agua (DMA).
2. El desarrollo y adaptación del índice CARLIT (CARtografía LIToral), el cual está basado en el cartografiado de las comunidades de la zona litoral y sublitoral somera para la estimación del estado ecológico de las aguas costeras del Mediterráneo andaluz, en el contexto de la DMA.
3. La Comparación los índices CARLIT y RSL en la zona de transición entre el Atlántico y el Mediterráneo (Estrecho de Gibraltar y parte Occidental del Mar de Alborán).
4. El estudio del efecto de patrones oceanográficos de meso-escala en la biogeografía de las comunidades rocosas de macroalgas a lo largo del Mar de Alborán.
5. El estudio de los patrones genéticos de las especies formadora de hábitats pertenecientes al grupo *Cystoseira ericaecifolia* a lo largo del Sur de la Península Ibérica.

Estos objetivos se abordan a lo largo de los 5 capítulos que componen el presente trabajo de la siguiente manera:

Los capítulos 1 y 2 se centran en los dos primeros objetivos propuestos. El capítulo 1 está dedicado a la adaptación del índice RSL (Reduced Species List; Wells et al., 2007) a la costa atlántica andaluza (Bermejo et al., 2012). Y el capítulo 2 a la adaptación del índice CARLIT (Ballesteros et al., 2007) a la región mediterránea andaluza (Bermejo et al., 2013).

En el capítulo 3 se comparan ambos índices en la zona del estrecho y oeste del Mar de Alborán (Bermejo et al., 2014). El objeto de este ejercicio fue asegurar la coherencia y comparabilidad de los valores de calidad ambiental obtenidos por ambos índices.

El capítulo 4 se evalua la relación entre los patrones oceanográficos y las comunidades litorales y sublitorales superiores en el Mar de Alborán (Bermejo et al., sometido).

El capítulo 5 se centra en el estudio genético de las comunidades de *Cystoseira* del grupo *ericaecifolia* (*C. amentacea* var. *stricta*, *C. mediterranea* y *C. tamariscifolia*) utilizando microsatélites.

## Objectivos e estrutura da tese

Os principais objectivos desta tese de doutoramento foram:

1 . O desenvolvimento e adaptação do índice RSL (Reduced Species List), o qual é baseado no estudo da composição e riqueza específica das comunidades intertidais rochosas dominados por macroalgas para estimar o estado ecológico das águas costeiras andaluzes do Atlântico, no âmbito da Directiva do Quadro da Água (DQA).
2. O desenvolvimento e adaptação do índice CARLIT (Cartografia Litoral), com base no mapeamento das comunidades da zona litoral e sublitoral superficial para estimar o estado ecológico das águas costeiras andaluzes do Mediterrâneo, no contexto da DQA .
3 . A comparação entre os índices RSL e CARLIT na zona de transição entre o Atlântico e o Mediterrâneo (Estreito de Gibraltar e parte ocidental do Mar de Alborán) .
4. O estudo do efeito de padrões oceanográficos de meso-escala na biogeografia das comunidades rochosas de macroalgas ao longo do Mar de Alborán.
5. O estudo dos padrões genéticos de espécies formadoras de habitats pertencentes ao grupo *Cystoseira ericaecifolia* ao longo do sul da península Ibérica.

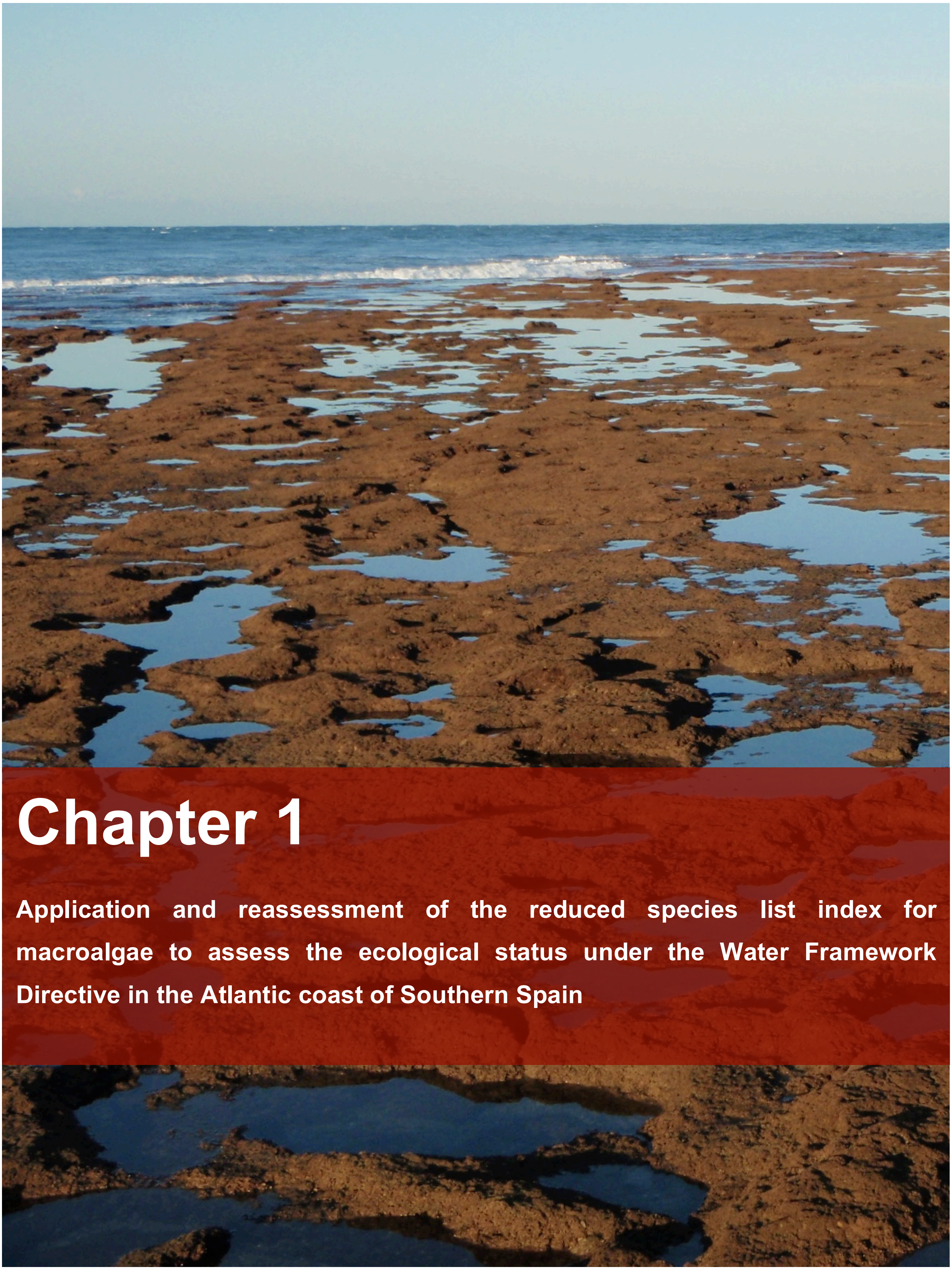
Estes objectivos são abordados em todos os 5 capítulos que compõem o presente trabalho da seguinte forma:

Os capítulos 1 e 2 centram-se sobre os dois primeiros objectivos propostos. O capítulo 1 é dedicado à adaptação do índice RSL (Reduced Species List; Wells *et al.,* 2007) para a costa Atlântica andaluza (Bermejo *et al*., 2012). E o capítulo 2 à adaptação do índice CARLIT (Ballesteros *et al.,* 2007) para a região do Mediterrâneo andaluz (Bermejo *et al.,* 2013).

No capítulo 3, os dois índices são comparados na zona do Estreito e a oeste do Mar de Alboran (Bermejo *et al*., 2014). O objectivo deste exercício foi garantir a coerência e a comparabilidade dos valores de qualidade ambiental obtidos por ambos os índices.

No capítulo 4 avalia-se a relação entre os padrões oceanográficos e as comunidades litorais e sublitorais superiores no Mar de Alboran (Bermejo *et al*., submetido).

O capítulo 5 centra-se no estudo genético das comunidades de *Cystoseira* do grupo *ericaecifolia* (*C. amentacea* var. *stricta*, *C. mediterranea* e *C. tamariscifolia*) usando microssatélites.



# Chapter 1

## Application and reassessment of the reduced species list index for macroalgae to assess the ecological status under the Water Framework Directive in the Atlantic coast of Southern Spain

*"Pocas regiones hay tan dignas de un estudio detenido, desde el punto de vista botánico, como la provincia de Cádiz. Centinela avanzado de la Península Ibérica, casi tocando con las próximas costas africanas, su flora presenta un carácter especial que no puede confundirse con ninguna otra; y si es así en lo que á flora terrestre se refiere, aún es más en cuanto con la flora marina se relaciona. En sus costas se confunden las aguas del Mediterráneo y el Atlántico, y las algas de ambos mares son arrojadas por las tempestades á sus playas. Es el país de promisión para el algólogo..."*

Romualdo González Fragoso
(Plantas Marinas de la Costa de Cádiz, 1886)

Trafalgar daybreak. October 2008. Photograph by Ignacio Hernández Carrero

# Application and reassessment of the reduced species list index for macroalgae to assess the ecological status under the Water Framework Directive in the Atlantic coast of Southern Spain

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## ABSTRACT

According to the Water Framework Directive (WFD) macroalgae are one of the Biological Quality Elements proposed to assess the ecological status of coastal water bodies. In the case of the North East Atlantic coastal shores, two methodologies have been implemented (RSL – reduced species list – in the U.K.; CFR – quality of rocky bottoms – in the Spanish Cantabric Sea). However, the ecological differences between these shores and the Atlantic coasts of Southern Spain imply a reassessment of these indices when applied to this water body. In this study, the RSL index has been reassessed for the rocky shores of the Atlantic coast of Andalusia (south-western Spain). In addition, an ecological and a morphological approximation to this index have been compared. After successive field sampling in the period 2006–2010, a reduced species list was developed for this shore. Based on anthropogenic pressures (water turbidity, nutrients, metal concentration and the distance to sources of stress), 19 sites along the coast were classified in five quality status (high, good, moderate, poor and bad) as proposed in the WFD. According to this classification the RSL index was calibrated. Finally, the results of the reassessed RSL-index were compared with the water quality. Overall, most of the elements yielded a significant relationship with the water quality and showed significant differences among the ecological quality classification. The less significant boundary among ecological status is the one lying between good and high. The results showed that both approximations of the RSL index were suitable to assess the ecological status, being the ecological approximation more suitable. Furthermore, the data analysis pointed out the existence of two coastal fringes with a different intertidal composition of algal species: Atlantic Cádiz and the Strait of Gibraltar.

Keywords: Biological indicators; Macroalgae; North East Atlantic; Rocky shore; Water Framework Directive; Water Quality.

## INTRODUCTION

Multiple activities producing very different stressors concur in coastal areas. Most of the national and international institutions have identified population density, urbanization, agriculture, tourism, industry, fisheries and marine transport as the main pressures on the coastal zone (Casazza et al., 2002; EEA, 1999; UNEP, 1996). These pressures can change the aquatic conditions producing different forms of pollution (e.g. acidification, eutrophication, heavy metals, invasions by alien species, pollution by organic compounds and by organic matter) and degrade the environment. In this sense, one of the main reasons that explain the regression of marine nearshore ecosystems is the organic and nutrient enrichment as a consequence of domestic wastes (Flechter, 1996). Hering et al. (2010) identified the eutrophication as the most important pressure in European marine ecosystems, being the reduction of nutrient loads the main restoration measure. This pressure can change the underwater light regime and substrate type (Nielsen et al., 2002; Schubert and Forster, 1997) implying a simplification of the architectural complexity of the communities (Arévalo et al., 2007). In addition, the increase of heavy metals introduced via polluted rivers, marine outfalls and through the deliberate dumping of wastes in coastal waters contributes to the overall deterioration of coastal ecosystems. In fact, anthropogenic releases of some heavy metals in aquatic ecosystems have been estimated to be up to three orders of magnitude greater than the natural inputs (Chase et al., 2001; Gheggour et al., 2002; Schindler, 1991). For these reasons, nutrients, turbidity and heavy metals are usually used to define the water quality. In this sense, a considerable effort has been made by the international community to monitor the distribution of nutrients, turbidity and heavy metals in the sea and to determine their effects on marine ecosystems. For instance, in the case of Andalusia (southern Spain) this monitoring has been carried out since 1988 onwards. However, analyses of water samples may give accurate, but local and transient information. Furthermore, this approach cannot determine the long-term effect of these pollutants on benthic communities (Licata et al., 2004).

Bioindicators have several noteworthy advantages compared to physico-chemical indicators. The most important is that bioindicators are a direct measurement of the pollution effects in organisms, which is often the main goal when assessing the effect of a pollutant. Secondly, bioindicators may indicate the long-term effects of pollutants in benthic communities when they cannot be measured or have disappeared from the environment (Licata et al., 2004). In addition, the use of bioindicators avoids drawbacks associated with a direct survey of contaminants in water samples, such as the need to periodically repeat numerous water drawings because of continuous movement of the waters and the fluctuation in contaminant levels (Ostapczuk et al., 1997). Therefore, the use of bioindicators can yield a more integrated response than physico-chemical indicators do.

Thus, the Water Framework Directive (WFD, 2000/60/EC) supports the use of biological indicators to assess water quality. Furthermore, to prevent further deterioration of marine habitats, WFD requires the assessment of the ecological status of surface waters to implement management plans. In the case of coastal water bodies, the ecological status has to be evaluated using different biological quality elements (BQEs; phytoplankton, macroalgae, marine angiosperms and benthic invertebrates), and supported by hydromorphological and physico-chemical quality elements. For the purposes of the WFD, the European coastal waters have been divided in relevant eco-regions (Mediterranean, Baltic, Black Sea and Atlantic) that include different biogeographic regions and subregions. In these regions, the coastal waters have been classified according to environmental characteristics to delimit different types (IES, 2009). In spite of this practical classification, it is of little value to develop a single indicator, even based on the same BQE, to assess the ecological status of all coastal waters of the same eco-region. There are biogeographical differences in these large eco-regions, which may not be acknowledged by indices that are developed in particular areas. For instance, in the case of the BQE macroalgae, Guinda et al. (2008) pointed out that although the WFD considers the Northeast Atlantic as an entire eco-region, the large marine ecosystems project (LME), initiated to support the global objectives of Agenda 21, clearly distinguishes the Iberian coastal marine ecosystem from northern coastal areas (EEA, 2006). Accordingly, when a biological indicator designed in one sub-region is used in others, this indicator may be reassessed.

The use of macroalgae as bioindicators to assess pollution in the marine environment has been proved successful in many ecological studies (e.g. Borowitzka, 1972; Díez et al., 1999; Gorostiaga and Díez, 1996). The sedentary condition of attached macroalgae integrates the effects of long-term exposure to nutrients and/or other pollutants resulting in a decrease or even disappearance of the most sensitive species and their replacement by highly resistant, nitrophilic or opportunists species (Díez et al., 1999; Murray and Littler, 1978; Tewari and Joshi, 1988). Therefore, macroalgal communities arise as a useful tool to analyze changes in water quality (Fairweather, 1990). Furthermore, as macroalgal communities provide habitat and canopy cover for a wide variety of intertidal organisms (e.g. Pavia et al., 1999), changes in these communities will have significant effects on shore ecosystems (e.g. Hereu, 2004). For these reasons, the WFD proposed, among others BQE (see above), the use of composition and abundance of macrophyte communities to develop bioindicators to assess ecological quality of European coastal waters.

So far, two indices, based on the study of macroalgal communities along intertidal rocky shores, have been proposed for Atlantic coastal waters: reduced species list (RSL; Wells, 2008; Wells et al., 2007) and quality of rocky bottoms (CFR; Guinda et al., 2008; Juanes et al., 2008).

The RSL index utilises five elements to describe ecological status: species richness of a reduced species list; proportion of red algae; proportion of green algae; ESG (ecological status group) ratio, and proportion of opportunist species (Wells et al., 2007). The RSL index is based on species occurrence while CFR index uses the relative abundance of species. This fact is very important when results are analyzed, because the sensibility and spatio-temporal scale depend on it. For this reason RSL is less sensitive but more robust and can be used in meso-scale studies (Bermejo, 2009). Furthermore, the RSL index is less subjective and more precise than the CFR index (Guinda et al., 2008). These characteristics suggest that the RSL index may be more suitable to assess the ecological status of coastal waters. In spite of this, the preliminary results obtained for this index in the northern coast of Spain were worse than the result obtained for CFR when a pollution gradient was assessed (Guinda et al., 2008). However, the same authors proposed that to achieve a good calibration and validation of both indices, further analyses should be carried out at a different geographical location and against different types of pollution sources.

Some elements used in the RSL-index have been previously discussed (Arévalo et al., 2007; Guinda et al., 2008). For instance, the classification of species in two ESG, based on the functional form groups of macroalgae proposed by Littler and Littler (1980) and Littler et al. (1983) may have some limitations, as functional forms were originally suggested to predict productivity and other ecological attributes (e.g. grazing resistance, competitive abilities, reproductive effort); however, the resistance to pollution cannot be directly deduced from morphological features of the species (Arévalo et al., 2007). This has sometimes led to assign a particular species to different ESG (e.g., Corallina, ESG-I by Orfanidis et al., 2001 and ESG-II by Ballesteros et al., 2007) or to give them an opportunistic character (e.g. Ceramium, considered opportunist by Guinda et al., 2008 and non-opportunist by Wells et al., 2007). On the other hand, the proportion of rhodophyta evidenced problems in adjusting due to the biogeographical and ecological differences between northern cold and southern temperate waters (Guinda et al., 2008).

Therefore, in this framework, this study pursues a double objective: (i) to apply and reassess the RSL index to the Atlantic coast of southern Spain and (ii) to compare the values of this index with the previous classification of the water quality based on concentration of nutrients, metals turbidity and distance to sources of stress at a spatial meso-scale.

## MATERIALS AND METHODS

From March of 2008 to April of 2010, 19 sites located along the Atlantic coast of southern Spain were sampled (Fig. 1). The field samplings were carried out during spring and summer, coinciding with the peak growth of littoral communities (Ballesteros, 1992). In each site, a stratified sampling, registering all subhabitats, was carried out to obtain amacroalgal species list (Wells et al., 2007). Each sampling lasted approximately 1 h and was carried out during the low tide along 50–60m width of the whole rocky intertidal shore. When identification of specimens in situ was impossible, they were taken to the laboratory for a detailed observation. The taxonomic nomenclature used followed AlgaeBase (Guiry and Guiry, 2010). At each sampling site, physical characterization of the shore was estimated according to Wells et al. (2007).

### *Reduced species list*

A reduced species list for Atlantic coasts of Andalusia was elaborated from the full species list obtained at each site. According to Wells et al. (2007) this list was composed of species with the most significant contribution to the overall species composition of rocky shores in this geographical area; moreover, the number of species from this RSL was directly proportional to the total species richness at different localities. This list acts as a surrogate to the production of a full species list, which requires a higher level of taxonomic expertise. Therefore, the species or taxa included must meet three requirements: (i) they must be easily identifiable; (ii) taxa associations were considered when these macroalgae had the same ecological requirements and/or taxonomic identification was difficult (i.e. Delesseriaceae group included the genera *Acrosorium*, *Cryptopleura* and *Haraldiophyllum*); and (iii) these taxa must by perennials and not seasonals. For this reason, easily identifiable seasonal species were excluded from the list (i.e. *Liagora* spp.; *Hypnea musciformis*).

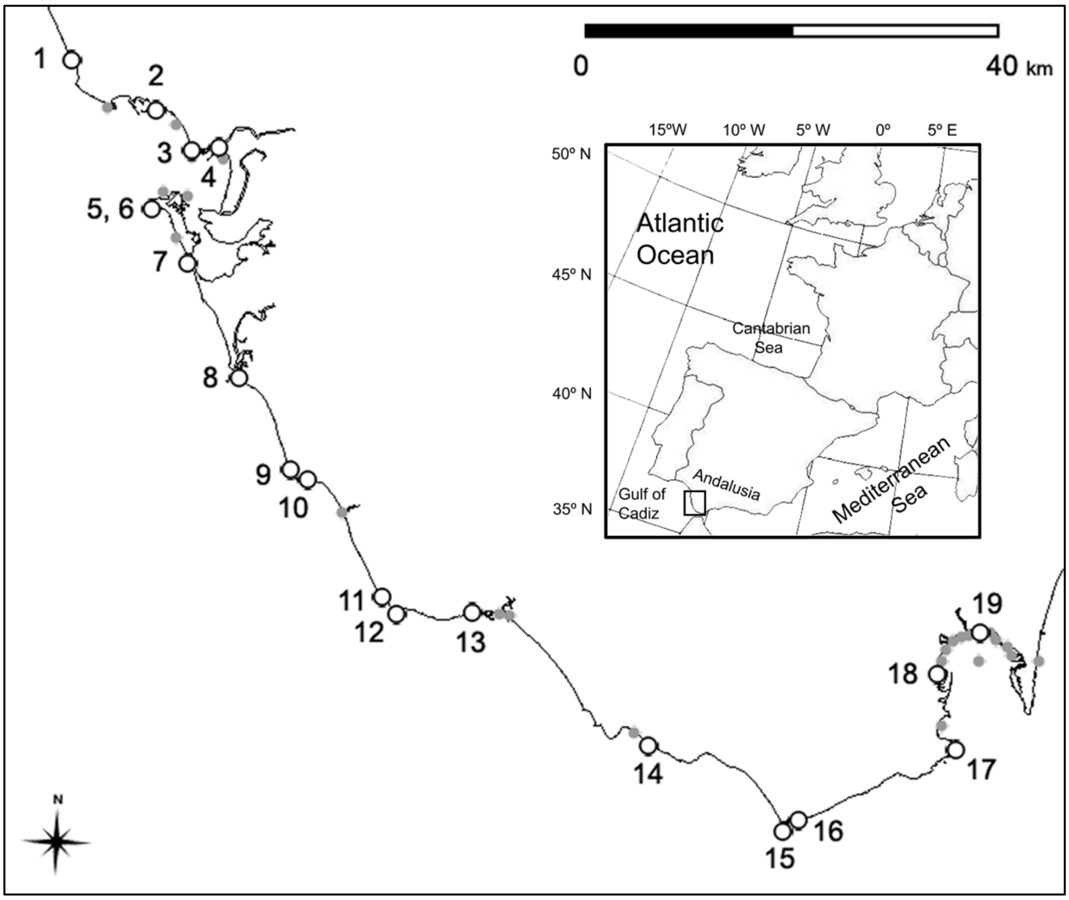


**Fig. 1.-** Geographical distribution of the different sampling points along the coast of Cadiz (Andalucía, southern Spain). 1- Punta Pegina, 2- Rota, 3- Aculadero, 4- La Puntilla, 5- Caleta (facing west from an isthmus), 6- Caleta (facing east from an isthmus), 7- El Chato, 8- Sancti Petri, 9- Roche, 10- Cala del Aceite, 11- Zahora, 12- Trafalgar, 13- Playa de la Hierbabuena, 14- Piscinas de Bolonia, 15- Isla de Tarifa, 16- Punta Camorro, 17- Punta Carnero, 18- Puerto de Algeciras, 19- Playa del Guadarranque. Black dots represent sample stations of “Plan de Policía de Aguas” for Andalusian shores.

To identify different ecological or biogeographic areas based on the species composition of macroalgal communities according to the RSL, a cluster analysis “between-group linkages” using Dice index was applied among sites (Dice, 1945). “Puerto de Algeciras (18)”, “La Puntilla (4)” and “Aculadero (3)” sampling sites were excluded from the cluster analysis because they presented some physical and ecological particularities (see below) that limited the growth of macroalgal communities. Moreover, “La Caleta left (5)” and “La Caleta right (6)”, and “Roche (9)” and “Cala del Aceite (10)” were aggregated due to the nearness of these sites and to facilitate the interpretation of the results.

*Reassessment of the RSL elements*

To apply the RSL index (Wells et al., 2007) in the southern coast of Spain the proportion of red algae was substituted for the number of red algae (Guinda et al., 2008), the ESG ratio was replaced with the proportion of ESG-I (Hernández, 2008; Ivesa et al., 2009) whereas two different approximations were proposed for the opportunists and for the ESG classification: (i) morphological approximation: to calculate the proportion of ESG-I, the reduced species list was classified in ESG-I and ESG-II (Orfanidis et al., 2001). When species were previously classified, this indication was followed (Guinda et al., 2008; Juanes et al., 2008; Orfanidis et al., 2003; Orlando-Bonaca et al., 2008). The same criterion was followed to classify the species as opportunists (Guinda et al., 2008; Orfanidis et al., 2003; Orlando-Bonaca et al., 2008). (ii) Ecological approximation: to estimate the different elements of the index, species were classified in two ecological groups based on ecological abilities (Grime, 1977) and according to ecological and sintaxonomic considerations previously described (Arévalo et al., 2007; Giaccone et al., 1993; Giaccone et al., 1994a,b). To classify species as opportunists, the criterion of Wells et al. (2007) was followed (UKTAG, 2009). In addition, based on data from the Atlantic coast of Cadiz, and according to the scoring system proposed by Wells et al. (2007) a new "correction factor" was calculated to obtain the species richness. In this case, chalk shore was removed of the scoring system because this substrate does not exist on the western coast of Andalusia (Vergara et al., 2006). According to Wells (2008), the application of the shore description is not as straight forward as the rest of the components as it only acts as a correction for the level of species richness, and not the proportions of green, red, and opportunist or the ESG ratio. Its inclusion into the element as a single component bares too much the weighting for the system; therefore it only needs to be incorporated into the final species richness value.

A preliminary quality status classification of the sites in five classes was established based on the distance to sources of stress and physico-chemical variables provided by the Water Policy Plan (WPP) for the Andalusian coast between 2004 and 2008. These sources of stress must be considered because high nutrients or turbidity values can be either natural or anthropogenically induced; therefore, the use of these distances can reflect this fact. The following sources of stress were taken into account: mouth of rivers, harbours, industries and urbanizations (e.g. López y Royo et al., 2009; Pinedo et al., 2007). Distances to the sources were calculated over aerial photographs using GIS. On the other hand, the WPP monitoring network provided data of available nutrients in the water column (ammonium, nitrate, nitrite, phosphate), turbidity (total suspended solids) and metal content index (MCI) in selected sites along Andalusian coast (Fig. 1). The MCI is calculated as the geometric mean of Cr, Ni, Mn, Cu, Cd, Pb, As, Hg and Zn concentrations and provided an overall estimation of the metallic content of the sites. In some cases a linear interpolation (Eq. (1)) was applied to calculate data in sites further away than 3 km from the nearest WPP sample station (Fig. 1):

$$\mathbf{C_S = C_1 + (C_2 - C_1) \times \left(\frac{D_{1,S}}{D_{1,2}}\right)} \tag{1}$$

where $C_S$ is the concentration in the interpolated site; $C_1$ is the concentration in the WPP sample station 1; $C_2$ is the concentration in WPP sample station 2; $D_{1,S}$ is the distance between the WPP sample station 1 and the interpolated site; and $D_{1,2}$ is the distance between sampling stations 1 and 2.

Based on these distances to sources of stress and physicochemical variables, a cluster analysis was applied using the Ward method and squared Euclidean distance. To avoid a bias in the classification and to assure that all variables have the same weight, they were normalized and re-scaled between 0 and 1 for the different elements. To clarify the cluster analysis, an assessment of anthropogenic pressures was done according to López y Royo et al. (2009). Considering the cluster and the pressure analysis, the quality status of the sampled sites was classified as high, good, moderate, poor and bad, as proposed in the WFD. Finally, this preliminary classification was later used to establish the boundary levels among the different ecological status classes (ESCs) for each element used in the index. The mid point between the upper and lower points of variance from adjacent quality classes was used to adjust the range of the different elements that compose this index according to Eqs. (2) and (3) (Wells, 2008):

$$\mathbf{LC = \frac{(X_1 + S_1) + (X_2 + S_2)}{2}} \tag{2}$$

when the value of the element increased with increasing EQR

$$\mathbf{LC = \frac{(X_1 - S_1) + (X_2 + S_2)}{2}} \tag{3}$$

when the value of the element decreased with increasing EQR

where LC is the limit between two adjacent ESC; $X_1$ is the mean of lower ESC; $S_1$ is the variance of lower ESC; $X_2$ is he mean of upper ESC; and $S_2$ is the variance of upper ESC.

The value of the ecological quality ratio (EQR) for the RSL index was calculated for each station considering the range of the different elements calibrated previously from Atlantic shores of Andalusia and the following equations proposed by Wells (2008). Eq. (4) was used when the value of the element increased with increasing EQR (species richness, proportion of red algae and proportion of ESG-I), while Eq. (5) was used when the value of the element decreased when EQR increased (proportion of green algae and proportion of opportunists):

$$\textbf{EQR} = \left\{\left(\frac{\textbf{value – lower CR}}{\textbf{CW}}\right) \textbf{x EQR BW}\right\} + \textbf{lower EQR BR} \tag{4}$$

$$\textbf{EQR = upper EQR BR -} \left\{\left(\frac{\textbf{value – lower CR}}{\textbf{CW}}\right) \textbf{x EQR BW}\right\} \tag{5}$$

where CR was the class range, CW the class width, BR was the EQR band range and BW was the EQR band width.

*Response of the RSL elements*

Finally, to analyze the response of the different elements in accordance with the water quality, a correlation analysis was performed between the gross value of the elements used to calculate the RSL index and the quality water value (QW). The QW was defined as the maximum value between MCI and eutrophication status, previously normalized and re-scaled between 0 and 1. Eutrophication status was obtained as the mean value of all physico-chemical variables related with the eutrophication (ammonium,nitrate, nitrite, phosphate and total suspended solids). In addition, a correlation analysis was performed between the gross values of the elements used to calculate the RSL index and the gross values of physico-chemical variables. Furthermore, oneway Analysis of Variance (ANOVA) was used to test the effects of pressures in RSL elements. In this case, bad (Puerto de Algeciras; 18) and poor (Aculadero; 3; La Puntilla; 4) statuses were excluded because the number of sites for these statuses was less than 3. To comply with Shapiro–Wilk normality test the proportion of green seaweeds was cube transformed. Thus, the factor water quality was fixed in three levels: moderate, good and high. All elements were homocedastic and Tukey test post hoc analysis was applied. In all cases, significance was set at 5% probability.

## RESULTS

*Reduced species list*

The RSL for the Atlantic coast of Andalusia consisted of 56 species/genera of macroalgae: 12 green, 14 brown and 30 red algae (Table 1). In the case of the morphological approximation, 20 species were classified as ESG-I, 36 and ESG-II and 8 as opportunists, whereas in the ecological approximation 26 species were classified as ESG-I, 30 as ESG-II and 5 as opportunists. In both approximations all the green algae were considered as ESG-II excepting *Flabellia petiolata*. In addition, most of the greens were considered opportunists. Comparing both approximations it can be seen that one brown and nine red algae were classified in different way. In 8 of these 10 cases, the divergences between both approximations consisted in the classification as ESG-I by the ecological approximation of species classified as ESG-II by the morphological approximation.

**Table 1-** Reduced species list of macroalgae for the Atlantic southern Spanish coast, Ecological Status Group (ESG) and opportunistic character.

| CHLOROPHYTA | ESG morph. | ESG eco. |
|---|---|---|
| *Bryopsis spp.* | II (o) | II |
| *Chaetomorpha spp.* | II (o) | II (o) |
| *Cladophora spp.* | II (o) | II |
| *Codium spp* erect* | II | II |
| *Codium spp* encrusting** | II | II |
| *Codium bursa* | II | II |
| *Derbesia spp.* | II (o) | II (o) |
| *Flabellia Petiolata* | I | I |
| *Pedobepsia simplex* | II | II |
| *Enteromorpha spp.* | II (o) | II (o) |
| *Ulva spp.* | II (o) | II (o) |
| *Valonia utricularis* | II | II |

| OCHROPHYTA | ESG morph. | ESG eco. |
|---|---|---|
| *Cladostephus spongiosus* | II | I |
| *Colpomenia sinuosa* | II | II |
| *Cystoseira compressa* | I | I |
| *Cystoseira spp.* | I | I |
| *Cystoseira usneoides* | I | I |
| *Dictyota dichotoma* | II | II |
| *Dictyopteris polypodioides* | II | II |
| *Fucus spiralis* | I | I |
| *Halopteris spp.* | II | II |
| *Saccorhiza polyschides* | I | I |
| *Padina pavonica* | I | I |
| *Laminaria ochroleuca* | I | I |
| *Ectocarpus & Sphacelaria* | II (o) | II (o) |

| RHODOPHYTA | ESG morph. | ESG eco. |
|---|---|---|
| *Delesseriaceae**** | II | I |
| *Asparagopsis armata* | II | II |
| *Botryocladia botryoides* | I | I |
| *Caulacanthus ustulatus* | II | I |
| *Ceramium spp.* | II (o) | II |
| *Chondracanthus Acicularis* | II | II |
| *Corallina sp.* | I | II |
| *Gelidium microdon* | II | I |
| *Gelidium spinosum* | II | II |
| *Gelidium corneum* | II | I |
| *Gelidium pusillum* | II | II |
| *Gymnogongrus & Ahnfetiopsis* | I | I |
| *Halopithys incurva* | I | I |
| *Halurus equisetifolius* | II | II |
| *Hildenbrandia rubra* | I | I |
| *Jania rubens* | I | I |
| *Laurencia obtusa* | II | II |
| *Lithophyllum byssoides* | I | I |
| *Lithophyllum dentatum* | I | I |
| *Lithophyllum incrustans* | I | I |
| *Nemalion helminthoides* | II | I |
| *Lomentaria articulata* | II | II |
| *Osmundea pinnatifida* | II | I |
| *Osmundea hybrida* | II | II |
| *Peyssonnelia spp.* | I | I |
| *Plocamium cartilagineum* | II | II |
| *Pterocladiella capillacea* | II | II |
| *Pterosiphonia complanata* | II | II |
| *Rhodymenia & Schottera* | I | I |
| *Sphaerococcus coronopifolius* | II | II |

(o) Species considered as opportunists

* Erect Codium: C. tomentosum, C. fragile, C. vermilara and C. decorticatum

**Encrusting Codium: C. adhaerens and C. effusum.

*** Deleseiraceae: Acrosorium uncinatum, Cryptopleura ramulosa or Haraldiophyllum bonnemaisonnii.

According to the data obtained for this RSL along the rocky shores of the Atlantic coast of Andalusia, two ecological regions were identified based on the results of the Hierarchical-Cluster analysis of presence–absence of the species in the sampling sites (Fig. 2). The first region corresponded to the Strait of Gibraltar; the second corresponded to the western shores (Atlantic region) of the Cadiz coast. The boundary between both regions was situated between the localities 13 and 14 (Fig. 1) near Barbate. Accordingly, a total of 50 species of the RSL were found in the Strait of Gibraltar region and 49 species in the Atlantic region. The former evidenced a higher percentage of brown algae (31%) than the later (23%). In contrast, the proportion of red algae was similar in both regions (Strait of Gibraltar 49%; Atlantic 52%). The two ecological regions shared 42 species, with 8 species exclusive to the Strait of Gibraltar region and 7 exclusive to the Atlantic one.

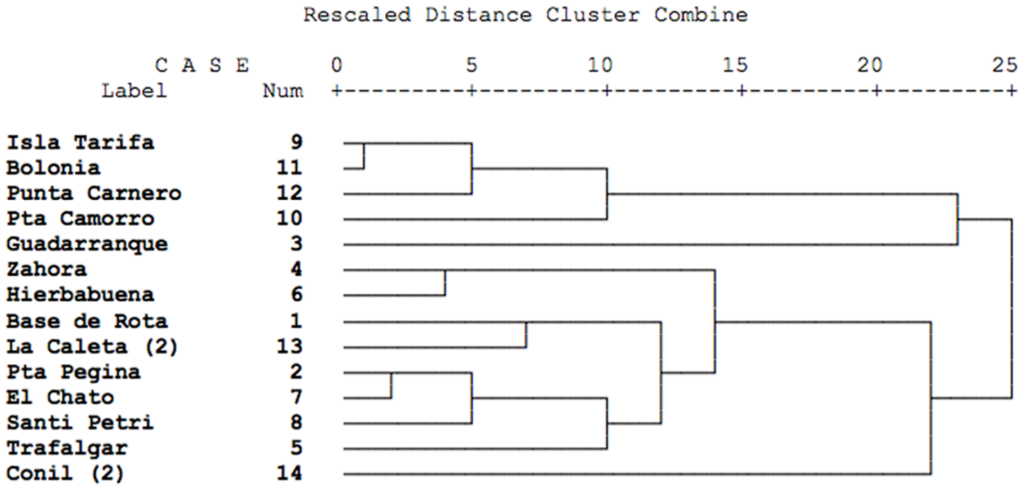


**Fig 2.-** Dendrogram depicting mutual similarities of flora of the different sites sampled. (2) – Agregate site (2 sampling stations).

*Reassessment of the RSL elements*

The species richness (species from the RSL; Fig. 3, Eq. (6)) showed a significant correlation with the shore description (p-value = 0.011; r = 0.492; n = 26). Thus, based on the scoring system of the shore characteristics, a correction factor (CF) for species richness was calculated from the non-linear relationship between the species richness and the scores of the shore description (Fig. 3; Eqs. (6) and (7)). This factor was based around an average shore description of 13, which corresponded to an expected richness of 23 species (Eq. (6)). The CF was calculated as the ratio between the expected species richness for the mean shore description of Andalusian Atlantic shores (23 species), and the expected species richness for any particular case (Eq. (7)). Finally, the corrected species richness was calculated by multiplying the observed species richness by the correction factor (Eq. (8)):

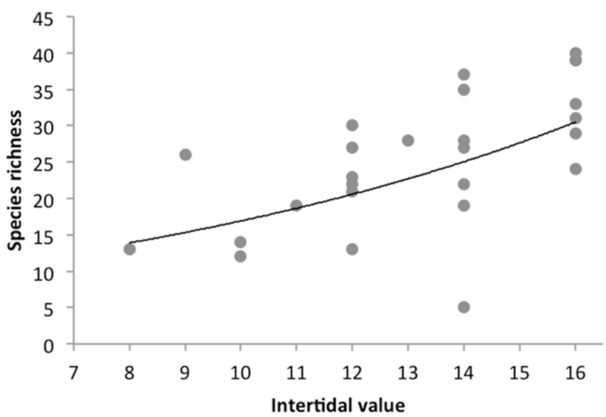


**Fig. 3**.- Exponential model for the relationship between shore description value and species richness.

$$\textbf{Expected species richness} = 6.3371 \times e^{0.0982 \cdot \textbf{Shore score}} \quad (6)$$

$$\textbf{CF} = \frac{23}{\textbf{expected species richness}} \quad (7)$$

$$\textbf{Corrected species richness} = \textbf{CF} \times \textbf{observed species richness} \quad (8)$$

The identification and analysis of physico-chemical variables and the distance to sources of stress led to a classification of the sampling sites in five quality status groups (Fig. 4; Table 2):

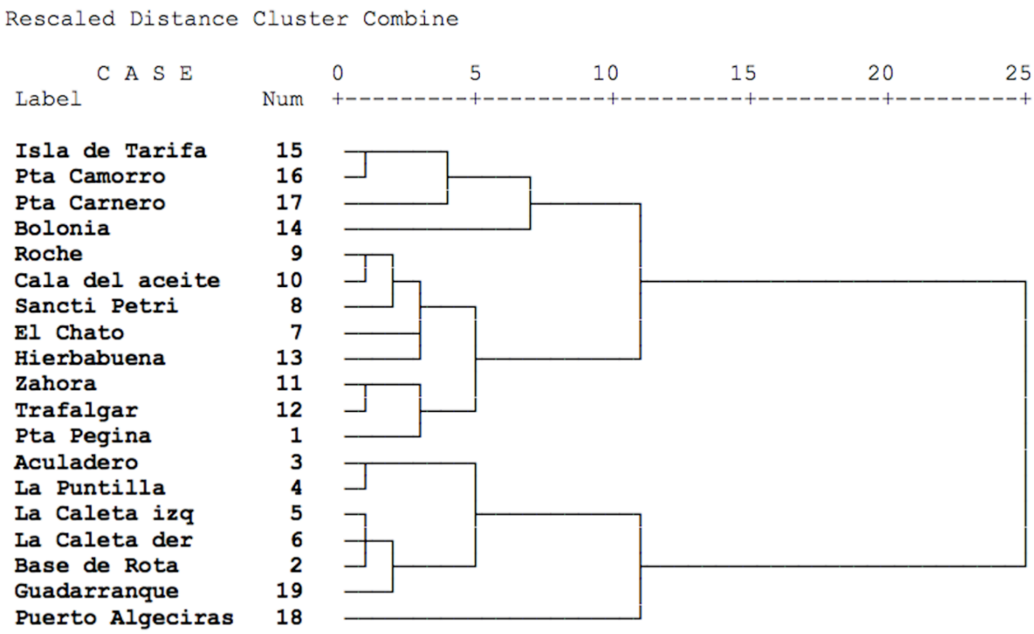


**Fig 4.-** Dendrogram depicting mutual similarities of the water quality of the different sites sampled.

High (4 sites) for Punta Carnero (17), Punta Camorro (16), Isla de Tarifa (15) and Piscinas de Bolonia (14, see site codes in Table 1). These sites showed the lowest values for nutrients, suspended solids and metal concentration; moreover, they were located in “El Estrecho” Natural Park where the marine and coastal environment is globally less influenced by anthropogenic pressures. Except Punta Carnero that showed high anthropogenic pressures due to the proximity of Algeciras harbour (6.72 km), the other places was subjected to low (15, 16) and none (14) pressures.

Good (8 sites) for Punta Pegina (1), El Chato (7), Sancti Petri (8), Roche (9), Cala del Aceite (10), Zahora (11), Trafalgar (12) and Hierbabuena (13). In general terms, these places showed lower values for physico-chemical variables than moderate, poor and bad ecological status, and were further to sources of stress. Anthropogenic pressures were assessed as low (1, 7) and moderate (8, 9, 10, 11, 12, 13) in these localities.

Moderate (4 sites) for the rocky shores of Rota (2), La Caleta (5, 6) and Guadarranque (19). In this group, except Rota (2) that showed moderate anthropogenic pressures, the other sites (5, 6, 19) were subjected to industrial (19) or urban (5, 6) high pressures.

Poor (2 sites) for El Aculadero (3) and La Puntilla (4). These sampled points are close to the Guadalete River subjected to high anthropogenic pressures related to this river and the presence of a large shipyard and Cadiz harbour in the nearness. Moreover, the physico-chemical variable showed higher values than those in moderate status.

Bad (1 site) for Puerto de Algeciras (18). This site showed the highest value for nutrients, suspended solids and heavy metal concentration. The existence of a discharge point of sewage outfall without treatment and the proximity of the massive Algeciras harbour suggested that environmental conditions here were clearly the worst among all sampling sites prospected.

**Table 2-** Mean ± standard deviation of the different physico-chemical variables and distances to sources of stress used to define a preliminary classification.

| Class | $NH_4^+$ (μM) | $NO_3^-$ (μM) | $NO_2^-$ (μM) | $PO_4^{3-}$ (μM) | SS ($mg\cdot L^{-1}$) |
|---|---|---|---|---|---|
| Bad | 5.24 | 13.58 | 0.28 | 0.81 | 15.67 |
| Poor | 3.18 ± 0.53 | 10.02 ± 0.19 | 0.46 ± 0.06 | 0.39 ± 0.04 | 14.42 ± 0.59 |
| Moderate | 1.06 ± 0.71 | 8.06 ± 0.45 | 0.30 ± 0.04 | 0.33 ± 0.14 | 14.70 ± 1.45 |
| Good | 0.88 ± 0.29 | 9.03 ± 1.48 | 0.28 ± 0.06 | 0.23 ± 0.09 | 13.23 ± 1.62 |
| High | 1.29 ± 0.82 | 7.25 ± 0.00 | 0.17 ± 0.02 | 0.31 ± 0.02 | 9.49 ± 0.24 |
| **Class** | **Urban. (Km)** | **Harbour (Km)** | **River (Km)** | **Industry (km)** | **ICM ($ng\cdot L^{-1}$)** |
| Bad | 0.00 | 0.40 | 3.10 | 2.9 | 0.00070 |
| Poor | 0.00 ± 0.00 | 0.95 ± 0.21 | 1.85 ± 0.35 | 6.25 ± 0.35 | 570 ± 50 |
| Moderate | 0.08 ± 0.15 | 0.27 ± 0.34 | 5.60 ± 3.01 | 6.12 ± 4.49 | 530 ± 20 |
| Good | 1.19 ± 0.78 | 4.98 ± 4.58 | 8.11 ± 5.39 | 29.63 ± 13.29 | 490 ± 40 |
| High | 1.26 ± 1.38 | 6.07 ± 6.87 | 25.60 ± 12.25 | 28.10 ± 14.07 | 440 ± 10 |

From this classification of water quality, the boundaries between classes of ecological status for the different elements of the RSL index were established (Table 3). This was achieved by the mid point, rounded off to the five multiple in elements expressed in percents, between the upper and lower error bars (calculated from standard deviation) of adjacent quality status classes. However, when these elements did not present the expected trend for some ecological status (proportion of opportunist for both approach and proportion of ESG-I from morphological approxi- mation) the limits were calculated as the midpoint between the lower limit of the lower status and the upper limit of the higher status.

**Table 3-** The metric scoring system with classification status ranges for macroalgae species richness, red algae, ESG-I, green algae and proportion of opportunists.

| Common elements | Bad | Poor | Moderate | Good | High |
|---|---|---|---|---|---|
| Corrected species richness | < 10 | 11 - 19 | 20 - 26 | 27 - 29 | > 29 |
| Number of red seaweeds | < 5 | 6 - 9 | 10 - 13 | 14 - 18 | > 18 |
| Proportion of green seaweeds | > 0.55 | 0.55 - 0.35 | 0.35 - 0.25 | 0.25 - 0.20 | < 0.20 |
| **RSL - morphological** | | | | | |
| Proportion of ESGI | < 0.25 | 0.25 - 0.30 | 0.30 - 0.325 | 0.325 - 0.35 | >0.35 |
| Proportion of opportunists | > 0.35 | 0.35 - 0.25 | 0.25 - 0.20 | 0.20 - 0.15 | < 0.15 |
| EQR-RSL | < 0.20 | 0.20 - 0.35 | 0.35 - 0.60 | 0.60 – 0.75 | > 0.75 |
| **RSL - ecological** | | | | | |
| Proportion of ESGI | < 0.15 | 0.15 - 0.25 | 0.25 - 0.35 | 0.35 - 0.40 | > 0.40 |
| Proportion of opportunists | > 0.30 | 0.30 - 0.15 | 0.15 - 0.10 | 0.10 - 0.05 | < 0.05 |
| EQR-RSL | < 0.20 | 0.20 - 0.40 | 0.40 - 0.60 | 0.60 - 0.75 | > 0.75 |

*Response of RSL elements*

The regression analysis between the different elements used to calculate the RSL index and the water quality (Table 4) indicated that species richness was one of the most sensitive, showing the expected trend and significant correlations with the QW estimated according to physico-chemical parameters ($R^2 = 0.564$; $p < 0.001$). On the other hand, the corrected species richness also showed a significant linear regression with the water quality ($p = 0.028$), although with a lower coefficient of determination ($R^2 = 0.253$).

**Table 4-** Coefficients of determination for the regression analysis between the elements of the RSL index and the water quality * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001.

| Common elements | $R^2$ | |
|---|---|---|
| Species richness | 0.564*** | |
| Corrected species richness | 0.253* | |
| Number of rhodophyta | 0.540*** | |
| Proportion of red seaweeds | 0.313* | |
| Number of green seaweeds | 0.161 | |
| Proportion of green seaweeds | 0. 550*** | |
| **Approximation elements** | **Morphological** | **Ecological** |
| Number of ESG-I | 0.415** | 0.641*** |
| Number of ESG-II | 0.463*** | 0.392** |
| Number of opportunists | 0.001 | 0.058 |
| Proportion of ESG-I | 0.541*** | 0. 735*** |
| Proportion of opportunists | 0.581*** | 0.588*** |
| EQR-RSL | 0.554*** | 0.581*** |

The proportion of green algae also showed a significant regression, increasing from unpolluted to polluted sites ($R^2$ = 0.550; $p < 0.001$). This trend was not attributed to the reduction in then number of green algae, as it can be seen by its lack of regression ($R^2$ = 0.161; $p > 0.05$). In the same manner as the proportion of greens, the proportion of opportunists decreased when the water quality increased ($R^2$ = 0.581 and $p < 0.001$; for the morphological approximation and $R^2$ = 0.588 and $p < 0.001$; for the ecological one), and this tendency was not attributed to the reduction in the number of opportunists ($R^2$ = 0.001 and $p > 0.05$; morphological approximation, $R^2$ = 0.058 and $p > 0.05$; ecological one). In contrast, the proportion of red algae was one of the least sensitive elements, showing a lower strength of the straight-line relationship between this element and the water quality ($R^2$ = 0.313; $p = 0.013$). There was a clear gradient between the number of red algae and the water quality ($R^2$ = 0.540; $p < 0.001$). However, it was not reflected in the proportion of red algae, because the number of species increased as well; thus, the proportion of reds did not show a clear variation with the water quality.

The proportion of ESG-I showed the expected trend, increasing with the water quality, and showing a significant linear regression for both the morphological and ecological approximations ($R^2$ = 0.514 and $p = 0.001$; morphological and $R^2$ = 0.735 and $p < 0.001$; ecological). However, the ecological approximation evidenced a better relationship than the morphological one. In both approximations, the number of ESG-I ($R^2$ = 0.415 and $p = 0.003$; morphological and $R^2$ = 0.641 and $p < 0.001$; ecological) and ESGII ($R^2$ = 0.463 and $p = 0.001$; morphological and $R^2$ = 0.392 and $p = 0.004$; ecological) species yielded a direct and significant relationship with the improvement in water conditions.

The regression values between the water quality and the final EQR value of the RSL index for the morphological ($R^2$ = 0.554; $p < 0.001$) and ecological ($R^2$ = 0.581; $p < 0.001$) approximations were quite similar; showing in both cases a direct linear regression with water quality. Hence, it can be stated that the integration of all information in the final score for the RSL showed a significant trend.

The correlation matrix between the gross values of the different physico-chemical parameters used to define the water quality, and the gross values of the elements used to calculate the RSL (Table 5) showed significant correlations for most of the cases. The concentration of nitrite and of suspended solids (SS) were the less correlated parameters with the different elements used to assess the ecological status. Conversely, the concentration of ammonium and the metallic content index (MCI) were the best correlated with the elements used in RSL-index.

In the case of the elements used to calculate the RSL, the matrix indicated that all of them showed the expected trend (Table 5). Moreover, the elements "number of reds" and "the ecological approximation for proportion of ESG-I" showed significant correlations with all physico-chemical parameters. In contrast, in general terms the corrected species richness showed the lowest correlations.

**Table 5-** Correlation matrix of the gross values of the elements used in RSL and the gross values of the different parameters used to define the water quality. Suspend Solids (SS), Metallic Content Index (MCI). * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001.

| **Common elements** | **$NH_4^+$** | **$NO_3^-$** | **$NO_2^-$** | **$PO_4^{3-}$** | **SS** | **MCI** |
|---|---|---|---|---|---|---|
| Corrected species richness | -0.839*** | -0.435* | -0.117 | -0.759*** | -0.240 | -0.747*** |
| Number of red seaweeds | -0.747*** | -0.699*** | -0.492* | -0.493* | -0.537** | -0.743*** |
| Proportion of green seaweeds | 0.788*** | 0.666** | 0.303 | 0.674** | 0.520* | 0.795*** |
| **RSL - morphological** | | | | | | |
| Proportion of ESG-I | -0.766*** | -0.674** | -0.110 | -0.813*** | -0.430* | -0.671*** |
| Proportion of opportunists | 0.883*** | 0.704*** | 0.215 | 0.780*** | 0.403* | 0.744** |
| EQR-RSL | -0.885*** | -0.618** | -0.378 | -0.789*** | -0.513* | -0.811*** |
| **RSL - ecological** | | | | | | |
| Proportion of ESG-I | -0.743*** | -0.761*** | -0.479* | -0.656** | -0.706*** | -0.820*** |
| Proportion of opportunists | 0.905*** | 0.710*** | 0.340 | 0.711*** | 0.401* | 0.705*** |
| EQR-RSL | -0.833*** | -0.661** | -0.449* | -0.672** | -0.572** | -0.812*** |

The EQR-RSL for both approximations showed very similar results, both of them having significant correlations with all the physico-chemical parameters, except in the case of nitrites for the morphological approximation. These results confirmed that this index integrated all the ecological information.

As it can be seen from the ANOVA results of the gross values of each element used in the RSL index (Table 6), there were significant differences in the mean values between moderate, good and high status for 4 of the 7 elements analysed. The proportion of ESG-I (morphological approximation), proportion of opportunists (ecological approximation) and corrected species richness showed the worst response and was not sensitive to the quality status. In addiction, the Tukey test revealed that the proportion of opportunist (morphological approximation) showed differences between moderate and good status but not between moderate and high, thus the trend was not the expected. On the other hand, the proportion of green seaweeds showed the best results and evidenced significant differences between moderate and good, and moderate and high status. Furthermore, none of the elements showed differences between good and high quality status. Finally, the most remarkable findings were that: (i) the RSL-index showed, for both approximations, a better response than the elements separately showing significant differences between moderate and good, and moderate and high ESC, and (ii) the inexistence of significant differences between good and high status, in the post hoc analysis for RSL values.

**Table 6-** Results of the ANOVA analysis between the different Ecological Status Class (ESC). Bad status was excluded from the analysis. Proportion of opportunists was transformed to comply with normality test (squared root transformation). Mean and standard deviation (SD) for each ESC and the results of the post hoc Tukey test are also indicated.

| **Common elements** | **ESC** | **Mean** | **SD** | **ANOVA (sig.)** | **Tukey** | |
|---|---|---|---|---|---|---|
| | Moderate | 24 | 5 | | **X** | |
| Corrected species richness | Good | 29 | 6 | 0.280 | **X** | |
| | High | 27 | 4 | | **X** | |
| | Moderate | 13 | 1 | | **X** | |
| Number of red seaweeds | Good | 15 | 3 | 0.010 | **X** | **X** |
| | High | 19 | 2 | | | **X** |
| | Moderate | 0.32 | 0.05 | | **X** | |
| Proportion of green seaweeds | Good | 0.23 | 0.04 | 0.001 | | **X** |
| | High | 0.20 | 0.03 | | | **X** |
| **RSL-morphological** | | | | | | |
| | Moderate | 0.33 | 0.06 | | **X** | |
| Proportion of ESG-I | Good | 0.34 | 0.05 | 0.856 | **X** | |
| | High | 0.33 | 0.05 | | **X** | |
| | Moderate | 0.19 | 0.06 | | **X** | |
| Proportion of opportunists* | Good | 0.11 | 0.04 | 0.049 | | **X** |
| | High | 0.14 | 0.04 | | **X** | **X** |
| | Moderate | 0.55 | 0.01 | | **X** | |
| EQR-RSL | Good | 0.71 | 0.07 | 0.024 | | **X** |
| | High | 0.73 | 0.10 | | | **X** |
| **RSL-ecological** | | | | | | |
| | Moderate | 0.33 | 0.06 | | **X** | |
| Proportion of ESG-I | Good | 0.36 | 0.06 | 0.022 | **X** | **X** |
| | High | 0.45 | 0.06 | | | **X** |
| | Moderate | 0.09 | 0.03 | | **X** | |
| Proportion of opportunists* | Good | 0.05 | 0.03 | 0.174 | **X** | |
| | High | 0.07 | 0.03 | | **X** | |
| | Moderate | 0.56 | 0.08 | | **X** | |
| EQR-RSL | Good | 0.70 | 0.08 | 0.007 | | **X** |
| | High | 0.78 | 0.08 | | | **X** |

Overall, the RSL index based on the ecological approximation was more sensitive to the quality status than the RSL index based on the morphological approach (Table 7), with 79% of the sites showing the same classification. The RSL based on the morphological approximation classified a lower number of the sites (68%) in the same ESC that the ecological status given by the preliminary classification. On the other hand, the percentage of localities classified in the same way using both approximations was 74%. Furthermore, differences between RSL approximations and preliminary quality status never produced differences in ecological status higher than one ESC. Some localities ("Punta Pegina" (1) and "El Chato" (7)) evidenced the same ecological status under the two approximations of the RSL index but this status was different taking into account the preliminary classification.

**Table 7-** Estimated classifications of the water quality for each sampling site and final EQR-RSL results obtained by each alternative. See name of localities in table 1.

| Locality | Ecological-EQR | Ecological-ESC | Morphological-EQR | Morphological-ESC | Preliminary Quality |
|---|---|---|---|---|---|
| 1 | 0.74 | GOOD | 0.68 | GOOD | GOOD |
| 2 | 0.67 | GOOD | 0.68 | GOOD | MODERATE |
| 3 | 0.32 | POOR | 0.32 | POOR | POOR |
| 4 | 0.30 | POOR | 0.34 | POOR | POOR |
| 5 | 0.56 | MODERATE | 0.56 | MODERATE | MODERATE |
| 6 | 0.49 | MODERATE | 0.54 | MODERATE | MODERATE |
| 7 | 0.84 | HIGH | 0.83 | HIGH | GOOD |
| 8 | 0.72 | GOOD | 0.73 | GOOD | GOOD |
| 9 | 0.74 | GOOD | 0.76 | HIGH | GOOD |
| 10 | 0.55 | MODERATE | 0.63 | GOOD | GOOD |
| 11 | 0.64 | GOOD | 0.64 | GOOD | GOOD |
| 12 | 0.67 | GOOD | 0.64 | GOOD | GOOD |
| 13 | 0.71 | GOOD | 0.77 | HIGH | GOOD |
| 14 | 0.87 | HIGH | 0.85 | HIGH | HIGH |
| 15 | 0.78 | HIGH | 0.77 | HIGH | HIGH |
| 16 | 0.81 | HIGH | 0.70 | GOOD | HIGH |
| 17 | 0.68 | GOOD | 0.60 | GOOD | HIGH |
| 18 | 0.08 | BAD | 0.06 | BAD | BAD |
| 19 | 0.51 | MODERATE | 0.42 | MODERATE | MODERATE |

## DISCUSSION

There are marked differences in the community features and the characteristics of the phycological flora composition along the Atlantic coasts of southern Iberian Peninsula (Fischer-Piette, 1958). This has been attributed to the influence of large rivers in the most western part, and differences in solar irradiance between the Strait of Gibraltar and the surroundings of Cádiz Bay (Fischer-Piette, 1958; Seoane-Camba, 1965). Despite these biogeographic differences, the response of the RSL index was suitable and it was not necessary recalculate boundaries between the different status classes for the different elements in the two regions (Strait of Gibraltar and Atlantic). This fact could be explained by the similarity between their flora (76% taxa of RSL were common in both regions) and because the two regions are represented by similar numbers of species in the proposed reduced list (50 for the Gilbraltar Strait and 49 for the Atlantic region).

The results presented in this study suggested that the RSL index maybe a suitable indicator to assess the ecological status of Atlantic coastal waters of southern Spain. In this sense, all the elements used to calculate the RSL index were sensitive to the water quality, as shown by the significant relations given in Tables 4 and 5. Overall, the integration of all elements in the RSL index showed a better response than the elements separately. Moreover, both alternatives of the RSL-index showed significant differences between moderate and good, and moderate and high ESC. In contrast, significant differences were not found between good and high ecological status. Therefore, the two adapted alternatives of RSL index for the Atlantic coasts of southern Spain responded adequately when they were used to estimate the ESC, although the ecological alternative was more sensitive to the water quality and must be chosen when the assessment is going to be carried out.

However, some questions arise from the results: Is this index sensitive enough for accomplishing the requirements of the WFD? Are all the elements used in RSL index suitable to assess the ecological status in the Atlantic coast of Southern Spain? Does this index integrate the environmental implication and importance of each indicator in the Atlantic coast of Andalusia? These questions will be discussed below.

*Is the RSL index sensitive enough for accomplishing the requirements of the WFD?*

Neither of the two alternatives to the RSL index can distinguish significantly between good and high water quality. However, considering that the WFD points out that all European surface waters should achieve the objective of a good ecological status by 2015, the most important fact is that proposed indices may discriminate between moderate and good, and moderate and high ecological status, as this index does. Moreover, bearing in mind a dynamic point of view on ecological succession (Orfanidis et al., 2005), the relationship between the taxonomic composition of the seaweed community and the physico-chemical water characteristics or pressures is not univocal. For instance, it is well documented that elevated concentrations of nitrogen and phosphorus in the water column do not necessarily indicate highly eutrophic conditions; neither do low concentrations necessarily indicate absence of eutrophication (Cloern, 2001). In this sense, the locality of “El Chato”, classified good according to physico-chemical data, was previously classified as high under subjective ESC based on expert knowledge of each of the sites irrespective of their species numbers and considering the proximity and magnitude of direct and indirect effluent discharges (Bermejo, 2009), and as low pressures subjected according López y Royo et al. (2009) criteria. These facts suggest that water quality may not always be accurately enough to discriminate, at least, between good and high ecological status.

*Are all the elements used in RSL index suitable to assess the ecological status in the Atlantic coasts of Southern Spain?*

Species richness showed a higher correlation with the water quality than corrected species richness. This result could be related with the fact that thenumberof high and good ESC is bigger than the number of low ESC (Fig. 3). It produces a bias that affect negatively to the corrected species value and it could be one of the reasons that explain the worse results obtained with the corrected species richness. Another matter is the fact that turbidity in the Atlantic coast of southern Spain was correlated with the ecological status in a lot of sampling sites. This occurs because the majority of the dumping activities take place in rivers, which also produces natural turbidity in the coast. For this reason, it was not possible to correct the effects of natural turbidity in macroalgae communities in the Atlantic coast of Southern Spain. Other issues related with this consideration are: (i) sometimes it is impossible to distinguish between natural and anthropogenic turbidity when they occur at the same time; (ii) the water turbidity can be very different at short time scales; (iii) it can be difficult to establish the limits between turbid and clear waters. Furthermore, the ecological differences between the northern and southern coast of Europe suggest that the factors considered in the scoring system related to physical structure of the intertidal and number of subhabitats should be reassessed to the Atlantic coasts of Andalusia. Therefore, the intertidal scoring system should be re-calculated for the southern Atlantic coast of Spain.

The results regarding the proportion and number of red macroalgae are in accordance with the results given by Guinda et al. (2008) for the northern coast of Spain, where the correlation between number of red algae and the water conditions was higher than that using the proportion of reds. This fact could be related to differences of the intertidal algae community composition between northern cold waters, where brown algae are dominant, and southern temperate waters, where red algae predominate (Boaventura et al., 2002; Fischer-Piette, 1963; Lüning, 1990). In this sense, many brown macroalgal species in the northern European coasts are large, cartilaginous and relatively hardy, and more likely to remain constant independently of environmental conditions (Wells et al., 2007). In contrast, a significant and direct correlation between the water quality and the number of brown algae ($R^2 = 0.490$; $p < 0.001$) was observed in the southern coast of Spain. Hence, these biogeographical and ecological differences, and the increase in species richness in relation to the improvement of water conditions, could explain why less satisfactory results were recorded when the proportion of red algae was considered. Furthermore, two different biogeographic zones have been described in the Atlantic coast of Andalusia (Seoane-Camba, 1965). The zone near the Strait of Gibraltar is characterised by amore “northern” phycological flora. In this sense, based on RSL data obtained from this study, this zone showed a R:P (rhodophyta:phaeophyceae) index (Feldmann, 1937) of 1.77 while this ratio in the Atlantic zone was computed as 2.67. This index is used to classify the flora in a latitudinal gradient, showing an inverse relation to latitude (R/P < 2 for cold-temperate zones; R/P > 4 for tropical zones; Báez et al., 2004). Therefore, considering the special condition of the shores of southern Spain, the element "proportion of red algae" was replaced by its absolute number, which is less sensible to the effect that biogeographic changes and the increment of species richness have in the proportion of red algae.

With respect to the proportion of green algae, Guinda et al. (2008) found similar results analysing the different elements of the RSL index along a pollution gradient in the north of Spain. Moreover, this element showed similar values in Cadiz and the British Isles to establish the Ecological Quality Ratio; in addition, when the RSL proposed by Wells et al. (2007) is analyzed, 83% of the green algae (Flores-Moya et al., 1995a), 63% of the reds (Conde et al., 1996) and 50% of brown algal species (Flores-Moya et al., 1995b) were also recorded in the Andalusian coast. These facts suggest that: (i) green algae show, in the European Atlantic coast, a more cosmopolitan distribution than red and brown ones; and (ii) green algae show similar ecological functions along the European coasts. According to Littler and Littler (1980) and Littler et al. (1983) chlorophytes can behave as opportunists or early colonizers, because they are very tolerant to environmental disturbances and stress situations. It is noteworthy that green species can thrive in simple substrates like small rocks or non-specific hard substrates, and they are present in virtually all water conditions. Therefore, green algae depend less on biogeographic factors, water quality or intertidal structure than red and brown algae. The proportion of green algae decreases in parallel to the improvement of environmental conditions, when late-successional brown and red species thrive in a large number. Hence, the similarity between the results obtained for the different regions, and the highly significant correlation with the water quality for this untransformed element, suggest that it could be very useful when results from different areas in the same eco-region are compared.

The ESG-I ratio is based on the assignment of any species found in a sample to a "late successional species" (ESG-I) or a "opportunist species" (ESG-II) group according to its morphology (Orfanidis et al., 2001), and based on the functional-form group model of macroalgae described by Littler and Littler (1980). The functional form group hypothesis was originally proposed to predict, from morphological features of the species, the productivity and other ecological attributes (e.g. grazing resistance, competitive abilities, reproductive effort), but not resistance to pollution (Arévalo et al., 2007). Although ESG-I ratio can be a preliminary approximation for indices based on abundance, or when a functional classification is used instead of a taxonomic one, when a reduced species list exists it is better to classify the species according to ecological abilities based on Grime's theory (1977) and according to local ecological and sintaxonomic data (Padilla and Allen, 2000; Arévalo et al., 2007; Tables 4 and 5). Besides, it is preferable to use the proportion of ESG-I instead of ESG-I ratio, because in the first case its value is limited between 0 and 1, and this fact is very important when the index must be calculated or calibrated (Hernández, 2008; Ivesa et al., 2009). In this case, the classification of the macroalgal species based on ecological and sintaxonomic data to calculate the proportion of ESG-I showed a significant correlation with the water quality. In contrast, the classification of different species based on morphological data to calculate the proportion of ESG-I evidenced a worse coefficient of determination. Furthermore, Guinda et al. (2008), using an ecological approximation to define the ESG groups, observed that although the results improved, these were insufficient to justify the usefulness of this indicator in the northern coast of Spain. In contrast, this element has shown a suitable response in the southern coast of Spain, and then its use is clearly justified.

Opportunistic species are those that present high growth rates and can thrive in ruderal sites (Littler et al., 1983) where great variations of physico-chemical variables exist. Nevertheless, some differences have been observed in the classification according to different authors (Juanes et al., 2008; Orfanidis et al., 2001; UKTAG, 2009), which can be very important for the final response of the element for two reasons: (i) proportion of opportunists use few species and the absence of a species produce a large change in the final result because biomass is not considered in this index; (ii) some species considered opportunists by some authors (Juanes et al., 2008; Orfanidis et al., 2001) did not show an opportunistic behaviour in the southern coast of Spain (e.g. *Bryopsis* spp., *Cladophora* spp., *Ceramium* spp.). In this case, differences were not found between the results for the proportion of opportunists based on morphological data (Juanes et al., 2008; Orfanidis et al., 2001) or in a more ecological approach (UKTAG, 2009). As an example, Arévalo et al. (2007) and Wells et al. (2007) considered filamentous or sheet-like groups such as most of the members of the order Ceramiales, which only thrive in good to high ecological status sites, as opportunists for the morphological classification. Considering the opportunist definition, these species should not be included in this group. However, bearing in mind a morphological classification, these species are classified as opportunist. In contrast, the ecological classification, based on plant strategies in response to perturbations, did not classify these species as opportunist.

The results found in this study and others (Guinda et al., 2008), and the considerations about the proportion of ESG-I and opportunists species (Arévalo et al., 2007; Wells et al., 2007) strengthen the idea that the use of an ecological approximation is better than a morphological one in order to classify the different species into ecological groups. Moreover, the accurate classification of different species is specially important in methods based on the taxonomic composition of the community, as all species have the same importance in the final result; in contrast, an erroneous classification of species that represent residual biomass in the communities does not produce a large error in indices based on species abundance. In this study and in others cases (Arévalo et al., 2007; Guinda et al., 2008; Wells et al., 2007) the most conflicting group was the red algae; the majority of misclassifications between ecological groups correspond with this taxonomic division. Nonetheless, the resistance to pollution can be considered as a continuous; hence the adscription of a species to ESG-I or ESG-II is a key issue and may lead to disagreement. One possible solution could be classifying species at a bigger scale. For instance, the BENTIX index for macroinvertebrates (Simboura and Zenetos, 2002) classifies species in three groups (“sensitive”, “indifferent” or “tolerant”). Macroalgae could also be classified as “ruderals”, “stress-tolerants” and “competitors” according to Grime (1977). Assigning values to each group (i.e. ruderals = 0, stress-tolerants = 0.5 and competitors = 1), and dividing the summation between the species richness gives an element similar to the proportion of ESG-I. This element is also limited between 0 and 1.

*Does this index integrate the environmental implication and importance of each element?*

The EQR-RSL values for the morphological and ecological alternatives showed the expected trend with water quality, and yielded one of the highest coefficients of determination in the regression analysis (Table 4). Moreover, both EQRs presented a significant correlation with all elements used to define the water quality (except nitrites in the case of morphological approximation; Table 5), being these alternatives especially sensitive to the elements related with eutrophication (ammonium, nitrates, phosphates) and heavy metal contamination (metal content index). In addition, the ANOVA and Tukey's analysis showed significant differences between moderate and good, and moderate and high ecological status (Table 6). In general terms the RSL showed the best response, because although the proportion of ESG-I (ecological alternative) showed the highest correlation with the physico-chemical variables, this element could not distinguish between moderate and good status, which is very important to comply with WFDs requirements.

The high correlations with elements related to the eutrophication (ammonium, nitrate, phosphate) and heavy metal contamination (metal content index) suggested that the RSL index is sensitive to pollution. In the case of the eutrophication, the negative effects of this pressure at a community level have been proved as a recurrent topic in the literature (e.g. Arévalo et al., 2007; Borowitzka, 1972; Seridi et al., 2007). However, although there are many studies about the effects of heavy metals in macroalgae at the organism level (e.g. Haug et al., 1974; Haritonidis and Malea, 1995; Villares et al., 2001), due to the nature of these pollutants, it is more difficult to track these effects at the community level, being this topic very scarce in the literature (e.g. Castilla, 1996; Sales, 2010). Furthermore, in southern andalusian coasts, nutrients and heavy metals showed significant correlations ($r = 0.844$; $p < 0.001$), and the effects of these variables cannot be discriminated; for this reason this results must be taken carefully.

*Beyond the WFD*

The adaptation and application of RSL-index will improve the present knowledge of phycological flora of the southern Atlantic Spain. In fact, during the present work 6 species have been cited for the first time in Cádiz, 3 in the region (Andalusia), and the presence of Solieria chordalis has been confirmed in Andalusia (Bermejo et al., 2010). The application of the RSL-index will also help the understanding of the composition, structure and functioning of the ecosystem in each site, being possible the monitoring of ecological changes in the long term (Wells et al., 2007). These data can be a baseline for measuring the response of the distribution of these species to global change (Boaventura et al., 2002). This fact is particularly important considering the special condition of the Iberian limits between the Atlantic Ocean and the

Mediterranean Sea. This database can also provide valuable information to identify community features that can be important for management and conservation programmes (Underwood, 1991), as the presence of threatened or invasive species, or particular sites from a biogeographic point of view.

In conclusion, the RSL-index showed consistent results in relation to those expected from the water quality analytical monitoring. However, this index did not discriminate clearly between good and high status classes. To achieve a better calibration and validation of this index, further analyses and intercalibration exercises should be carried out to adjust the intertidal scoring system along the Andalusian coast.

### *Author Contributions*

Conceived and designed the experiments: RBL, JJV, IH. Analyzed the data: RBL. Wrote the manuscript: RBL, IH, JJV.

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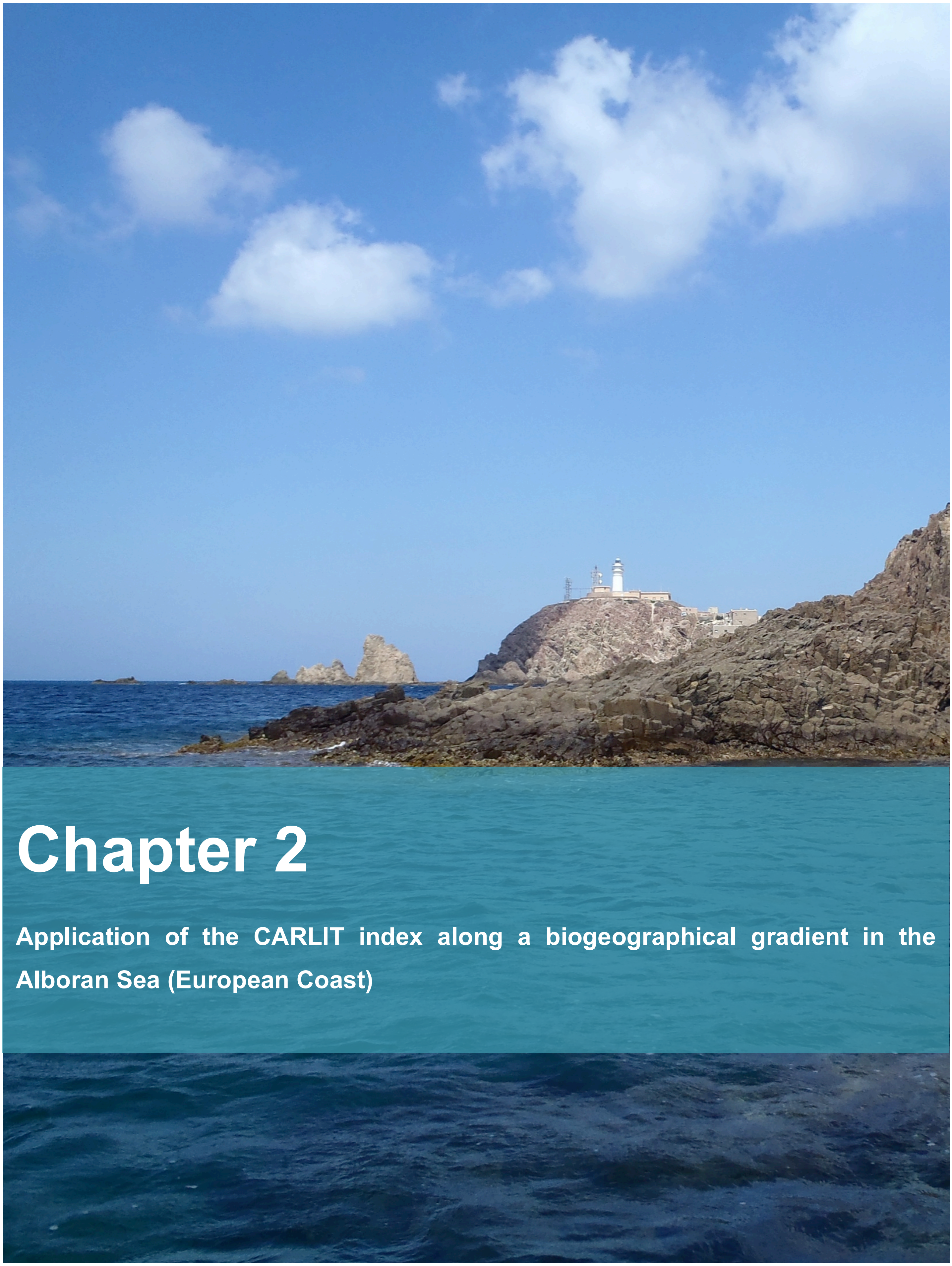

# Chapter 2

## Application of the CARLIT index along a biogeographical gradient in the Alboran Sea (European Coast)

*"Yo,*
*que en la piel tengo el sabor*
*amargo del llanto eterno,*
*que han vertido en ti cien pueblos*
*de Algeciras a Estambul,*
*para que pintes de azul*
*sus largas noches de invierno.*
*A fuerza de desventuras,*
*tu alma es profunda y oscura."*

Joan Manuel Serrat
(Mediterráneo, 1971)

Cabo de Gata. Julio 2011. Photograph by Antonio Bermejo Lacida

# Application of the CARLIT index along a biogeographical gradient in the Alboran Sea (European Coast)

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## ABSTRACT

An index, based on littoral communities assemblages (CARLIT), was applied to assess the ecological status of Northwestern Mediterranean coastal waters, following the requirements of the European Water Framework Directive. The biogeographical particularities of the Alboran Sea suggested a reassessment of this index, and that was the main objective of this work. Due to these biogeographical particularities, two regions were proposed in the studied region, with new reference conditions for each region. Subsequently, by means of a multivariate analysis, littoral community abundances and the CARLIT index were compared with factors related to geomorphology, biogeography and anthropogenic pressures. Overall, the biogeographical component determined the distribution of littoral communities. In contrast, the ecological status yieded by the index only was significantly related to anthropogenic pressures. The results pointed out that the reassessment of the CARLIT index was suitable to evaluate the ecological status of the Alboran Sea.

Keywords: Biological indicators; Littoral communities; Alboran Sea; Water Framework Directive; Macroalgae; CARLIT index.

## INTRODUCTION

Most of the international environmental institutions have identified population density, urbanization, agriculture, tourism, industry, fisheries and marine transport as the main pressures on the coastal zone (Casazza et al., 2002; EEA, 1999; UNEP, 1996). These pressures can change aquatic habitats producing different forms of pollution (e.g. overgrazing, eutrophication, heavy metals, invasions by alien species, organic compounds) and degradation of the environment. The effects of this environmental degradation have been observed in littoral ecosystems, involving the disappearance of sensitive ecosystem engineer species, simplifying the architectural complexity of the communities (e.g. Arévalo et al., 2007; Orfanidis et al., 2001), and homogenizing ecosystems (e.g. Airoldi and Beck 2007; Coll et al., 2010). In this sense, different seaweeds of the order Fucales and seagrasses, some of the most important bioengineering species in the Mediterranean phytal zone (Feldmann, 1937; Giaccone, 1973), are suffering a general decline (Delgado et al., 1999; Diaz-Almela et al., 2007; Serio et al., 2006; Thibaut et al., 2005). These macrophytes provide habitat and canopy cover for a wide variety of organisms (Mangialajo et al., 2008; Vergés et al., 2009; Vizzini, 2009); consequently, any impact in these communities will have significant effects on shore ecosystems (e.g. Hereu, 2004). Therefore, habitat destruction or degradation is considered the most important threat to the diversity, structure, and functioning of marine coastal ecosystems and to the goods and services they provide in the Mediterranean Sea (Claudet and Fraschetti 2010; Coll et al., 2010; Lotze et al., 2006).

The European Water Framework Directive (WFD 2000 ⁄ 60 ⁄ EC) requires the use of biological elements to assess the ecological status of a particular Water Body with the goal of maintaining and improving aquatic environments, avoiding further degradation. For coastal waters in the context of the WFD one out of the three biological quality elements (BQEs) proposed are benthic macrophytes. In this context, two indices, based on the study of macroalgal communities along intertidal rocky shores, have been proposed for Mediterranean coastal waters: the cartography of littoral and upper-sublittoral benthic communities (CARLIT; Ballesteros et al., 2007) and the Ecological Evaluation Index (EEI; Orfanidis et al., 2001, 2011).

The CARLIT index estimates the ecological status (ES) of a given water body from the cartography of the commonest littoral and upper-sublittoral communities along rocky shores. The communities are sorted in different sensitivity levels (SL) according to ecological and syntaxonomic considerations previously described (Ballesteros et al., 1984; Bellan-Santini, 1968; Belsher, 1977; Boudouresque, 1985; Pinedo et al., 2007) and the ES is calculated considering the length of coast occupied for each community. On the other hand, the EEI index (Orfanidis et al., 2001, 2011) is based on point measurements (replicates of quadrats) of the relative percentage of cover of late-successional species and opportunistic ones. Our preliminary studies showed that both indices are sensitive to anthropogenic pressures in the Andalusian coast (Bermejo et al., unpublished), but CARLIT showed some methodological advantages. For instance, the application of CARLIT generates a cartography of littoral and upper-sublittoral communities; which, in itself, can be an important tool for the management of coastal areas, especially in marine protected areas (García-Gómez et al., 2003), and for conservation programmes of threatened and protected species such as *Posidonia oceanica* or *Cystoseira* spp. Moreover, the CARLIT methodology uses a continuous measure, which may be more accurate for the purpose of the WFD. In this sense, the considered spatial scale reduces the uncertainty in the ecological assessment associated with the high horizontal and depth-related heterogeneity shown by macrophytic communities, which have been identified as the most important source of misclassifying of ES (Mascaró et al., 2013). In addition, the CARLIT follows a non-destructive methodology, which is essential for preservation, considering that recolonization of rocky bare substrates for some late successional species is very slow (Mangialajo et al., 2012; Thibaut et al., 2005) and repetitive, destructive samplings could be a threat to local populations. On the other hand, the simultaneous use of both flora and fauna makes this index more sensitive, providing better evidences of changes in the community structure (Díez et al., 2012; Underwood, 1996). For these reasons, since habitat destruction is possibly the most important threat to the biodiversity in the Mediterranean Sea (Claudet and Fraschetti 2010; Coll et al., 2010; Lotze et al., 2006) and a global phenomenon (Novacek and Cleland, 2001; Pimm et al., 1995) occurring at extended spatial scales (Claudet and Fraschetti 2010), the scale used by CARLIT makes this methodology very useful to assess and monitor Mediterranean coasts. Due to all these reasons, the CARLIT index has already been acknowledged in Spain, France, Italy and Croatia (MED-GIG, 2011)

The geographic location of the southern Iberian Peninsula involves singular biogeographic and ecological conditions, receiving climatic influences from the Atlantic Ocean and the Mediterranean Sea. Accordingly, Pérès and Picard (1964) divided the Mediterranean Sea in four subregions, being Alboran sea one of those. Many marine ecologists have highlighted the particularities of the Alboran Sea, as it is considered a soft transition between the Mediterranean and the Atlantic (Báez et al., 2004; Ballesteros et al., 2007), where some typical Mediterranean assemblages are frequent scarce or absent, and North Atlantic macroalgal species such as *Cystoseira tamariscifolia*, *Fucus spiralis*, *Fucus vesiculosus and Laminaria ochroleuca* are present (Conde 1989; Flores-Moya et al., 1995). For these reasons, Ballesteros et al. (2007) stated clearly that reference conditions developed for the Northwestern Mediterranean coast were not valid for the Alboran Sea.

Thus, in this framework, this study pursues three objectives: i) to define proper reference conditions to apply the CARLIT index in the Alboran Sea; ii) to assess the sensitivily of CARLIT to anthropogenic pressures in the coastal zone, and the influence of natural variability in the results yielded by the index; and iii) to estimate the ecological status of the coastal water bodies of the Alboran Sea (European shores) using this methodology.

## MATERIAL AND METHODS

### *Sampling sites and procedure*

This study was carried out in 37 sites along the coast of southern Spain in the Alboran Sea, where more than 60 kilometres of rocky shores and exceptionally sheltered sedimentary coasts dominated by seagrasses or macroalgae were sampled (Fig. 1). Shores were visited from June to August 2011, coinciding with the peak growth of littoral communities (Ballesteros, 1992). The sampling survey consisted of a run of the different stretches of coast on foot and snorkelling. Each stretch of coast was divided in sectors based on littoral and upper-sublittoral communities (or combination of both types of communities; Table 1) and geomorphological categorized information obtained in the field (slope, morphology and natural/artificial substrate; see Ballesteros et al., 2007). The initial and final points of the different sectors were marked using a Geographical Positioning System (GPS; Magellan Triton 400). The minimal length of coast surveyed was 20 metres (according to X. Torras, com. pers.). Subsequently, using a Geographical Information System (GIS) and orthophotographs from the REDIAM (net of environmental information of the government of Andalusia; Southern Spain), these sectors were divided again considering the coastline orientation and the degree of wave exposure measured as the perpendicular distance to the nearest coast. Finally, the length of each sector was measured. The final result is a partition of the rocky shoreline in several sectors defined according to littoral and upper-sublittoral communities and geomorphological characteristics, as in Ballesteros et al. (2007). The final result is a partition of the rocky shoreline in several sectors defined according to littoral and upper-sublittoral communities and geomorphological characteristics, as in Ballesteros et al. (2007): in this case, the geomorphological characteristics considered were defined based on coastline morphology, coastline slope, coastline orientation, substrate nature and degree of wave exposure.

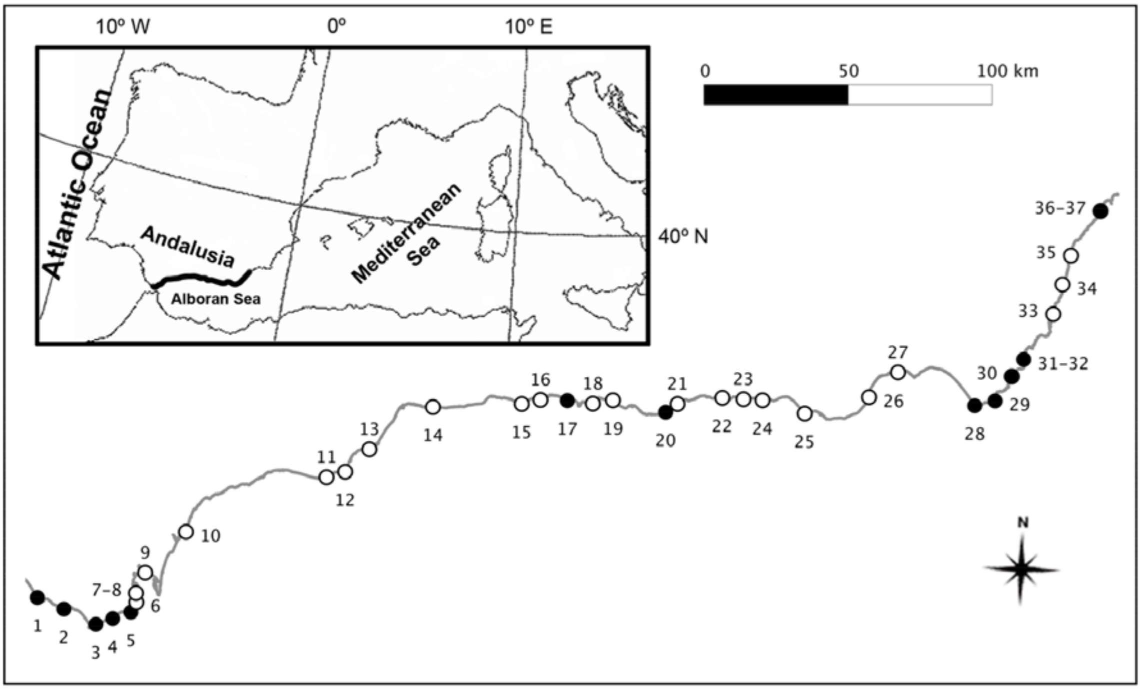



**Fig. 1.-** Geographical distribution of the different sampling points along the coast of Mediterranean coast of southern Spain. **1-** Camarinal; **2-** Punta Paloma; **3-** Punta Camorro; **4-** Guadalmesí; **5-** Cala Arenillas; **6-** Punta Carnero; **7-** Bahía de Algeciras; **8-** Puerto de Algeciras; **9-** Guadarranque; **10-** Torreguadiaro; **11-** Calahonda; **12-** Faro de Calaburras; **13-** Torrequebrada; **14-** La Araña y Rincón de la Victoria; **15-** Torrox; **16-** Nerja; **17-** Maro y Cerro Gordo; **18-** Peñon del santo (Almuñecar); **19-** Caletón (Salobreña); **20-** Cala Rijana; **21-** Castel de Ferro; **22-** Cala del Ruso; **23-** La Alcazaba; **24-** Adra; **25-** Guardias Viejas; **26-** Roquetas de Mar; **27-** Playa de las olas (Almería); **28-** Cabo de Gata; **29-** San José; **30-** Isleta del Moro; **31-** Cala Carnaje; **32-** El Playazo (Rodalquilar); **33-** El Algarrobico; **34-** Playa de Mojácar; **35-** Villaricos; **36-** Cala Panizo; **37-** San Juan de los Terreros. Open dots- sites that not accomplish with reference conditions; Black dots- sites that accomplish with reference conditions.

The CARLIT index (Ballesteros et al., 2007) is based on the cartography of littoral and upper-sublittoral communities of the rocky shoreline in different sectors, each one characterized by a community or combination of communities with a sensitively level according to ecological and sintaxonomic considerations previously described (Ballesteros et al., 1984; Bellan-Santini, 1968; Belsher, 1977; Boudouresque, 1985; Pinedo et al., 2007). Due to the influence of the natural geomorphological variability in the development of several littoral and sub-littoral communities (Ballesteros, 1992), the CARLIT index proposes different reference conditions for each Geomorphological Relevant Situation (GRS), which permit the correction of this natural variability. These GRSs are defined as the combination of the most relevant geomorphological factors determining the littoral and upper-sublittoral communities in reference sites with no, or very minor, disturbance from human activities. Thus, the environmental quality assessment of a stretch of coast is estimated as a ratio between the environmental quality in a particular site and the environmental quality in a reference site with similar geomorphological characteristics. For a detailed description of the CARLIT procedure see Ballesteros et al (2007).

**Table 1-** Summarized description and sensitivity levels of the main community categories distinguished in the monitored coasts (modified from Ballesteros et al., 2007).

| Code | Community | SL |
|---|---|---|
| Ac | Dense populations of *Astroides calycularis* | 20 |
| Lb | Build-ups of *Lithophyllum byssoides* | 20 |
| SG 5 | Seagrasses meadows* | 20 |
| OC | Populations of *Cystoseira*** different C. compressa and *C. ericaefolia* group | 20 |
| Ce 5 | Continuous belt of *Cystoseira ericaefolia* group*** | 20 |
| Ce 4 | Discontinuous belt of *C. ericaefolia* group | 19 |
| SG 3 | Abundant patches of dense stands of seagrasses | 15 |
| SG 5- | *C. nodosa* or *Z. noltii* meadows on death matte of *P. oceanica* | 15 |
| Ce 3 | Abundant patches of dense stands of *C. ericaefolia* group | 15 |
| Fs | Populations of *Fucus spiralis* | 15 |
| Ce 2 | Abundant scattered specimens of *C. ericaefolia* group | 12 |
| Cc | Populations of *C. compressa* | 12 |
| SG 3- | Abundant patches of *C. nodosa* or *Z.noltii* on death matte of *P. oceanica* | 12 |
| SG 1 | Rare scattered stands of seagrasses | 10 |
| Ce 1 | Rare scattered specimens of *C. ericaefolia* group | 10 |
| SG 1- | Rare scattered stands of *C. nodosa* or *Z. noltii* on death matte of *P. oceanica* | 8 |
| Co | Belt of *Corallina spp.* without *C. ericaefolia* group | 8 |
| J | Belt of *Haliptilon/Jania spp.* without *Cystoseira ericaefolia* group | 8 |
| My | Belt of mussels (*Mytilus spp.*) without *Cystoseira ericaefolia* group | 6 |
| Li | Belt of *Lithophyllum incrustans* and other encrusting corallines | 6 |
| Ul | Upper sublittoral belts of *Ulva spp.* and *Cladophora spp.* | 3 |
| Cy | Communities dominated by Cyanobacteria and *Derbesia tenuissima* | 1 |

* Seagrasses- *Cymodocea nodosa* (Cn), *Posidonia oceanica* (Po) or *Zostera noltii* (Zn).
** Others *Cystoseira*- *C.elegans* (Cel), *C.foeniculacea* (Cf), *C.sauvegeuana* (Cs), *C.spinosa* (Csp), *C.mauritanica* (Cm).
*** *Cystoseira ericaefolia* group– *Cystoseira tamariscifolia*, *C.amentacea* var. *stricta* and *C.mediterranea*.

The ecological differences between the Alboran Sea and the Northwestern Mediterranean imply a reassessment and redefinition of GRSs (Ballesteros et al., 2007). On the other hand, in the Alboran Sea the presence and the abundance of several littoral and upper-sublittoral communities respond mostly to the presence of a biogeographical natural gradient (Conde, 1989; Báez et al., 2004; Ballesteros and Pinedo, 2004). In this sense, to reduce the influence of this natural gradient in the final result of the CARLIT index, the studied area was divided in different ecological or biogeographical regions with proper reference conditions. Therefore, before to apply CARLIT index, ecological or biogeographical regions were identified and reference conditions were redefined for each identified region.

*Identifying ecological regions in European Alboran coast*

The ecological differences between the Alboran Sea and the Northwestern Mediterranean imply the inclusion of several common community categories from the former in the original Northwestern Mediterranean list (Ballesteros et al., 2007). Moreover, due to the ecological importance and the sensitivity of seagrasses to anthropogenic pressures, the degree of development of seagrass meadows was also considered to improve the sensitivity of the methodology (Table 1).

To identify ecological or biogeographic regions based on the percentages of rocky shore occupied by perennial littoral and upper-sublittoral communities, a cluster analysis using group average linking of Bray-Curtis similarity index (Bray and Curtis, 1957) was applied among the 37 sites. Subsequently, an analysis of species contribution to similarity (SIMPER; Clarke and Gorley, 2006) was carried out to detect which species contributed most to the dissimilarity among the identified regions.

*Reference conditions*

Reference sites were chosen from marine protected areas and sectors that fulfilled the criteria proposed by the Mediterranean Geographical Intercalibration Group (Med-GIG) for macroalgae: i) population density with settlements lower than 1000 ind/km2 in the next 15 km and/or more than 100 habitats/km2 in the next 3 km within that area (winter population); ii) no more than 10% of artificial coastline; iii) no harbour (more than 100 boats) within 3 km; iv) no beach regeneration within 1 km; v) no industries within the 3 km; vi) no fish farms within the 1 km; vii) no desalination plants within 1 km; and viii) no evidence of *Cystoseira* forest regression due to other unconsidered impacts. In this case, the reference sites (black dots, Fig. 1) chosen were the undisturbed areas of El Estrecho Natural Park (1-5), Maro-Cerro Gordo Natural Area (17), Cala Rijana (20), Cabo de Gata Natural Park (28-32), Cala Panizo (36), and San Juan de los Terreros (37).

To define reference conditions in Alboran Sea, the five geomorphological factors for reference sites were combined, obtaining different real situations characterized by one unique combination of geomorphological categories (e.g. high continuous coast, vertical, south, natural, > 1000 m). Based on the percentage of coast occupied by each community category for each real situation, an Analysis of Similarity (ANOSIM) and a non-metric multidimensional scaling (MDS) analysis (Clarke and Warwick, 2001) were performed to identify the most relevant geomorphological factors for each region. Subsequently, a value of Ecological Quality (EQ) was assigned for each identified GRS in reference places according to eq.1, being these values considered the highest possible (i.e., reference condition).

$$EQ = \Sigma (l_i * SL_i) / \Sigma l_i \qquad (1)$$

where EQ is the environmental quality of a particular stretch of coastline; $l_i$ is the length of the coastline occupied by the community category I and $SL_i$ is the sensitivity level of the community category i.

*Ecological assessment*

According to the WFD, the ecological status has to be expressed in terms of ecological quality ratios (EQR). This ratio indicates the relationship between the value of the BQE (i.e., macroalgae) recorded for a given water body and the value for this element in the reference conditions applicable to that body, yielding a value between zero and one, with high ecological status represented by values close to one. In this case, the EQR was calculated for the stretch of coast studied according to eq. 2 (Ballesteros et al., 2007).

$$EQR = ( \Sigma(EQ_{SSi}/EQ_{RSi})*l_i) / \Sigma l_i \qquad (2)$$

where i is the situation; $EQ_{SSi}$ is the EQ in the study site for the situation i; $EQ_{RSi}$ is the EQ in the reference sites for the situation i; and $l_i$ is the coastal length in the study coast for the situation i.

*Response of CARLIT to natural and anthropogenic pressures*

To test the sensitivity of CARLIT along Alboran Sea, values of 10 environmental variables related to the morphology of the coastline, biogeographic factors and anthropogenic pressures, were obtained for each sampled locality. Variables related to the morphology of coastline (% artificial coast; % of coastline constituted by blocks; % of coastline constituted by high coast; and % of coastline constituted by low coast) were measured in situ. The mean temperature of seawater was obtained from REDIAM. The tidal range in each locality was obtained from the annual tide table of 2011 elaborated by the “Instituto Hidrográfico de la Marina Española”. The percentage of urban, agricultural (irrigated), industrial and natural land was calculated on ca. 9 $km^2$ between the coastal line sampled and 3 km inland. When the stretch of the surveyed coast was shorter than 3 km, this was located in the middle of the corresponding coastal fringe. The software used for this purpose was QGIS and the necessary geographical information was obtained from REDIAM. Subsequently, using the data for these ten variables a Principal Component Analysis (PCA) was used to reduce the number of variables avoiding repetitive information. The PCA solution was rotated, using Varimax rotation method, to make easier the interpretation of the results. Finally, this information was used to assess the response of CARLIT to natural and anthropogenic pressures, performing a Spearman correlation analysis between the percentages of coastline occupied for each community class found and the EQR value of CARLIT, and the obtained principal components.

On the other hand, to validate and make comparable our results with previous studies (e.g. Bermejo et al., 2012; López y Royo et al., 2011; MED-GIG, 2011), the response of CARLIT against antropogenic pressure was examined with two indices: Land Use Simplified Index (LUSI; Flo et al., 2011) and the methodology proposed by López y Royo et al. (2009). Both indices are based on the surface occupied for urban, agriculture and industrial activities, providing the former a quantitative solution, and a qualitative solution the latter. Finally, the response of CARLIT to anthropogenic pressures was evaluated: i) using a Pearson correlation analysis between the EQR value of CARLIT and total value of LUSI; and ii) performing one-way Analysis of Variance (ANOVA) to test the effects of human pressures estimated according to López y Royo et al. (2009) in the EQR. In the ANOVA, all classes complied with Shapiro–Wilk normality test and were homocedastic. A post hoc analysis (Tukey test; Zar 1984) was applied. In all cases, significance was set at 5% probability.

*Ecological Status of water bodies from European Alboran coast*

Based on the stretches of coast studied, an assessment of the Ecological Status (ES) was performed in 2 Atlantic water bodies and 18 Mediterranean ones, which were previously defined by the Government of Andalusia. The criteria of correspondence between the EQR and ES were the same that in Ballesteros et al. (2007): 0-0.25 (bad), 0.25-0.40 (poor), 0.40-0.60 (moderate), 0.60-0.75 (good) and 0.75-1 (high).

## RESULTS

*Defining ecological regions in European Alboran coasts*

The new littoral and upper-sublittoral communities, which are proposed to be included in the classification list of CARLIT for the Mediterranean coast of southern Spain, are reported in Table 1. It includes now: the vulnerable Cnidaria, *Astroides calycularis*, which is highly sensitive to pollution and it is included in the red list of invertebrate species in Andalusia (Moreno et al., 2008), thus the maximum sensitivity level (SL=20) was assigned to this specie; and *F. spiralis*, which is present in the western Alboran region, where it has the distribution limit in the Mediterranean European coast (Conde, 1989), and it seems be more sensible to high pollution levels than in others areas (López-Rodríguez et al., 1997; Wilkinson et al. 2007); for these reasons the sensitivity level was set at 15 (SL= 15); Moreover, to improve the sensitivity of the CARLIT index, seagrasses were divided in six categories: 5, 5-, 3, 3-, 1 and 1-, according to a decreasing coverage and sensitivity level (SL= 20, 15, 15, 12, 10 and 8, respectively).

According to the percentage of coast occupied for the littoral and upper-sublittoral community categories in each site, three outliers and two ecological regions were identified corresponding to an arbitrary slice in the dendrogram arising from the cluster analysis at a Bray–Curtis similarity of 55% (Fig. 2). The first region corresponded to the western Alboran Sea (from 1 to 20); the second one corresponded to the eastern shores of Alboran Sea (from 20 to 37). The boundary between both regions was situated between the localities 20 and 25, and temperature seems to play an important role in this division (Fig. 2). Accordingly, the SIMPER analysis (Table 2) showed that *P. oceanica*, *Cystoseira ericaefolia* and *Mytilus* spp. were the species that more contributed to the dissimilarity between the two regions (approximately 20% each one). In western Alboran Sea, *P. oceanica* was rare and *C. ericaefolia* was less frequent than in eastern Alboran waters; these species were found forming stands and scattered belts. In contrast, *Mytilus* was an important component of littoral communities in the western area. In the Eastern region, *C. ericaefolia* and *P. oceanica* were present more frequently and generally forming dense continuous belts and meadows, whereas *Mytilus* was rare. Moreover, the absence of *F. spiralis* and *Lithophyllum byssoides* in the Eastern region must be highlighted, along with *Cystoseira mauritanica*. In the western Alboran Sea, neither *Cystoseira elegans*, *C. sauvegeuana* nor *C. spinosa* were found. On the other hand, the three outliers can be attributable to high levels of pollution in site 8 (Algeciras harbour), high percentage of artificial coast in site 24 (Adra), and the special conditions of the intertidal in site 34 (Mojácar, where the intertidal zone was composed for scattered metric and decimetric blocks).

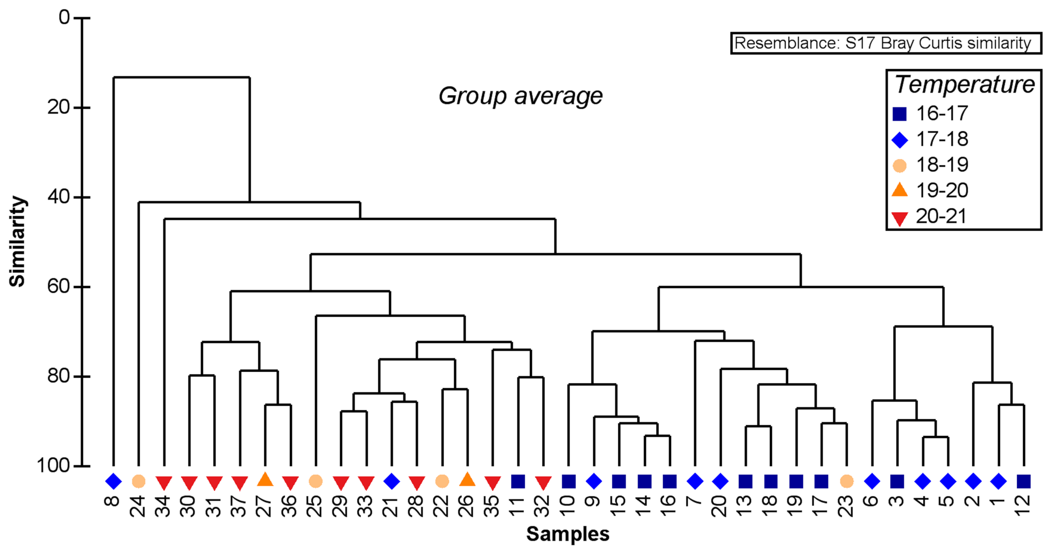



**Fig 2.-** Dendrogram depicting mutual similarities of littoral and sub-littoral communities of the different sampled locations. Coloured dots refer to the mean sea surface temperature (ºC) in each locality.

**Table 2-** Results of SIMPER on benthic communities contributing to average dissimilarity between ecological regions. Average dissimilarity = 50.31.

| Community | Western area<br>Av. Abundance | Eastern area<br>Av. Abundance | % Contribution |
|---|---|---|---|
| *P. oceanica* | 0.02 | 0.49 | 21.49 |
| *C. ericaefolia* | 0.22 | 0.51 | 18.38 |
| *Mytilus spp.* | 0.36 | 0.03 | 18.11 |
| *Corallina spp.* | 0.90 | 0.82 | 12.10 |
| *C. compressa* | 0.05 | 0.17 | 7.98 |
| *L. byssoides* | 0.13 | 0.00 | 6.45 |
| *C. nodosa* | 0.01 | 0.15 | 6.40 |

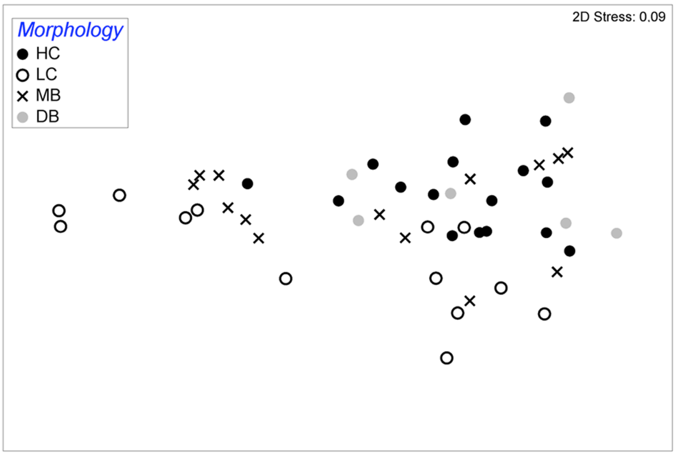
*Defining reference conditions*

In western Alboran region (1-20), combining the five geomorphological factors used for reference sites, 50 different real situations were found. The results of the ANOSIM (Table 3) and MDS analysis showed that “coastline morphology” (Fig. 3) and “degree of wave exposure” (Fig. 4) were the most important variables determining the community categories in reference sites of this region, being the second variable more relevant. For coastline morphology, the most important differences were found between high coast (HC) and low coast (LC) (Fig. 3). In addition, LC situations showed a higher dispersion than HC ones. In the case of degree of wave exposure, “>1000 meters” and “between 0 and 500 metres” of perpendicular distance to the nearest coast were the commonest. On the other hand, “500-1000 metres” situation was rare. For this reason, only two categories of “degree of wave exposure” were considered: “>1000 meters” and “<1000 metres” of perpendicular distance to the nearest coast. Therefore, for the western Alboran coast, eight GRS were obtained combining these variables (Table 4).

**Table 3-** Results of ANOSIM for each geomorphological factor and area, based on different situations resulting from all the available combinations of the geomorphological variables considered in reference sites in Western and Eastern Alboran region according to the percentage of coast occupied by each community category for each situation. Bold letters mean significant correlations at: * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001.

| Geomorphological factor | Western area<br>Global R statistic | Eastern area<br>Global R statistic |
|---|---|---|
| Coastal morphology | **0.133**** | **0.231***** |
| Slope | -0.023 | -0.060 |
| Natural/Artificial | n/a | -0.255 |
| Orientation | 0.086 | 0.081 |
| Wave exposure | **0.186***** | **0.144**** |

In the case of eastern Alboran (21-37), 51 different real situations were obtained from the combination of the geomorphological factors. As in western Alboran, “coastline morphology” (Fig. 5) and “degree of wave exposure” (Fig. 6) were the most important variables determining the community categories found in the reference sites. However, in this case “coastal morphology" showed a higher ANOSIM R-value (Table 3) suggesting a larger influence of this factor in the communities. Also in this region, degree of wave exposure was based on the two categories considered above. Therefore, for the eastern Alboran coast eight GRS were obtained (Table 4).



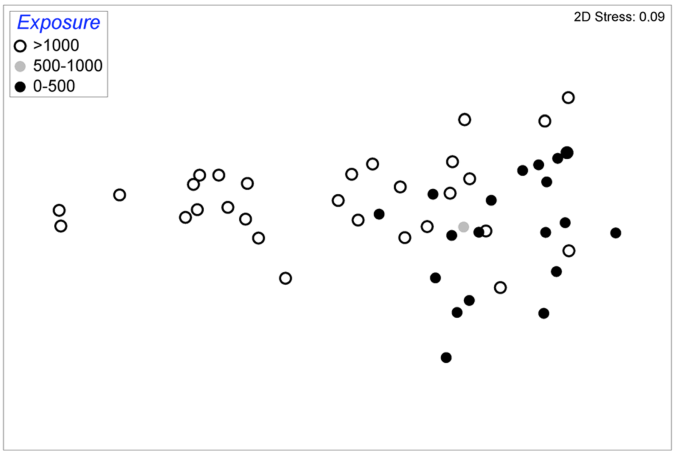
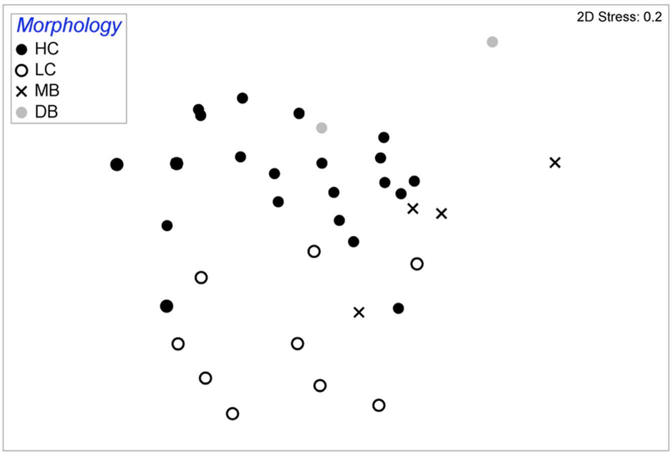




**Fig. 3 (left).-** MDS analysis showing the distribution of different situations resulting from all the available combinations of the geomorphological variables considered in reference sites in Western Alboran region according to the percentage of coast occupied by each community category for each situation. High coast, low coast, metric blocks and decimetric blocks are indicated with different symbols and colours.

**Fig. 4 (right).-** MDS analysis showing the distribution of different situations resulting from all the available combinations of the geomorphological variables considered in reference sites in Western Alboran region according to the percentage of coast occupied by each community category for each situation. The categories of "wave exposure" are indicated with different colours.



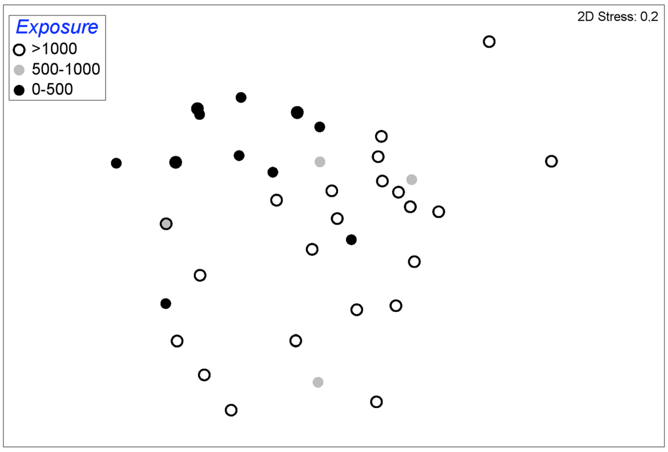




**Fig. 5 (left).-** MDS analysis showing the distribution of different situations resulting from all the available combinations of the geomorphological variables considered in reference sites in East Alboran region according to the percentage of coast occupied by each community category for each situation. High coast, low coast, metric blocks and decimetric blocks are indicated with different symbols and colours.

**Fig. 6 (right).-** MDS analysis showing the distribution of different situations resulting from all the available combinations of the geomorphological variables considered in reference sites in eastern Alboran region according to the percentage of coast occupied by each community category for each situation. The categories of "wave exposure" are indicated with different colours.

Table 4 shows the EQ values for each GRS in the two regions. Overall, eastern Alboran showed higher values than western Alboran, especially in exposed localities. It is remarkable that HC showed the lowest EQi value in the Western region; by contrast, it obtained the highest EQi in the Eastern region. The DB was the most rare, less abundant GRS for both regions (less than 5% of surveyed references shores).

**Table 4-** Ecological quality values (EQi) calculated for the four geomorphological relevant situations in reference conditions for each ecological region. HC- High Coast; LC- Low Coast; MB- Metric Blocks; DB- Decimetric Blocks. Exp- >1000 m: No Exp- <1000 m.

| GRS | $EQ_i$ Western | Long | $EQ_i$ Eastern | Long |
|---|---|---|---|---|
| Exp HC | 10.2 | 2900 | 19.3 | 1190 |
| Exp LC | 17.3 | 13550 | 17.9 | 3070 |
| Exp MB | 14.6 | 7760 | 18.8 | 630 |
| Exp DB | 11.6 | 1080 | 16.0 | 190 |
| No Exp HC | 12.0 | 930 | 16.9 | 1230 |
| No Exp LC | 14.7 | 1020 | 12.9 | 770 |
| No Exp MB | 9.6 | 690 | 8.0 | 110 |
| No Exp DB | 7.1 | 90 | 8.0 | 30 |

The comparison of MDS results showed that the most important differences regarding coastal morphology were between HC and LC for both regions (Fig. 3 and 5). Nonetheless, in the Western region, the dispersion of points was high for LC and low for HC (Fig. 3). Conversely, in the Eastern region the dispersion for HC and LC points was similar (Fig. 5). In the case of “degree of wave exposure” (Fig. 4 and 6) the most important differences were found between “>1000 metres” and “between 0-500 metres”, being the dispersion higher in “>1000 meters” in both regions.

*Response of CARLIT to natural and anthropogenic pressures*

The results of the PCA, carried out with ten parameters related to geomorphology, biogeography and human pressures in all the sampling stations, showed a decreased, but relatively closed contribution of the first four axes (C1 = 28.9%; C2 = 19.4%; C3 = 18.8%; and C4 = 15.5%) to the explanation of total variance (82.6%). As it can be seen in Table 5, the first component (C1) was positively correlated to the percentage of artificial coast, the percentage of coastline constituted by blocks and the percentage of surface occupied by agriculture, and inversely related to the percentage of natural surface, thus, it is related to morphological and agriculture pressures. The second component (C2) was directly related to the mean temperature and inversely to the tidal range, both parameters could be considered related to biogeography. The component C3 was positively related to the percentage of low coast and negatively to the percentage of high coast, showing geomorphological information. The fourth component (C4) was directly related to the percentage of urban and industrial surface, and negatively to the percentage of natural surface, being related to urban and industrial pressures.

**Table 5-** The coefficients of the rotated principal components, using varimax rotation method, for the ten variables.

| Variable | C1 | C2 | C3 | C4 |
|---|---|---|---|---|
| % Artificial coast | **0.791** | 0.053 | 0.112 | -0.038 |
| % Blocks | **0.655** | 0.380 | 0.409 | -0.363 |
| % High coast | -0.352 | -0.267 | **0.784** | 0.321 |
| % Low coast | -0.249 | -0.095 | **-0.957** | 0.038 |
| Tidal range | -0.361 | **0.778** | -0.267 | 0.178 |
| Temperature | -0.142 | **-0.887** | -0.141 | -0.077 |
| % Urbanization | 0.297 | 0.486 | -0.149 | **0.582** |
| % Natural | **-0.882** | -0.162 | 0.117 | -0.499 |
| % Industrial | -0.131 | 0.087 | 0.189 | **0.820** |
| % Agriculture | **0.846** | -0.239 | -0.075 | -0.094 |

The Spearman correlation matrix between the four components and the percentage of coastline occupied by the different categories of communities showed that C2 was the component that showed the highest number of significant correlations, with 15 out of 29 community classes (Table 6). In contrast, C3 was the component that presented the lowest number of correlations to the community classes; 4 out of 29. The C1 and C4 yielded significant relationship with 10 and 6 communities respectively.

The EQR values of water bodies showed significant correlations with components C1 (rho = -0.486, p-value <0.001) and C4 (rho = -0.322, p <0.05), but not with C3 and C2 (Table 6). Thus, CARLIT index seems be more correlated to agriculture pressures and morphological alteration of rocky shores, than to urban and industrial pressures. However, when the correlation analysis was performed separately in the two regions, it was observed that: i) in western Alboran, C2 (rho = 0.385; p-value < 0.05) and C4 (rho = -0.726; p-value < 0.001) correlated significantly with EQR; ii) conversely, in eastern Alboran correlation was only significant with C1 (rho = -0.705; p-value < 0.001), pointing out differences in anthropogenic pressures between western Alboran, where urban and industrial pressures were higher, and eastern Alboran, where agriculture pressures and morphological alteration of rocky shores were more relevant.

The comparison between CARLIT and the indices of anthropogenic pressures confirmed that CARLIT was sensitive to human pressures in the European coast of Alboran Sea. In this sense, the ANOVA (Table 7) indicated that there were significant differences in the mean values of EQR between high, moderate, low and none anthropogenic pressure levels assessed according to López y Royo et al. (2009). The Tukey´s test revealed that a high-pressure level showed significant differences with the others. Overall, this high level was related to the presence of industrial activities or industrial harbours. The moderate-pressure level evidenced significant differences with the absence of pressures, but not with low-pressure levels. The low-pressure level only showed significant differences with the high-pressure one. On the other hand, the Pearson correlation between EQR values and LUSI index was significant (r = -0.652; p-value <0.001). Furthermore, the correlation strength was higher in the western Alboran (r = -0.799, p-value <0.001) than in Eastern region (r = -0.436, p-value < 0.05).

**Table 6-** Spearman correlation matrix of the principal components obtained, and the different communities used to calculate the CARLIT index. Bold letters mean significant correlations at: * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001.

| | C1 | C2 | C3 | C4 |
|---|---|---|---|---|
| Cy | -0.062 | -0.106 | -0.214 | -0.188 |
| My | **0.285*** | **0.457**** | 0.149 | **0.488***** |
| Li | -0.136 | **0.313*** | 0.005 | 0.253 |
| Ul | **0.386**** | -0.200 | 0.194 | 0.245 |
| Co | **-0.375*** | 0.196 | 0.141 | -0.224 |
| Lb | **-0.528***** | **0.526***** | -0.246 | **-0.449**** |
| Fs | **-0.387**** | **0.579***** | -0.239 | **-0.389**** |
| Ce 1 | 0.048 | **0.335*** | -0.040 | -0.239 |
| Ce 2 | -0.206 | -0.013 | -0.041 | -0.111 |
| Ce 3 | 0.081 | -0.037 | -0.085 | -0.226 |
| Ce 4 | -0.027 | **-0.292*** | 0.052 | -0.089 |
| Ce 5 | -0.245 | **-0.657***** | -0.020 | 0.163 |
| Cc | -0.228 | **-0.425**** | -0.120 | -0.171 |
| Cel | **-0.292*** | **-0.367*** | 0.045 | -0.135 |
| Cf | -0.001 | -0.152 | **-0.357*** | 0.121 |
| Ch | **-0.456**** | 0.202 | -0.198 | **-0.351*** |
| Cs | -0.057 | **-0.347*** | **-0.376*** | 0.075 |
| Csp | 0.031 | -0.265 | -0.265 | 0.078 |
| Cm | **-0.518***** | **0.540***** | -0.220 | **-0.463**** |
| Po 1 | 0.125 | 0.043 | 0.020 | -0.033 |
| Po 3 | **0.312*** | **-0.514**** | -0.270 | -0.157 |
| Po 5 | **-0.394**** | **-0.590***** | 0.135 | 0.052 |
| Cn 1 | 0.119 | -0.258 | -0.192 | -0.033 |
| Cn 3 | -0.252 | **-0.409**** | -0.174 | 0.132 |
| Cn 3- | 0.094 | 0.172 | -0.125 | 0.219 |
| Cn 5 | -0.064 | **-0.348*** | **-0.359*** | 0.025 |
| Cn 5- | 0.018 | -0.214 | 0.117 | 0.064 |
| Zn 1 | -0.078 | -0.156 | 0.016 | -0.062 |
| Ac | -0.037 | 0.023 | **0.380*** | -0.116 |
| EQR | **-0.486***** | -0.159 | 0.015 | **-0.322*** |

**Table 7-** Results of the ANOVA analysis between the different anthropogenic pressure levels obtained according to López y Royo et al. (2009) and EQR for CARLIT. Mean and standard deviation (SD) for each pressure level and the results of the post hoc Tukey test are also indicated.

| **Pressure level** | **Mean** | **SD** | **ANOVA (sig.)** | **Tukey** | | |
|---|---|---|---|---|---|---|
| High | 0.56 | 0.15 | | | | **X** |
| Moderate | 0.76 | 0.14 | <0.001 | | **X** | |
| Low | 0.85 | 0.09 | | **X** | **X** | |
| None | 0.94 | 0.07 | | **X** | | |

*Ecological Status of European coasts of Alboran Sea*

Among the 20 water bodies assessed (Table 8; Fig. 7), 11 yielded a high ES, 6 were estimated as good, 2 as moderate and 1 as poor. In the water bodies with moderate or poor ES, the longitude of rocky shore transects studied was lower than 900 m, and all cases were located in Algeciras Bay, where it exists a conspicuous environmental degradation due to urban and industrial pressures (Bermejo et al., 2012; Díaz-de Alba et al., 2011; Morales-Caselles et al., 2007). When localities were considered instead water bodies, 21 showed a high ES, 11 were estimated as good, 4 as moderate and 1 as poor. The locality with poor ES (9) and two of these four localities with moderate ES (7 and 8) were sited in Algeciras Bay. The other two localities with moderate ES were located in Torrox (12) and Villaricos (32), in water bodies that were estimated as in good ES.

**Table 8-** Values of EQR, LUSI, pressure level (PL) assessed according López y Royo et al. (2009) and longitude of coast sampled in meters corresponding to the 37 localities studied, and EQR and ES values to 20 water bodies from Andalusia for the year 2011. *-Reference site.

| Site | EQR | LUSI | PL | Long | WB | EQR | ES |
|---|---|---|---|---|---|---|---|
| 1* | 0.94 | 0 | None | 2490 | 1 | 0.94 | High |
| 2* | 0.93 | 0 | None | 4690 | | | |
| 3* | 0.82 | 1 | Low | 4570 | 2 | 0.88 | High |
| 4* | 0.89 | 0 | Low | 4910 | | | |
| 5* | 0.85 | 1 | Moderate | 3600 | 3 | 0.85 | High |
| 6 | 0.74 | 2.5 | High | 2100 | 4 | 0.74 | Good |
| 7 | 0.49 | 5 | High | 830 | 5 | 0.49 | Moderate |
| 8 | 0.41 | 5 | High | 180 | 6 | 0.41 | Moderate |
| 9 | 0.37 | 3.75 | High | 800 | 7 | 0.37 | Poor |
| 10 | 0.88 | 1 | Moderate | 1220 | 8 | 0.82 | High |
| 11 | 0.73 | 3 | Moderate | 1880 | | | |
| 12 | 0.98 | 2 | Moderate | 660 | | | |
| 13 | 0.78 | 3.75 | Moderate | 880 | 9 | 0.78 | High |
| 14 | 0.70 | 3 | Moderate | 1690 | 10 | 0.70 | Good |
| 15 | 0.44 | 3 | Moderate | 370 | 11 | 0.62 | Good |
| 16 | 0.69 | 4 | Moderate | 900 | | | |
| 17* | 0.80 | 1 | Low | 6730 | 12 | 0.80 | High |
| 18 | 0.80 | 1.5 | Moderate | 1230 | 13 | 0.80 | High |
| 19 | 0.67 | 2 | High | 1500 | 14 | 0.67 | Good |
| 20* | 0.89 | 1.25 | Low | 1030 | 15 | 0.78 | High |
| 21 | 0.82 | 2 | Low | 3210 | | | |
| 22 | 0.70 | 3 | Moderate | 710 | | | |
| 23 | 0.63 | 1.25 | Low | 950 | | | |
| 24 | 0.74 | 5 | Moderate | 1400 | 16 | 0.70 | Good |
| 25 | 0.66 | 3.75 | Moderate | 1700 | | | |
| 26 | 0.68 | 4 | Moderate | 710 | 17 | 0.80 | High |
| 27 | 1.00 | 2.5 | Moderate | 450 | | | |
| 28* | 0.90 | 0 | None | 1310 | 18 | 0.92 | High |
| 29* | 0.90 | 1.25 | Low | 1600 | | | |
| 30* | 0.99 | 1.25 | None | 1170 | | | |
| 31* | 1.00 | 0 | None | 600 | | | |
| 32* | 0.79 | 0 | None | 490 | | | |
| 33 | 0.68 | 0 | High | 1190 | 19 | 0.63 | Good |
| 34 | 0.95 | 1 | Low | 900 | | | |
| 35 | 0.45 | 3 | High | 1930 | | | |
| 36* | 1.00 | 1 | None | 560 | 20 | 0.94 | High |
| 37* | 0.92 | 2 | Low | 1490 | | | |

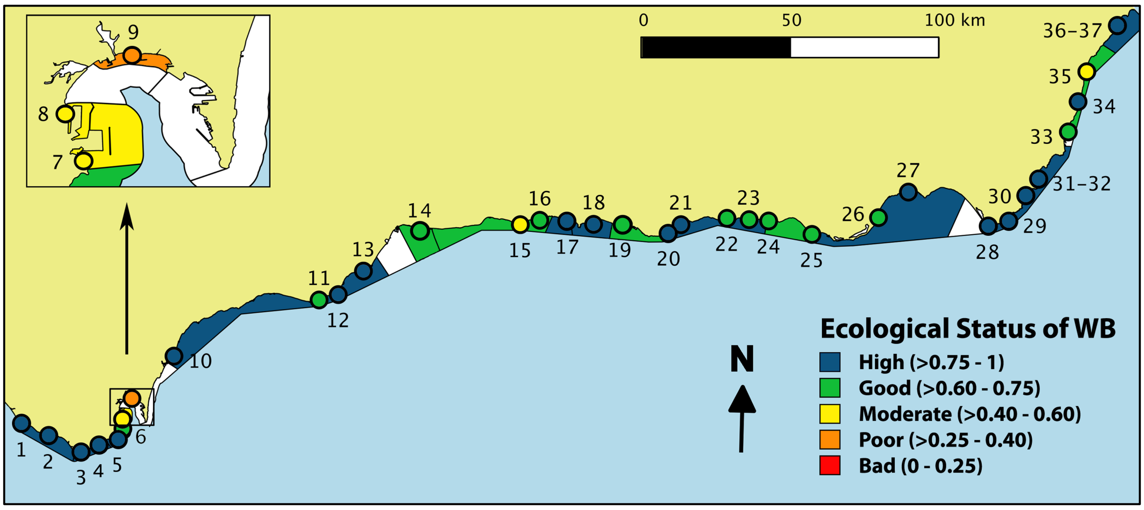



**Fig. 7.-** Ecological Status of coastal water bodies along the Mediterranean coast of Andalusia in 2011.

## DISCUSSION

The CARLIT index is a useful tool to estimate the ecological status in the Northwestern Mediterranean Sea (Asnaghi et al. 2009; Ballesteros et al. 2007; Mangialajo et al. 2007). However, this index has some limitations that must be considered: i) The CARLIT index cannot assess shorelines which are completely sandy (Ballesteros et al., 2007); ii) the assessment of coastlines with low percentages of rocky shores may be undervalued due to the lower structural complexity of their upper-sublittoral macroalgal stands, which often lack extensive *Cystoseira* assemblages (Ballesteros et al, 2007); iii) This index has been developed for Mediterranean rocky shores with narrow tidal ranges. In oceanic environments where the tidal range is wider and the period is semidiurnal, this methodology can hardly be applied and it must be modified (Mangialajo et al., 2007). In addition, according to Ballesteros et al. (2007) the biogeographical differences between Alboran and Northwestern Mediterranean Sea precluded the use of reference conditions developed for the Mediterranean, so specific reference conditions had to be developed for the Alboran Sea. Furthermore, the position of the Alboran Sea, in transition between the Atlantic and the Mediterranean, confers to these water bodies particular dynamic and physico-chemical characteristics (Cano, 1977, 1978); this is also influenced by the special orographic features of this area, which produces complex oceanographic and meteorological conditions (García-Lafuente et al., 1998; García-Lafuente and Ruiz 2007). These particular conditions determine the distribution of seaweeds along the Alboran Sea (Conde, 1989), which can be considered as a soft transition between the Mediterranean and the Atlantic region (Báez et al., 2004; Ballesteros and Pinedo, 2004). For these reasons, besides considering different reference conditions than those in Northwestern Mediterranean, the Alboran Sea must be divided into different regions with own specific reference conditions to avoid the influence of this biogeographical gradient.

Considering the limitations of CARLIT and the special biogeographical conditions of the Alboran Sea, some questions must be addressed: Is the CARLIT methodology suitable to assess the ES in Mediterranean coastal waters of Andalusia? How many regions must be considered to define the reference conditions? Is the index sensitive enough to anthropogenic pressures in Alboran?

*Is CARLIT methodology suitable to assess the ES in the European coastal waters of Alboran Sea?*

The Mediterranean coast of Andalusia is divided into 35 water bodies defined according to their typology and their anthropogenic pressures and impacts (Fig. 7), as proposes the WFD. Seven water bodies are transitional and CARLIT cannot be applied. Furthermore, nine of the remaining water bodies are completely devoid of natural rocky shores. It is remarkable that six out of nine water bodies without natural rocky shore were very small and highly modified -mainly inner parts of harbours and marinas- and they do not represent the ecological quality of open waters. For these reasons, Ballesteros et al. (2007) did not consider these types of water bodies. Nevertheless, if highly modified waters are considered, these 6 water bodies could be assessed using the CARLIT index because artificial rocky shores and man-made structures, where some littoral and upper-sublittoral communities could be found, are present (Blanfuné et al., unpublished). Thus, 19 (or 25 if harbours and marinas are considered) out of 28 coastal water bodies could be assessed in Andalusia, comprising most of the areas of the European coasts of the Alboran Sea.

However, due to special environmental and ecological conditions of the Alboran Sea, some methodological modifications are needed to apply the index. The tidal range, the swell, the structural complexity of intertidal, and the low development of intertidal assemblages hinder the sampling using a boat in the western Alboran. Thus, samplings were carried out on foot and snorkelling. Furthermore, the rocky shores of western Alboran Sea show a naturally lower structural complexity of their littoral and upper-sublittoral (20-30 centimetres) macroalgal stands, which often lack extensive *Cystoseira* spp. assemblages or *Posidonia* meadows (Table 2). To assess accurately this region it was necessary to increase the sensibility of CARLIT. This was addressed by increasing the spatial resolution of the index (20 m instead 50 m as in Ballesteros et al, 2007), and the depth surveyed (2 m instead of the 20-30 cm considered in Northwestern Mediterranean). In this way, additional sublittoral communities were monitored making the index more sensitive due to the increase in data availability, which might be useful for the management of sublittoral threatened organisms (e.g. *P. oceanica*, *A. calycularis*, *Cystoseira* spp.). However, this procedure implies higher costs in sampling effort and time consuming, which may reduce spatial scale by the survey of random representative subsectors of coast for each water body instead of covering the whole coastline (Ballesteros et al., 2007). These modifications can also be useful to apply the index in other places where the tidal range is higher than in the Mediterranean Sea. In this sense, the CARLIT index was successfully applied in two Atlantic water bodies (up to 1.8 metres of maximum tidal range; water bodies 1 and 2, Table 8). On the other hand, when reference conditions in eastern Alboran, which is more similar to the Northwestern Mediterranean coast than western Alboran, were compared to references conditions proposed by Ballesteros et al. (2007), higher ES values were recorded for the water bodies in the Alboran Sea (Table 4). This fact could be explained by the methodological modifications stated above. In our study, smaller stands were considered as meadows (20 metres) in comparison to those considered by Ballesteros et al. (2007) (50 meters), which could produce an EQ overestimation (Mangialajo, com. pers.). Moreover, the greater water depth considered led to the inclusion of seagrass meadows more frequently in the data set, therefore increasing EQ.

*How many regions must be considered to define the reference conditions in Alboran Sea?*

In this study two ecological or biogeographical regions were found: western and eastern Alboran (Fig. 2; Table 2). The limit was placed between the localities 20 (Cala Rijana) and 25 (Guardias Viejas). In this sense, Álvarez-Cobelas et al. (1989), based on marine floristic composition, also identified an edge close to these localities (see Fig. 1). Similar results were found based on population genetic for very different organism like oysters (Saavedra et al., 1993), mussels (Quesada et al., 1995), seagrasses (Alberto et al., 2008) or fishes (Bargelloni et al., 2003) between Mediterranean and Atlantic populations. This limit seems to be related to the Almeria-Oran oceanographic front (Tintore et al., 1988), which acts as hydrogeographical barrier between Atlantic and Mediterranean.

In this case, the boundary localities chosen were Cala Rijana (20) and Castel de Ferro (21) because of the biggest change in landscape were observed between these localities. Temperature and tidal range seem to be key factors to explain the differences in distribution and development of the most littoral and upper-sublittoral communities along the Alboran Sea (Table 6; Fig. 2). The main differences in landscape between these regions were due to the species *P. oceanica*, *C. ericaefolia*, *Mytilus* and, to a lesser extent, *Corallina* spp. Considering that oligotrophic conditions favoured the development of *P. oceanica* and *C. ericaefolia* assemblages (Arévalo et al., 2007; Giaccone et al., 1993; Giaccone et al., 1994a,b; Pinedo et al., 2007), and the oppose behaviour is expected for *Mytilus* beds (Arévalo et al., 2007; Ballesteros et al., 2007; Pinedo et al., 2007). The higher abundance of *Mytilus* and the lower development *C. ericaefolia* and *P. oceanica* assemblages besides the lower mean seawater temperature of western Alboran must be related to deep-water upwellings, which are common in this area (Rodríguez, 1990). It is remarkable that the presence of these upwellings was also proposed to explain the dominance of big suspension feeders in deep waters of “La Herradura” (Fig. 1, site 17; Cebrián and Ballesteros, 2004). All these facts reflect the importance of the upwellings in Alboran Sea determining the spatial patterns of distribution and abundance of these littoral and sublittoral habitat-forming species, which play a fundamental role in the ecosystem determining different biotic interaction and abiotic environmental conditions.

Nevertheless, others hydrodynamic factors seem to be also important for the development of littoral and upper sublittoral communities. The differences between reference conditions defined for the western and eastern Alboran Sea (Fig. 3, 4, 5 and 6; Table 4) suggest different hydrodynamic conditions. In this way, the Eastern region yielded higher EQ values for reference conditions than the Western one for HC, metric and decimetric blocks (MB and DB) in wave exposed conditions. This fact was related to the stronger hydrodynamic conditions (waves and tides) in the western Alboran. For example, the high waves that produce the movement of blocks and make the settlement harder for littoral organisms (e.g. Juanes et al. 2008) are more frequent in western Alboran than in the Eastern region. Furthermore, the higher development of communities dominated by *C. ericaefolia* is also probably favoured to the lower tidal range in the Eastern region (e.g. Mangialajo et al., 2012). Therefore, these facts point out to the existence of a natural gradient along Alboran Sea and revealing the importance of considering these two regions when CARLIT is applied.

*Is CARLIT index sensitive enough to anthropogenic pressures?*

The results obtained in this study suggest that CARLIT is a suitable indicator to assess the ES of Mediterranean coastal waters in the European coasts on the Alboran Sea. The lack of correlation of EQR values (Table 6) with geomorphological and biogeographical components (C3 and C2), and the significant relationship with anthropogenic pressures (C1 and C4) indicated that the index avoided the effects of natural pressures and it is sensitive to anthropogenic ones. Nevertheless, the heterogeneous distribution of human activities between eastern and western Alboran and the integrated response of littoral and sublittoral rocky shore communities to pressures make difficult the assessment of the sensitivity of CARLIT to anthropogenic pressures separately. For these reasons, the LUSI index and the approach by López y Royo et al. (2009) were more reliable for this purpose. Furthermore, these methodologies have been previously used to assess the response of different ecological indices (e.g. Bermejo et al., 2012; López y Royo et al., 2011; MED-GIG, 2011).

The high correlation between EQRs and LUSI and the significant differences in EQR (Table 7) for the different pressure levels assessed according to López y Royo et al. (2009), support the idea that CARLIT was sensitive to anthropogenic pressures in the European coasts of the Alboran Sea. Moreover, the EQR and the ecological status obtained with CARLIT in the Strait of Gibraltar (localities 1-9) were similar to the results obtained by Bermejo et al. (2012) using the RSL index for macroalgae (Wells et al., 2007) although CARLIT usually yielded slightly higher EQR values (Table 9) and some disagreements were identified for lower ecological status. These results are in accordance with those obtained in the intercalibration process for the North Atlantic Geographical Intercalibration Group (NEA-GIG), where a modification of the RSL boundaries suggested by Bermejo et al. (2012) was proposed to avoid an underestimation in the final EQR value (NEA-GIG, 2011). In any case, further analyses and intercalibration exercises should be carried out to confirm this issue. On the other hand, the correlation between EQRs and LUSI was lower in the Eastern region than in the Western one. This could be explained by: i) differences in the resilence of communities (e.g. Viaroli et al., 2008); ii) differences in the effect and the intensity of agriculture and urban pressures; iii) differences in the gradient of human pressures considered in Eastern and Western regions; iv) physico-chemical conditions (e.g. local strong currents can disperse the pollutants faster, thus maintaining low levels of contaminants); and v) recent historical events of community degradation that produce indefinite or very long term (relative to human timescales) changes in marine ecosystems (e.g. Knowlton, 2004).

**Table 9-** EQR and ecological status values obtained for RSL (Bermejo et al., 2012) and CARLIT indices in six localities in the Strait of Gibraltar subregion.

| Locality | RSL | CARLIT |
|---|---|---|
| 1 | 0.86 – High | 0.94 – High |
| 2 | 0.83 – High | 0.93 – High |
| 3 | 0.77 – High | 0.87 – High |
| 4 | 0.78 – High | 0.89 – High |
| 5 | 0.67 – Good | 0.85 – High |
| 6 | 0.57 – Moderate | 0.74 – Good |
| 7 | 0.40 – Moderate | 0.49 – Moderate |
| 8 | 0.08 – Bad | 0.41 – Moderate |
| 9 | 0.52 – Moderate | 0.37 – Poor |

Overall, European coastal waters of the Alboran Sea evidenced good or high ES (Fig. 7; Table 8). Exceptionally, three water bodies in Algeciras Bay showed moderate or poor ecological status. This could be explained for natural characteristics of these water bodies, anthropogenic pressures and methodological causes: i) natural causes would be related to the geomorphology of the area, which have low hydrodynamic and high water renewal time, and thus is more susceptible to water pollution processes. In addition, two rivers flow into this Bay, affecting the water quality; ii) The bay is severely industrialised (Díaz-de Alba et al., 2011; Morales-Caselles et al., 2007; Morillo et al., 2007; Sánchez de la Campa et al., 2011), so that water bodies were classified as modified or highly modified; iii) The CARLIT index allows to localise small sewage outfalls and other environmental pressures at a reduced scale, which is extremely important in the establishment of accurate management plans (Ballesteros et al., 2007). However, when the ES was estimated for a particular water body, the EQR values can be dependent on the spatial scale. For instance, the presence of a degraded stretch of coast in a small water body will have noteworthy consequences on the final scores. In contrast, the same degraded stretch of coast in a greater water body could be unnoticed (Table 8, 35 – Villaricos, and 15 – Torrox).

*Beyond the WFD*

The application of CARLIT will aid in the understanding of the composition, structure and functioning of the coastal ecosystem, making feasible the monitoring of ecological changes in the long term (Wells et al., 2007). This data set can be a baseline for measuring the response of the distribution of considered species and communities to global change (Boaventura et al., 2002). This fact is particularly important considering: i) the special condition of Alboran Sea as the limit between the Atlantic Ocean and the Mediterranean Sea (Alberto et al., 2008; Conde, 1989); ii) that habitat destruction or degradation as the most important threat to the diversity, structure, and functioning of marine coastal ecosystems and the goods and services they provide in the Mediterranean Sea (Claudet and Fraschetti, 2010; Coll et al., 2010; Lotze et al., 2006); iii) that some of the species recorded are protected by specific national or international legislation (e.g. Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean).

In conclusion, although temperature and tidal range were the most important factors to explain the distribution of littoral and upper-sublittoral communities along the Alboran Sea, the CARLIT index was sensitive to anthropogenic pressures. In practise, two regions should be considered to accurately assess the ecological status of the European coastal waters of the Alboran Sea, encompassing the natural variations that occur over the coast. However, although the CARLIT index was sensible to anthropogenic pressures, the EQR values and the final ecological status can be dependent on the length of rocky shore in the water body assessed.

### *Author Contributions*

Conceived and designed the experiments: RBL, GdF, IH, JJV. Analyzed the data: RBL, GdF. Wrote the manuscript: RBL, JJV, IH.

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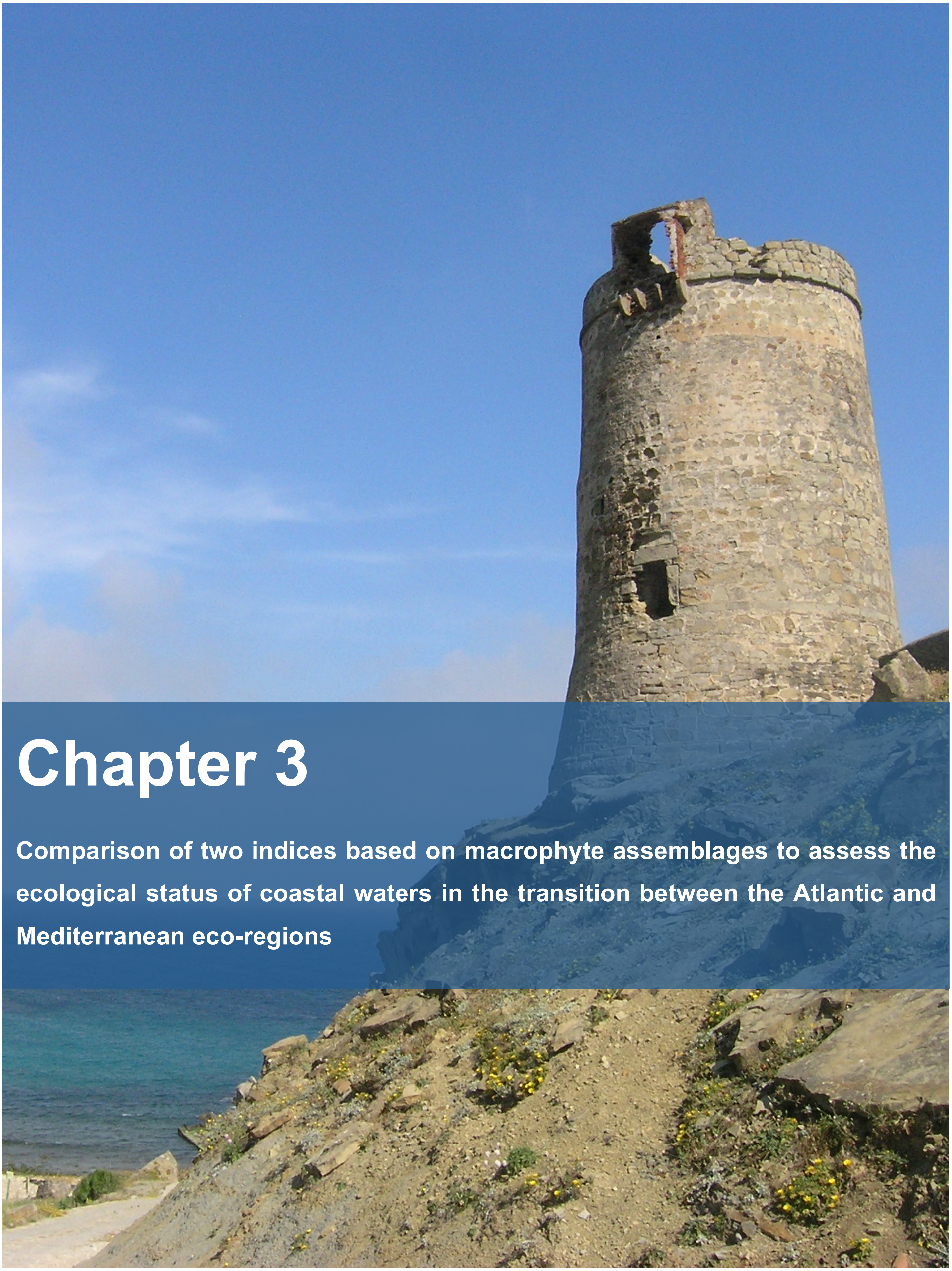

# Chapter 3

## Comparison of two indices based on macrophyte assemblages to assess the ecological status of coastal waters in the transition between the Atlantic and Mediterranean eco-regions

*"Y mientras tanto,*
*no hay nada más exacto que la vida*
*y sin embargo no la comprendemos."*

Miguel Sánchez Robles
(El Tiempo y la Sustancia, 2000)

Torre del Guadalmesí. Tarifa, Strait of Gibraltar. Junio 2011. Photograph by Antonio Bermejo Lacida

# Comparison of two indices based on macrophyte assemblages to assess the ecological status of coastal waters in the transition between the Atlantic and Mediterranean eco-regions.

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## ABSTRACT

Several indices based on the composition and abundance of aquatic flora have been developed to assess the ecological status of coastal waters along the European coasts in the context of the Water Framework Directive. This Directive pointed out the intercalibration of indices based on the same biological element within an eco-region to ensure the consistency and comparability among them. For a better management of coastal areas in the transition zone between two eco-regions, the comparison of indices developed for each eco-region may also be necessary. The aim of this work was to compare two indices based on macrophytes that have been proposed for two different and adjoining eco-regions: the RSL (Reduced Species List) in the Atlantic eco-region and the CARLIT (Cartography of littoral and upper sublittoral communities) in the Mediterranean. These indices were applied in 14 sites in the transition between the Atlantic Ocean and the Mediterranean Sea, where a wide range of anthropogenic pressures can be found, from high (Algeciras Bay) to almost negligible ("El Estrecho" Natural Park). Overall, both indices were sensitive to anthropogenic pressures and suitable to assess the ecological status. The comparison between indices suggested a bias in the assessment of the ecological status between good and high classes due to a different definition of high ecological status class between RSL and CARLIT. In addition, the most important disagreements between indices were found in the most degraded localities. The results showed, however, a high comparability between RSL and CARLIT despite their marked conceptual and methodological differences.

Keywords: Macroalgae; Atlantic Ocean; Mediterranean Sea; Water Framework Directive; RSL; CARLIT.

## INTRODUCTION

The European Water Framework Directive (WFD 2000/60/EC) requires the use of biological elements to assess the ecological status (ES) of a particular water body with the goal of maintaining and improving aquatic environments, avoiding further degradation. In the case of coastal water bodies (WBs), macroalgae have been one of the four biological quality elements (BQEs) proposed.

Macroalgal communities are sensitive to an array of anthropogenic pressures (e.g. Borowitzka, 1972; Gorostiaga and Díez, 1996; Díez et al., 1999), but they may be confounded by variability caused by natural environmental factors (Crowe et al., 2000), which may be major drivers of community structure (Moss, 2007). To avoid the influence of this natural variability in the ecological assessment, the WFD divided the European waters in relevant eco-regions (Mediterranean, Baltic, Black Sea and Atlantic) and coastal water bodies were classified according to environmental characteristics to define different types (IES, 2009). In spite of this practical classification, all the particularities of an entire eco-region cannot be taken into account (e.g. Guinda et al., 2008) and it is not possible to develop a single indicator, even based on the same BQE, to assess the ES of all coastal waters within the same eco-region. Furthermore, there is also the age-old problem of those carrying out the monitoring, which are often unwilling to change from their usual practices (Hering et al., 2010). As a result, several indices have been developed for each ecoregion for the same BQE in the framework of the WFD.

Although Member States are allowed to use their own national classification systems, adequate comparability and consistency has been sought through the process of intercalibration, undertaken by the different Member States within an eco-region (European Commission, 2000 – Annex V). In consequence, intercalibration between indices developed for different eco-regions is not necessary under the WFD. However, in border areas, the comparison between indices developed for different eco-regions can be useful for local management of coastal waters and for the identification of possible biases in the ES assessment. This becomes particularly important considering legal implications when a good ES is not reached for a water body (European Commission, 2000).

Due to the geographical position of the Strait of Gibraltar, on the boundary between the Atlantic and the Mediterranean eco-regions, up to seven indices based on the BQE macroalgae could be applied: five developed for the North East Atlantic ecoregion: CFR (Guinda et al., 2008; Juanes et al., 2008;), RSL (Wells et al., 2007), CCO (NEA GIG, 2011), RICQI (Díez et al., 2012), MarMAT (Neto et al., 2012); and two in the Mediterranean: EEIc (Orfanidis et al., 2001; Orfanidis et al., 2011) and CARLIT (Ballesteros et al., 2007). This diversity of indices based on macroalgal assemblages reflects the suitability of this BQE as bioindicator and the importance of biogeographical differences in the assessment of the ES. In Mediterranean coasts of Southern Spain, the CARLIT methodology was considered the most suitable (Bermejo et al., 2013). The index follows a non-destructive methodology and it generates useful cartography of littoral and upper-sublittoral communities (Ballesteros et al., 2007). In Atlantic coasts of Southern Spain, the RSL was the methodology chosen because it produces an appropriate assessment of the ecological status based on the macrophyte community features of this region (Bermejo et al., 2012).

The present work was intended to perform a direct comparison between a Mediterranean (CARLIT) and an Atlantic (RSL) index in the transitional area between these two ecoregions. The goals of this study were: i) to check the sensitivity of both indices, RSL and CARLIT, to anthropogenic pressures assessed according to Mediterranean and North-East Atlantic

Geographical Intercalibration Groups (Med GIG and NEA GIG) and ii) to evaluate the comparability between these indices in the transition zone, in order to ensure the consistency of the ecological assessment in the Mediterranean and Atlantic coast of southern Spain.

## MATERIAL AND METHODS

### *Study area*

Samplings were conducted in the Strait of Gibraltar (Fig. 1), the boundary zone between the Atlantic and Mediterranean eco-regions, from May to July 2011 (excepting Algeciras harbour -2010-, and Torreguadiario -2012-), coinciding with the seasonal peak of growth in littoral macroalgal communities (González, 1994). The study was carried out in 14 sites covering a wide range of anthropogenic disturbances (Fig. 1): El Estrecho (Strait of Gibraltar) Natural Park (1-8), Algeciras Bay (9-11) and Western Alboran (12-14).

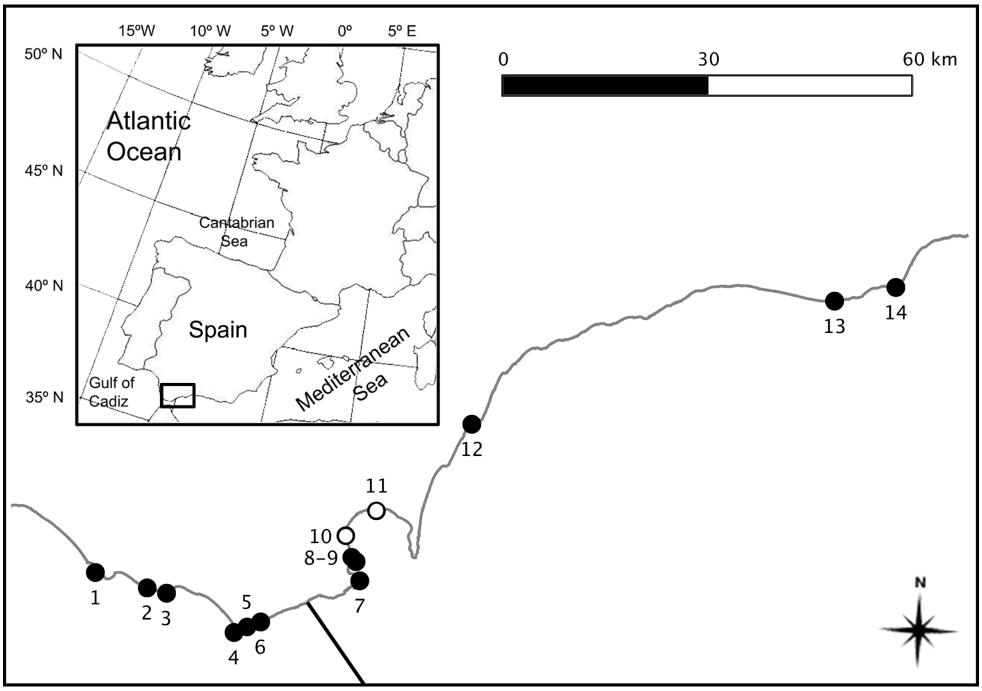


**Fig. 1.-** Geographical distribution of the different sampling points along the European Coast of the Strait of Gibraltar and Western Alboran Sea. The geographical border proposed by the WFD between Atlantic and Mediterranean ecoregions is indicated with the black line. **1-** Camarinal; **2-** Piscinas de Baelo; **3-** Punta Paloma; **4-** Tarifa island; **5-** Tarifa harbour; **6-** Punta Camorro; **7-** Punta Carnero; **8-** Punta San García; **9-** Algeciras Bay; **10-** Algeciras harbour; **11-** Guadarranque river mouth; **12-** Torreguadiaro; **13-** Cala de Mijas; **14-** Calaburras. Open dots- sites in highly modified water bodies; Black dots- sites in natural water bodies.

The Strait of Gibraltar Natural Park is the sector where the marine and coastal environment is less influenced by anthropogenic pressures. Only point source anthropogenic pressures associated with towns (i.e., Tarifa) and residential areas (sites 4-8), as well as diffuse pressures related to marine traffic and industrial activities from Algeciras Bay (sites 7 and 8) are found. In contrast, Algeciras Bay supports a high population density and it is heavily industrialised (Morales-Caselles et al., 2007; Morillo et al., 2007; Díaz-de Alba et al., 2011), being the most disturbed sector studied. Furthermore, the geomorphology of this sector determines a lower hydrodynamic exchange and favours higher water renewal times in comparison with more exposed zones, making the sector more susceptible to water pollution. Finally, anthropogenic pressures are lower in western Alboran than in Algeciras Bay. The human disturbances are associated with the urbanization of the coast. Despite this anthropogenic impact that may affect littoral and upper sublittoral communities, it is remarkable the presence of a Site of Community Importance (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora) due to the biogeographical importance of Calahonda (13) and Calaburras (14), which have been proposed as the limit between the Atlantic and the Mediterranean for the northern coast of Alboran Sea (Conde, 1989; González, 1994; García-Raso et al., 2010).

Although each sector has their own particularities due to the transitional character of this region, the general intertidal zonation pattern of benthic communities along the studied area can be divided into three main fringes: i) the upper littoral, which is dominated by the small barnacle *Chthamalus stellatus*, small caespitose red seaweeds (i.e. *Gelidium pusillum*, *Chondracanthus acicularis*, *Osmundea hybrida*), *Fucus spiralis*, and *Ulva* spp.; ii) the low littoral dominated by *Corallina* spp. and *Mytilus* spp.; and iii) the upper sublittoral characterized by *Cystoseira* spp., *Asparagopsis* spp., *Halopithys incurva*, *Sphaerococcus coronopifolius*, or *Halopteris scoparia* among others (Seoane-Camba, 1965; Conde, 1984; Pérez-Llorens et al., 2012).

*CARLIT and RSL indices*

In each site, two different methodological approaches to assess the ES of coastal waters based on macroalgal rocky-shore communities were applied and compared: the CARLIT (Ballesteros et al., 2007; Bermejo et al., 2013) and the RSL indices (Wells et al., 2007; Bermejo et al., 2012).

The RSL index was developed by Wells et al. (2007) for the coasts of the British Isles. This is a multimetric index based on species occurrence from a reduced species list. In this case, the index was applied considering the modifications proposed by Bermejo et al. (2012) for the Atlantic coast of southern Spain. The main modifications include the use of formal transects of 50-60 m instead undefined stretches of coast, the adaptation of the reduced species list (including two species of seagrasses: *Cymodocea nodosa* and *Posidonia oceanica*) and the scoring criteria for each of the five elements that compose this index (Corrected species richness, number of red algae, proportion of green algae; proportion of late successional species -ESG I-; and proportion of opportunist species) for the studied area. However, the ES classification of the different localities was fulfilled according to the further modifications posed by the NEA GIG (2011) resulting from the intercalibration process (Table 1) (European Commission, 2013).

**Table 1-** Boundaries for the classification of Ecological Status in the RSL (after NEA GIG, 2011) and CARLIT (Ballesteros et al., 2007) indices. ESC: Ecological Status Class. EQR: Ecological Quality Ratio.

| ESC | EQR RSL | EQR CARLIT |
|---|---|---|
| High | 1.00 - 0.75 | 1.00 - 0.75 |
| Good | 0.75 - 0.48 | 0.75 - 0.60 |
| Moderate | 0.48 - 0.40 | 0.60 - 0.40 |
| Poor | 0.40 - 0.20 | 0.40 - 0.25 |
| Bad | 0.20 – 0.00 | 0.25 – 0.00 |

The CARLIT index was developed by Ballesteros et al. (2007) for the NW Mediterranean Sea. This methodology is a unimetric index that estimates the ES from the cartography of the commonest littoral and upper-sublittoral communities along the entire rocky shores of a Water Body, which is divided in different sectors, each one characterized by a community category or combination of communities with a sensitivity level (SL). For this study, the CARLIT index was applied according to the adaptation proposed by Bermejo et al. (2013) for this area, which included new community categories and reference conditions. Moreover, this adaptation included some important methodological modifications from Ballesteros et al. (2007) such as: i) samplings were carried out by walking and snorkelling instead on boat; ii) the spatial resolution was increased (20 m instead 50 m); and, iii) the depth considered in our study was increased up to 2 m instead of 20-30 cm. Furthermore, as it was not possible to survey the whole rocky coastline, data were collected in continuous sampling units between 0.5 and 1.5 km, except for Algeciras harbour, where the length of natural rocky shore was only 180 m. The boundaries used for ES classification of the different localities are reported in Table 1 (Ballesteros et al., 2007; Bermejo et al., 2013).

*Anthropogenic pressures assessment*

Anthropogenic pressures were estimated in each site using the methodologies proposed by the NEA GIG and Med GIG. For the Atlantic eco-region, the NEA GIG (2011) proposed an anthropogenic pressures assessment system (NEA-PA). This methodology is based on a semi-quantitative assessment of three types of pressures (urban, industrial and diffuse discharges) considering the distance to punctual sources of pollution and the intensity of these pressures (e.g., population equivalent in the case of discharges). A total of 4 or 5 levels were defined (0 – no pressure to 4 – very high) for each type of pressure. In this case, the criteria used to define diffuse pressures were clarified based on López y Royo et al. (2009), and further limits for urban and industrial discharged were established (Table 2). The final value of the anthropogenic pressure was the sum of the three types of pressures considered. Theoretically, this index offers a quantitative value between 0 at the best and 11 in the worst situation.

Land Use Simplified Index "LUSI" (Flo et al., 2011) is the methodology used by the Med GIG (2011) to estimate anthropogenic pressures. This scoring system is based on the analysis of aerial images considering urban, agricultural and industrial surface, degree of water confinement in each site and the influence of rivers. The LUSI provides a quantitative estimation of pressures between 0 at the best and 8.75 in the worst situation.

**Table 2-** Scoring system used to assess the pressures related to industrial and urban discharges (extracted from Annex 1 of Lisbon NEA-GIG meeting), and diffuse pressures. High Risk pressures (HRP) were considered chemicals, paper mills and others included in the IPPC (International Plant Protection Convention).

| **Urban and industrial discharges** | | | | | |
|---|---|---|---|---|---|
| | | | *Distance (m)* | | |
| *Population equivalent* | 2500-1500 | 1500-500 | 500-100 | 100-50 | <50 |
| <2000 | 0 | 0 | 0 | 1 | 2 |
| 2000-10000 | 0 | 0 | 1 | 2 | 3 |
| 10000-15000 | 0 | 1 | 2 | 3 | 4 |
| >15000 (or HRP) | 1 | 2 | 3 | 4 | 4 |

| **Diffuse pressures** | | | | | |
|---|---|---|---|---|---|
| | | | *Distance (m)* | | |
| *Pressure* | >5000 | 5000-2500 | 2500-1000 | 1000-500 | <500 |
| River mouths | 0 | 1 | 2 | 3 | 3 |
| Industrial habours | 0 | 1 | 2 | 3 | 3 |
| Commercial harbours | 0 | 0 | 1 | 2 | 3 |
| Marinas | 0 | 0 | 0 | 1 | 2 |
| Land use in one km$^2$ | >90% Natural (0) | >50% Natural or >90% Agriculture (1) | | >50% Urban (2) | >90% Urban (3) |

*Data analyses*

To test the sensitivity of the elements used to calculate the RSL and CARLIT indices, and to identify possible disagreements between the methodologies used to estimate anthropogenic pressures, a Spearman correlation analysis was performed between the EQR values provided by both indices, and the total value of anthropogenic pressures. This analysis was also performed for the absolute values of the elements that compose the RSL and CARLIT indices. In all cases significance was set at 5% probability.

Additionally, the comparability of both indices was assessed, using a Pearson correlation analysis between the EQR values provided by both methodologies and the two approaches proposed in the intercalibration exercise: the boundary bias and the class agreement (European Commision, 2011).

The boundary bias was defined as the deviation in the relative positioning of relevant class boundaries (high-good and good-moderate) measured by the magnitude and direction of deviation by a class boundary of one method relative to the average boundary position derived from all methods participating in the exercise (European Commission, 2011). This value is expressed in class equivalents and it should not exceed 0.25 units.

To calculate the boundary bias, a linear equivalence model was performed based on the EQRs obtained by each method. In this case, the EQR value of the RSL was selected as the common metric or the dependent variable, considering that this index was previously intercalibrated in the NEA GIG for southern Spain (NEA GIG, 2011). Based on this regression, CARLIT boundaries were expressed in the common metric (EQR RSL) and the BIAS between both methodologies was calculated according to equation 1 (Option 2; Birk et al., 2011):

(1) BIAS = (lBPx - lABP) / (CWx)

where lBPx is the lower boundary position for the “x” index in the common metric for the studied class; lABP is the lower average boundary position considering all methods in the common metric; and CWx is the width of the class in which the lABP value would be classified for the studied method "x" (see more details in Birk et al., 2011).

On the other hand, the class agreement was performed using the absolute average class difference (AACD, European Commission, 2011). The AACD was calculated by averaging scores, considering the difference in class obtained and the number of classes between evaluations, i.e. a value of 0 is assigned to sites evaluated in the same class by both classification systems, 1 to sites with one class difference between classification systems, 2 to sites with two classes differences, and so on. The criterion proposed for sufficient comparability between classification systems is less than a half class difference (0.5) considering only upper class boundaries (high-good and good-moderate) according to the general principles of comparability analysis (European Commission, 2011).

## RESULTS

The values for LUSI and NEA-PA showed a high degree of correlation in the studied area ($\rho = 0.939$; $p < 0.001$). This suggests that anthropogenic pressures were estimated similarly in both ecoregions despite the differences between those methodologies. In general terms, RSL and CARLIT were sensitive to anthropogenic pressures, obtaining worse ecological quality values at the sites located in Algeciras Bay, where the marine and coastal environment is more disturbed by human activities, and the highest ecological quality values in the El Estrecho Natural Park, where the human pressures are lowest (Table 3; Fig. 2 a, b). Despite the marked differences between the two methodologies the obtained results were quite similar in the qualitative assessment of the ES. Nevertheless, it is important to notice that the EQR values yielded by CARLIT were overall higher than the EQR values yielded by RSL.

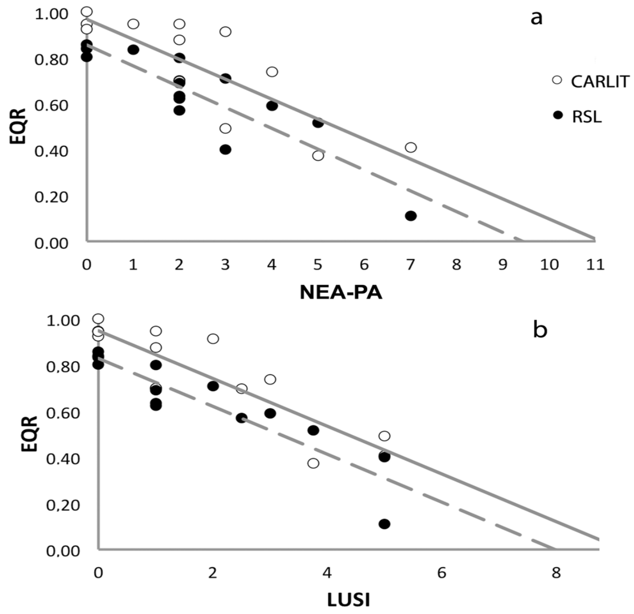


**Fig. 2 –** Relationship between the EQR results obtained by the RSL and CARLIT indices, and the anthropogenic pressures values assessed according to NEA GIG (NEA-PA) (A) and Med GIG (LUSI) (B).

**Table 3-** Scores of anthropogenic pressures according to the LUSI and NEA-PA indices, EQR and ES values rendered by RSL and CARLIT. Name of localities as in Fig 1. * - Highly modified water body. Localities with controversial results are shaded.

| Locality | NEA-PA | LUSI | EQR-RSL | EQR-CARLIT | ES-RSL | ES-CARLIT |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0.80 | 0.95 | High | High |
| 2 | 0 | 0 | 0.86 | 1.00 | High | High |
| 3 | 0 | 0 | 0.84 | 0.92 | High | High |
| 4 | 2 | 1 | 0.80 | 0.70 | High | Good |
| 5 | 2 | 1 | 0.62 | 0.70 | Good | Good |
| 6 | 1 | 0 | 0.83 | 0.95 | High | High |
| 7 | 2 | 1 | 0.69 | 0.95 | Good | High |
| 8 | 2 | 2.5 | 0.57 | 0.70 | Good | Good |
| 9* | 3 | 5 | 0.40 | 0.49 | Moderate | Moderate |
| 10* | 7 | 5 | 0.11 | 0.41 | Bad | Moderate |
| 11* | 5 | 3.75 | 0.52 | 0.37 | Good | Poor |
| 12 | 2 | 1 | 0.63 | 0.88 | Good | High |
| 13 | 4 | 3 | 0.61 | 0.74 | Good | Good |
| 14 | 3 | 2 | 0.75 | 0.91 | High | High |

*RSL index*

The values of all elements used to calculate the RSL index showed the worst values in Algeciras Bay whereas the best results were found in El Estrecho Natural Park (Table 4). The correlation analysis between the metrics considered in the RSL index, and anthropogenic pressures indicated that all elements were correlated significantly with anthropogenic pressures, according to the expected trend (Table 5). Proportion of green algae and proportion of opportunists were the elements with the lowest correlation coefficients. It is remarkable that the integration of all information in the final score for the RSL showed the highest value of Spearman coefficient for anthropogenic pressure values.

**Table 4-** Values of the elements used in the RSL index. * - Highly modified water body.

| Locality | Species richness | Corrected Species richness | Number of red algae | Proportion of green algae | Proportion of ESGI | Proportion of opportunists |
|---|---|---|---|---|---|---|
| 1 | 32 | 26 | 17 | 0.125 | 0.469 | 0.031 |
| 2 | 35 | 33 | 19 | 0.171 | 0.514 | 0.057 |
| 3 | 39 | 32 | 20 | 0.179 | 0.513 | 0.077 |
| 4 | 40 | 32 | 22 | 0.225 | 0.400 | 0.075 |
| 5 | 28 | 23 | 16 | 0.214 | 0.357 | 0.107 |
| 6 | 35 | 28 | 18 | 0.171 | 0.486 | 0.029 |
| 7 | 32 | 25 | 20 | 0.219 | 0.375 | 0.093 |
| 8 | 25 | 23 | 14 | 0.160 | 0.320 | 0.160 |
| 9* | 23 | 22 | 12 | 0.348 | 0.174 | 0.130 |
| 10* | 7 | 7 | 2 | 0.571 | 0.000 | 0.429 |
| 11* | 19 | 18 | 13 | 0.263 | 0.316 | 0.105 |
| 12 | 21 | 23 | 13 | 0.190 | 0.381 | 0.095 |
| 13 | 25 | 24 | 10 | 0.200 | 0.440 | 0.087 |
| 14 | 30 | 28 | 14 | 0.170 | 0.430 | 0.071 |

**Table 5-** Values of ρ coefficients for the Spearman correlation matrix between the elements of the RSL index and values of anthropogenic pressures according to LUSI and NEA-PA. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

| ***Elements*** | **LUSI** | **NEA-PA** |
|---|---|---|
| Corrected species richness | -0.792*** | -0.728** |
| Number of red algae | -0.787*** | -0.761** |
| Proportion of green algae | 0.615* | 0.645* |
| Proportion of ESG I | -0.837*** | -0.746** |
| Proportion of opportunist | 0.770** | 0.626* |
| EQR RSL | -0.890*** | -0.803*** |
| ***Gross elements*** | | |
| Species richness | -0.799*** | -0.764** |
| Number of green algae | -0.122 | -0.258 |
| Number of ESG I | -0.901*** | -0.860*** |
| Number of ESGII | -0.378 | -0.452 |
| Number of opportunist | 0.362 | 0.211 |

In the case of proportion of green seaweeds, the positive and significant correlation with anthropogenic pressures was not due to an increase in the number of green seaweeds in disturbed sites, as there was a lack of correlation of number of green seaweeds with anthropogenic pressures (Table 5). In the same manner, the proportion of opportunist species showed a direct correlation with the pressure level that was not related to an increase in the number of opportunist species in degraded sites. In contrast, the negative correlation between the proportion of ESG I and human disturbances was associated with a decrease in the number of late successional or perennial species, as showed by the significant and negative correlation between the number of ESG I and anthropogenic pressures. The number of opportunist and pollution tolerant species (ESG II; see Orfanidis et al. (2001) and Arévalo et al. (2007)) did not show a significant correlation with human pressures (ρ = -0.378 for LUSI; ρ = -0.452 for NEA-PA; $p > 0.05$), but showed a negative trend, decreasing when pressures were high. On the other hand, the corrected species richness and the species richness yielded similar results as the correction factor is based on the physical complexity of the intertidal, and this complexity was very similar among the sampled localities (data not shown).

*CARLIT index*

The proportion of coast covered by the main community categories found in the 14 sampling localities is presented in Table 6. Belts of mussels (*Mytilus* spp.) were scarce or absent in El Estrecho Natural Park. In this sector the macroalgae *Lithophyllum byssoides* and *Fucus spiralis* were important elements in upper littoral zone. *Cystoseira tamariscifolia* was also present, but this species did not form continuous belts. In contrast, in Algeciras Bay, the intertidal landscape was dominated by *Mytilus* spp. and *Corallina* spp., being sensitive species such as *Cystoseira* spp., seagrasses and *Lithophyllum byssoides* absent. Finally, in western Alboran there was a remarkable presence of stands of the seagrasses *Posidonia oceanica* and *Cymodocea nodosa* and a major development and density of *Cystoseira tamariscifolia* assemblages. In this sector, beds of *Mytilus* were also an important component of littoral communities.

**Table 6-** Proportion of coast dominated or co-dominated by the main community categories described in Bermejo et al. (2013) for the Alboran Sea.

| Locality | Ac | My | Lb | Li | Co | Ul | Cc | Cf | Ch | Cm | Ct 1-2 | Ct 3 | Ct 4-5 | Fs | Po 1 | Po 3 | Cn 1 | Cn 3- |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 0.00 | 0.00 | 0.80 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.52 | 0.28 | 0.12 | 0.88 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 3 | 0.00 | 0.00 | 0.45 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.21 | 0.75 | 0.25 | 0.75 | 0.00 | 0.45 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4 | 0.09 | 0.00 | 0.43 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| 5 | 0.09 | 0.00 | 0.43 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| 6 | 0.00 | 0.00 | 0.73 | 0.00 | 1.00 | 0.00 | 0.07 | 0.00 | 0.28 | 0.15 | 0.13 | 0.10 | 0.00 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 |
| 7 | 0.00 | 0.32 | 0.90 | 0.00 | 0.90 | 0.00 | 0.12 | 0.00 | 0.00 | 0.19 | 0.12 | 0.11 | 0.00 | 0.47 | 0.00 | 0.00 | 0.00 | 0.00 |
| 8 | 0.00 | 0.06 | 0.38 | 0.24 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 | 0.32 | 0.00 | 0.00 | 0.32 | 0.00 | 0.00 | 0.00 | 0.00 |
| 9 | 0.00 | 0.27 | 0.00 | 0.44 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11 | 0.00 | 1.00 | 0.00 | 0.76 | 1.00 | 0.86 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12 | 0.00 | 1.00 | 0.00 | 0.80 | 1.00 | 0.29 | 0.00 | 0.10 | 0.00 | 0.00 | 0.07 | 0.93 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 13 | 0.00 | 0.25 | 0.00 | 0.00 | 0.72 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.34 | 0.27 | 0.05 | 0.00 | 0.11 | 0.52 | 0.00 | 0.47 |
| 14 | 0.00 | 0.12 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08 | 0.52 | 0.08 | 0.12 | 0.00 | 0.52 | 0.05 | 0.00 |

Ac - dense populations of *Astroides calycularis*, My - belts of *Mytilus* spp., Lb - build-ups of *Lithophyllum byssoides*, Li - belts of *L. incrustants*, Co - belts of *Corallina* spp., Ul - upper sublittoral belts of *Ulva* spp., Cc - populations of *Cystoseira compressa*, Cf - populations of *C. foeniculacea,* Ch - populations of *C.humilis,* Cm - populations of *C. mauritanica*, Ct 1-2 - scattered individuals of *C. tamariscifolia*, Ct 3 - stands of *C. tamariscifolia*, Ct 4-5 - continuous belt of *C. tamariscifolia*, Fs - *Fucus spiralis*, Po 1 - scattered stands of *Posidonia oceanica*, Po 3 - dense stands of *P. oceanica*, Cn 1 - scattered stands of *Cymodocea nodosa*, Cn 3- - dense stands of *C. nodosa* on death matte of *P. oceanica*.

The stretches of coast surveyed were classified in seven different SL according to the littoral and upper sublittoral communities found (Table 7). The SL 19 was rare. For this reason, SL 19 and SL 20 were considered together for further Spearman correlation analysis. The minimum sensitivity level reached in this study for a stretch of coast was 6. Belts of mussels or encrusting corallines, without sensitive species, dominated these stretches. The proportion of coast dominated for very sensitive littoral and sublittoral communities (SL 19-20) was major in El Estrecho Natural Park, due to the presence of *Lithophyllum byssoides*, and in to a lesser extent, small patches of *Cystoseira mauritanica* and *C. humilis*. In Western Alboran, the highest sensitivity levels were associated with *Cystoseira tamariscifolia* belts. In contrast, very sensitive communities were absent in Algeciras Bay.

**Table 7-** Proportion of coast classified in a determined sensitivity level according to Bermejo et al. (2013). * - Highly modified water bodies.

| Locality | SL 6 | SL 8 | SL 10 | SL 12 | SL 15 | SL 19 | SL 20 |
|---|---|---|---|---|---|---|---|
| 1 | 0.000 | 0.000 | 0.242 | 0.154 | 0.604 | 0.000 | 0.000 |
| 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.196 | 0.000 | 0.804 |
| 3 | 0.000 | 0.000 | 0.000 | 0.246 | 0.000 | 0.000 | 0.753 |
| 4 | 0.000 | 0.568 | 0.000 | 0.000 | 0.000 | 0.000 | 0.432 |
| 5 | 0.000 | 0.568 | 0.000 | 0.000 | 0.000 | 0.000 | 0.432 |
| 6 | 0.000 | 0.088 | 0.000 | 0.000 | 0.048 | 0.000 | 0.864 |
| 7 | 0.000 | 0.097 | 0.000 | 0.000 | 0.000 | 0.000 | 0.903 |
| 8 | 0.203 | 0.419 | 0.000 | 0.000 | 0.000 | 0.000 | 0.378 |
| 9* | 0.593 | 0.407 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 10* | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 11* | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 0.041 | 0.033 | 0.820 | 0.000 | 0.107 |
| 13 | 0.000 | 0.115 | 0.165 | 0.086 | 0.583 | 0.000 | 0.050 |
| 14 | 0.000 | 0.000 | 0.076 | 0.000 | 0.515 | 0.409 | 0.000 |

The Spearman correlation analysis between the proportion of coast with a determined SL and the values of anthropogenic pressure (Table 8) showed that only the lowest and highest SL were significantly correlated with anthropogenic pressures. On the other hand, the integration of all information in the final EQR score for the CARLIT index showed the highest values of the Spearman coefficients for anthropogenic pressure values.

**Table 8-** Values of ρ coefficients for the Spearman correlation matrix between the proportion of coastline with different sensibility and final EQR CARLIT, and values of anthropogenic pressures according to LUSI and NEA-PA. * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001

| Sensitivity level | LUSI | NEA-PA |
|---|---|---|
| **6** | 0.772** | 0.640* |
| **8** | 0.188 | 0.087 |
| **10** | -0.068 | 0.021 |
| **12** | -0.354 | -0.348 |
| **15** | -0.329 | -0.222 |
| **19-20** | -0.653* | -0.571* |
| **EQR CARLIT** | -0.840*** | -0.782*** |

*Comparability between indices*

The EQR values estimated by the CARLIT and RSL indices showed a significant linear correlation ($r = 0.832$; $p < 0.001$; Fig. 3). However, it is important to notice that the EQRs yielded by CARLIT were overall higher than the EQRs yielded by RSL, being the average difference 0.11 EQR units. Considering this fact and the identical boundary for high-good ES proposed for RSL and CARLIT (Table 1), a bias was evident. To quantify this bias according to equation 1, the equivalence between CARLIT and RSL was calculated from a linear model ($R^2 = 0.69$), being EQR CARLIT values transformed to EQR RSL following equation 2.

(2) EQR RSL = 0.8* EQR CARLIT + 0.032

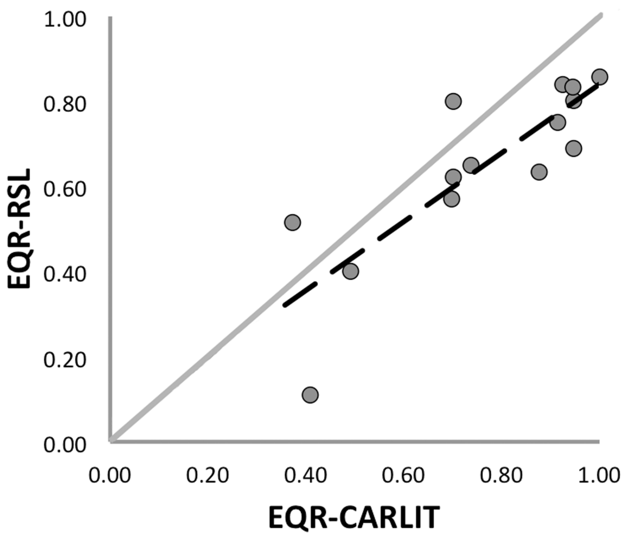


**Fig. 3 –** Linear regression between the EQR results obtained by the CARLIT and RSL indices (dashed line) and expected trend (solid line).

When boundary bias were analysed based on equation 1, it was found that the main disagreement between CARLIT and RSL was found between the high and good boundary classes, as the former index did not fulfil the criterion of comparability of less than 0.25 (bias = -0.276 class equivalent). The rest of comparisons fulfilled this criterion. In this sense, it is remarkable the small bias between good and moderate ES for CARLIT (bias = 0.116) and RSL (bias = -0.069), which reflects the agreement in the definition of the boundary between these ES classes.

The qualitative comparison of the results yielded by the two indices is shown in Table 3. Both indices showed the highest EQR and ES values in El Estrecho Natural Park, and the lowest values in Algeciras Bay. The localities with an ES lower than good were placed on Algeciras Bay (sites 9 and 10 for RSL; and sites 9, 10 and 11 for CARLIT). In this case, five discrepancies in the ES classification were identified in localities 4, 7, 10, 11 and 12. However, according to the general principles of comparability analysis, the discrepancy identified in site

10 was not considered to calculate the AACD. The estimated AACD value was 0.286, which showed that the class agreement between CARLIT and RSL was enough to accomplish with the proposed criterion of less than a half class difference (0.5) suggested in the intercalibration exercise for sufficient comparability.

## DISCUSSION

Different scoring systems to assess anthropogenic pressures have been proposed by Med GIG (LUSI) and by the NEA GIGs (NEA-PA). The use of two different scoring systems is due to both environmental particularities of each ecoregion and to the different approach chosen by each Intercalibration group. However, as both methods aim at quantifying the same anthropogenic pressures, the results are highly correlated, as showed by the high Spearman correlation.

There are marked differences between the RSL an the CARLIT indices, such as the spatial scale considered (dozens of meters vs. hundreds of meters), the biological level studied (species vs. communities), the aggregation strategy of the different elements (multimetric vs. unimetric), and on the definition of reference conditions (see below). Nevertheless, both methods were sensitive to anthropogenic pressures following the methodologies proposed by NEA and Med GIG, and were significantly correlated between them. In addition, RSL and CARLIT yielded comparable results according to the intercalibration guidelines (European commission, 2011), being the boundary bias between good and high ES the only conflictive result (bias > 0.25). Therefore, in general terms: i) both indices can be considered equally sensitive to anthropogenic pressures and they could be used to assess ES in the transitional area between Mediterranean and the Atlantic ecoregions; and ii) they yielded comparable and consistent results in ES classification of the studied localities.

At this point, different questions arise regarding possible explanations for the divergences and comparability between RSL and CARLIT indices.

### *How comparable are RSL and CARLIT indices?*

The results obtained in this work suggested a similar definition of the ES in relation to anthropogenic pressures. These indices have been developed based on the same conceptual framework: anthropogenic pressures can produce a decrease or even the disappearance of the most sensitive species, while the most tolerant taxa will remain (e.g. Borowitzka, 1972; Gorostiaga and Díez, 1996; Díez et al., 1999). In the case of the RSL index, this fact was reflected in the Spearman correlation between the gross elements and anthropogenic pressures, where only species richness and the number of ESG I species showed significant and negative correlations with the anthropogenic pressures. In the case of CARLIT index, the absence of very sensitive species in disturbed stretches of coast and the significant correlation between the proportion of coast occupied by sensitive communities and anthropogenic pressures also supported this idea. Furthermore, considering the methodological differences between RSL and CARLIT, this high correlation also suggested that the effects of significant anthropogenic pressures may be reflected at different biological levels (species (RSL) vs. community (CARLIT) levels) and spatial scales studied (dozens of metres (RSL) vs. hundreds of metres (CARLIT).

Regarding to the criteria followed in the intercalibration process, the boundary bias between high and good ES for CARLIT index was the only conflictive point (bias > 0.25). This bias reflects an important disagreement in the definition of the boundary between these ES classes for RSL and CARLIT. However, considering that the WFD points out that all European surface waters should achieve the objective of a good ecological status by 2015, the existence of a bias between high and good ES is not as important as a bias between moderate and good ecological status because of legal consequences from WFD. In any case, this bias can be attributed to an underestimation of ES by RSL between good and high categories. Previous studies support this idea based on: i) the problems to discriminate between good and high status classes pointed out by Bermejo et al., 2012; ii) the relatively high and positive bias obtained in the intercalibration process for both corrected boundaries (moderate-good bias = 0.215; good-high bias =0.225; NEAGIG, 2011); and iii) the fact that RSL never reached the EQR value of 1 in any reference site (maximum was 0.86).

*Which are the reasons for the divergences between RSL and CARLIT?*

The most important divergences between RSL and CARLIT were found in sites where the ES was good or high, and in places with a clear degradation of the littoral and upper sublittoral communities. In the first case the disagreement between RSL and CARLIT can be attributed to the underestimation of the ES by RSL between good and high classes, as mentioned above. On the other hand, the disagreements in degraded localities must be mainly related to the differences in the spatial scale considered and in the biological level studied for both indices.

The fact that RSL never reached the EQR value of 1, even in undisturbed localities used to define reference conditions, suggest that the problems to discriminate between good and high ES can be attributed to an incorrect definition of the “high” category, which produces an underestimation in the ES. This fact mainly explains the observed bias between good and high ES between RSL and CARLIT. In this sense, the high Spearman correlation between RSL and CARLIT ($\rho = 0.739$; p-value $< 0.01$), excluding degraded localities (lower than good ES; localities 9, 10 and 11) from the analysis, showed that the remaining localities were ranked in a similar way. The similar ranking of sites supports that differences between these indices are mostly explained by the definition of reference conditions and not by differences in the assessment concept. Therefore, the adjustment of the boundary class between good and high ES for RSL can overcome this bias for the studied area.

The major divergences in the ES classification were found in the degraded localities 10 and 11. Moreover, the three most degraded localities according to the EQR values were ordered in different ways by RSL (10 < 9 < 11) and CARLIT (11 < 10 < 9) with respect to the ES (Table 3). These disagreements can be partially attributed to the differences in the spatial scale considered and the biological level studied. In degraded places, where the species richness is usually low and some sensitive or opportunist species would be present with a residual biomass. The anecdotic presence of these species does not produce a large change in indices based on species abundance (i.e. CARLIT), but it could generate important changes in indices based on the taxonomic composition of the community (i.e. RSL) (Guinda et al., 2008; Bermejo et al., 2012). On the other hand, in places with punctual disturbances such as sewages, which could generate gradients in environmental conditions, the displacement of the sampling station some dozens of meters could produces important divergences in the final result in indices with a reduced sampling area (i.e. RSL) (e.g. Mascaró et al., 2013).

*Which are the pros and cons of the different approaches?*

The results suggested that both indices were sensitive to anthropogenic pressures and yielded comparable results. Thus, both indices can be considered equally adequate for the assessment of the ES in the context of the WFD in the transition zone between the Mediterranean and the Atlantic ecoregions. There are however strengths and drawbacks regarding the use of any of the two indices.

On one hand, the RSL considers a relatively small sampling size reducing the time and effort of the fieldwork, which is important in places where the tidal period determines the available sampling time. By these reasons, the index may be easier to apply, quicker and also cheaper, which can very important when large coastlines must be surveyed (López y Royo et al., 2011). This methodology could be useful to assess the ecological status and to monitor the impact of punctual sources of pollution. However, the representativeness of this approach is limited considering the size of WBs, the high spatial variability of analysed benthic communities (e.g. Underwood and Chapman, 1996; Benedetti-Cecchi et al., 2001), and the existence of gradients in punctual anthropogenic disturbances.

On the other hand, the spatial scale considered in CARLIT index reduces the uncertainty in the ES assessment (e.g. Mascaró et al., 2013). The simultaneous use of flora and fauna (Underwood, 1996; Díez et al., 2012), and the consideration of the qualitative abundance of habitat forming species (Guinda et al., 2008; O´Connor, 2013) give an idea about the structure and functioning of the community, providing better evidences of possible changes. In this sense, CARLIT will provide cartographic information to assess the evolution of marine coastal communities, which can be especially useful for conservation programmes of threatened and/or protected organisms such as seagrasses or *Cystoseira* species. Moreover, considering the purposes of the WFD, and that habitat destruction is possibly the most important threat to biodiversity (Pimm et al., 1995; Novacek and Cleland, 2001) occurring at extended spatial scales (Claudet and Fraschetti 2010), the cartography of the whole (or important part of) coastline maybe more accurate to assess the ecological status and to monitor coastal waters.

However, the application of CARLIT index can be an important challenge in coasts with wide intertidal ranges due to the limited sampling time available. Moreover, considering the necessary effort and time of the fieldwork, this methodology requires more man effort than RSL.

In conclusion, both indices were equally sensitive to anthropogenic pressures and they could be used to assess ES in the Mediterranean and the Atlantic waters of the Strait of Gibraltar, which may render an integrated assessment of the ES of the transition zone between the Atlantic and the Mediterranean. Moreover, despite the discrepancies found, the results obtained in this work pointed out that it is possible to reach comparable ecological assessment using indices that are substantially different, as RSL and CARLIT are, which can be very useful for the management of these coastal waters. Similar results were found by Lopez y Royo et al. (2011) between indices developed for *Posidonia oceanica* for the assessment of the ES in the Mediterranean. On the other hand, the most important disagreements between the two indices were found in disturbed places. The methodological differences in the spatial scale considered and the biological level studied must be important factors to explain these divergences for the ES.

The experimental intercalibration in border areas is a useful tool to ensure the consistency and comparability of indices, providing a common basis for the assessment and interpretation of ES and water quality (López y Royo et al., 2011). In this sense, the possibility to apply the RSL and CARLIT indices in the transition zone between the Mediterranean and Atlantic eco-regions leaves open the possibility of an experimental comparison between the Atlantic and Mediterranean eco-regions at a bigger scale.

### *Author Contributions*

Conceived and designed the experiments: RBL, LM, IH, JJV. Analyzed the data: RBL Wrote the manuscript: RBL, JJV, IH, LM.

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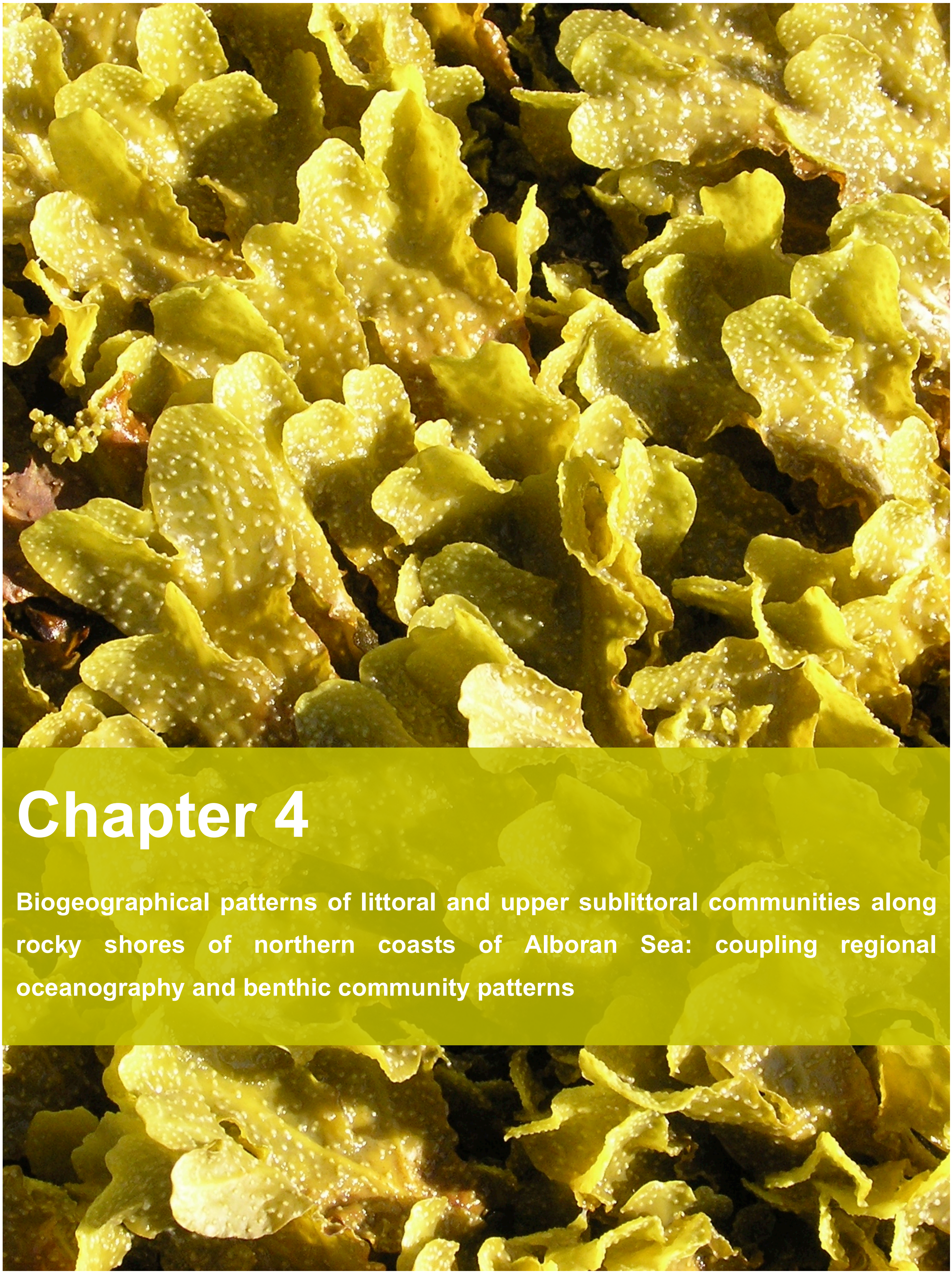

# Chapter 4

## Biogeographical patterns of littoral and upper sublittoral communities along rocky shores of northern coasts of Alboran Sea: coupling regional oceanography and benthic community patterns

*"The degrees of dissimilarity will depend on the migration of the more dominant forms of life from one region into another having been more or less effectually prevented, at periods more or less remote;- on the nature and number of the former immigrants;- and on the action of the inhabitants on each other in leading to the preservation of different modifications; the relation of organism to organism in the struggle for life being, as I have already often remarked, the most important of all relations. Thus the high importance of barriers comes into play by checking migration; as does time for the slow process of modification through natural selection."*

Charles Darwin
(The Origin Of Species, 1859).

Macro view of a stand of *Fucus spiralis*. Strait of Gibraltar. June 2011. Photograph by Antonio Bermejo Lacida

# Biogeographical patterns of littoral and upper sublittoral communities along rocky shores of northern coasts of Alboran Sea: coupling regional oceanography and benthic community patterns.

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**Submitted to:** Plos One

## ABSTRACT

Alboran Sea is the westernmost ecoregion of the Mediterranean Sea and is placed in the vicinity of Strait of Gibraltar, the only natural connection of Mediterranean Sea with global circulation. This ecoregion presents steep and highly variable environmental gradients, thus acting as a natural filter for species distribution in the Mediterranean. The main aim of this study was to evaluate the influence of regional oceanographic processes on spatial patterns of benthic communities along the northern coast of the Alboran Sea, proposing potential linking mechanisms between oceanography and community structure. The spatial structure of benthic communities along the northern coast of Alboran was assessed using data of landscape and species composition, covering the whole rocky shore. Spatio-temporal variability in coastal oceanography was assessed with satellite data of sea surface temperature and a high spatio-temporal resolution analysis based on Empirical Ortogonal Functions. Three biogeographical subregions were identified in northern Alboran coast: western, central and eastern. The coincidence of these regions with oceanographic patterns and the significant correlation between oceanographic and biological data suggest a strong link between community features and oceanographic phenomena. Overall, central subregion showed the minimum species richness, with the landscape dominated by filter-feeders and poorer and undifferentiated flora. In contrast, eastern and western subregions showed higher and similar values for species richness, with a landscape dominated by high productive macrophytes in western Alboran and slow-growing ones in eastern Alboran (a footprint for Atlantic and Mediterranean characteristics). The divergent character of central Alboran can be related to the alternating occurrence of upwelling episodes and the arrival of Mediterranean waters in this subregion, causing short time variations in physico-chemical properties and food availability. In conclusion, this study supports the existence of a steep community gradient between the Atlantic and the Mediterranean, which is mainly driven by regional oceanographic processes.

Keywords: Alboran Sea; Biogeography; Landscape; Littoral communities; Macroecology; Oceanography and community patterns.

## INTRODUCTION

The Mediterranean Sea is the largest and deepest semi-enclosed sea on Earth. This sea is an important marine biodiversity hot spot, as a consequence of its geological and climatic history (Coll et al., 2010; Picotti et al., 2014). Two milestones have been especially important shaping the present Mediterranean biodiversity: i) the Messinian salinity crisis (Krijgsman et al., 1999; Duggen et al., 2003), and ii) the climatic oscillations in the Quaternary (Bianchi & Morri, 2000; Hewitt, 2000, 2004). The closure of the Rifean and Baetic gateways about 6 M years ago left the Mediterranean Sea completely isolated. Its negative water balance transformed it into a series of hypersaline lakes (i.e. Messinian salinity crisis), producing a massive extinction of the previous biota. Afterwards, the opening of the Strait of Gibraltar (5.3 Ma) allowed the recolonisation of species of Atlantic origin. Since this date, the Strait of Gibraltar has been the unique natural communication of the Mediterranean Sea with the rest of global circulation. The alternation of the ice ages with the warm interglacials periods during the whole Quaternary (from 1.8 Ma to the present) resulted in different immigration waves of Atlantic biota of boreal or subtropical origin into the Mediterranean (Rodríguez, 1982; Bianchi & Morri, 2000; Figueroa et al., 2014). In this sense, the Alboran Sea played an important role in the conformation and maintenance of the Mediterranean biodiversity, both in the past and the present, as a selective barrier for Atlantic and Mediterranean species (e.g. Bargelloni et al., 2003).

The Alboran Sea is the westernmost Mediterranean ecoregion (Spalding et al., 2007). It is considered a transition area between the Mediterranean Sea and the Atlantic Ocean (Báez et al., 2004; Ballesteros et al., 2007). This is why some typical Mediterranean assemblages are scarce or absent whereas north-east Atlantic macroalgal species such as *Cystoseira tamariscifolia*, *Fucus spiralis* or *Laminaria ochroleuca* are present (Conde, 1989; Flores-Moya et al., 1995a). Different biogeographical limits have been recognised in this sea based on the presence-or-absence of species datasets or population genetic studies for particular taxa (Álvarez-Cobelas et al., 1989; Conde, 1989; Quesada et al., 1995; Alberto et al., 2008). However, the mechanisms and the importance of the linking processes between oceanographic conditions and biogeographical variations in species and communities remain unknown.

The integration of biogeography and ecology supposes an opportunity to link local and regional scales, improving the knowledge about the causes of distribution patterns at the community level (Briggs, 2007). Recent studies in various geographical locations have begun to integrate ecology with biogeography at mesoscale (tens to hundreds of kilometres), providing empirical evidences to support that geographical variations in structure and composition of intertidal benthic populations are strongly linked with oceanographic patterns (Bustamante & Branch, 1996; Broitman et al., 2001; Blanchette et al., 2008). Oceanographic processes control the distribution of food, nutrient, and propagules (Blanchette et al., 2006), and the patterns of hydrodynamics, temperature or salinity. These processes may affect in different ways marine benthic organism and their biological relationships, determining the composition and structure of benthic communities across regional scales (Schiel, 2004).

In the Alboran Sea the main forcing of the hydrodynamics is the Atlantic Jet and its intensity (Renault et al., 2012). This jet feeds a complex pattern of anticyclone oligotrophic gyres in this region: a more constant and stable Western Alboran Gyre (WAG) and a less frequent Eastern Alboran Gyre (EAG). The intensity of the Atlantic inflow varies seasonally, being reinforced during summer when the evaporation in the Mediterranean basins is maxima (Renault et al., 2012), and constituting a stable circulation system with both gyres (WAG and EAG). Associated to the gyres, coastal upwelling processes are described along the north-western coast of the Alboran Sea (Parada & Canton, 1998; Macías et al., 2008). Furthermore, upwelling processes are partly dependent on meteorological conditions, being enhanced during westerly winds situations (Macías et al., 2008). These complex oceanographic patterns increase the spatial and temporal variability in the area, generating environmental gradients, which could affect littoral and sublittoral assemblages and their spatial patterns.

The present study aims to analyse spatial patterns of littoral and upper sublittoral community structure and composition in relation to oceanographic conditions and coastal geomorphology along the northern coast of the Alboran Sea. The study suggests potential mechanisms linking the observed patterns of community structure with the prevailing oceanographic conditions.

## MATERIAL AND METHODS

### Field collection of data

This study was carried out between 2010 and 2013, in 27 sites along the northern coast of the Alboran Sea (Fig. 1). To reduce the influence of anthropogenic pressures, degraded sites were avoided according to Bermejo et al. (2013). The field samplings were carried out during spring and summer, coinciding with the peak of growth in littoral communities (González, 1994). At each site, a stretch of coast of different length was surveyed to define the littoral and upper sublittoral landscape according to Bermejo et al. (2013). Moreover, a stratified sampling was carried out to obtain a macrophyte species list, except sites 3, 10, 16, 20, 22, 23 and 26, where it was not possible for sampling time limitation.

*Landscape and coastal morphology.*

The sampling survey consisted in a run of the different stretches of coast, between 500 and 1500 m, on foot and snorkelling. Each stretch of coast was divided in sectors based on littoral and upper-sublittoral communities (Table 1) and coast morphology (categorized in Low Coast, High Coast and Blocks). The initial and final points of the different sectors were marked using a Geographical Positioning System (GPS) Magellan Triton 400. The minimal length of coast surveyed was 20 m. In each sector a value of abundance (Table 2) was recorded for each one of the 18 species considered in Table 1. Subsequently, using a Geographical Information System (GIS) and orthophotographs from the environmental information network of the autonomic government of Andalusia, southern Spain (REDIAM), the length of each sector and the perpendicular distance to the nearest coast was measured. Considering this distance, each sector was classified as exposed (distance >1000m) or non exposed (distance <1000 m). The final result is a partition of the rocky shoreline in several sectors defined according to littoral and sublittoral communities and geomorphological characteristics (Ballesteros et al., 2007; Bermejo et al., 2013). The final relative species abundance (RA) for each site was estimated according to equation 1.

$$\mathbf{RA = \Sigma(L \times Ab)/TL} \tag{1}$$

where L is the sector lenght, Ab is the abundance value according to table 2, and TL is the total length of the stretch of coast surveyed.

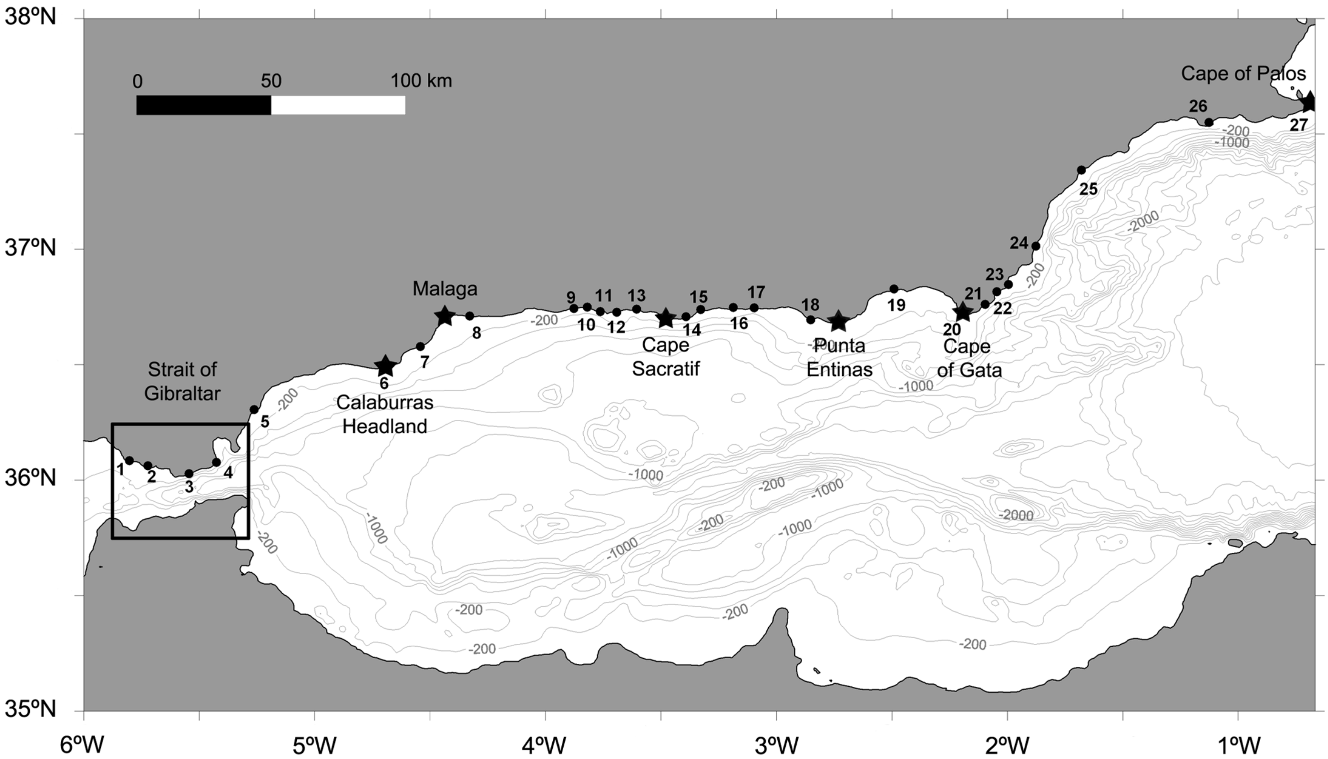


**Fig. 1.-** Geographical distribution of the sampling points along the northern coast of the Alboran Sea. **1-** Camarinal; **2-** Punta Paloma; **3-** Guadalmesí; **4-** Punta Carnero; **5-** Torreguadiaro; **6-** Calaburras; **7-** Torrequebrada; **8-** La Araña y Rincón de la Victoria; **9-** Nerja; **10-** Maro **11-** Cerro Gordo; **12-** Almuñecar; **13-** Salobreña; **14-** Cala Rijana; **15-** Castel de Ferro; **16-** Cala del Ruso; **17-** La Alcazaba; **18-** Guardias Viejas; **19-** Almería; **20-** Cabo de Gata; **21-** San José; **22-** Isleta del Moro; 2**3-** Cala Carnaje y El Playazo; **24-** El Algarrobico; **25-** San Juan de los Terreros; **26-** Cala Salitrona; **27-** Cabo de Palos. Open dots- sites that not accomplish with reference conditions; Black dots- sites that accomplish with reference conditions.

**Table 1-** Species list of sessile invertebrates, seagrasses and seaweeds considered for landscape description of Alboran Sea.

| Code | Species |
|---|---|
| | Sessile invertebrates |
| Ac | *Astroides calycularis* |
| My | *Mytilus* spp. |
| | Seagrasses |
| Cn | *Cymodocea nodosa* |
| Po | *Posidonia oceanica* |
| Zn | *Zostera noltii* |
| | Seaweeds |
| Cp | *Caulerpa prolifera* |
| Co | *Corallina* spp. |
| Ce | *Cystoseira elegans* |
| Ct | *Cystoseira ericaefolia** |
| Cc | *Cystoseira compressa* |
| Cf | *Cystoseira foeniculacea* |
| Chh | *Cystoseira humilis* var. *humilis* |
| Chm | *Cystoseira humilis* var. *myriophylloides* |
| Cm | *Cystoseira mauritanica* |
| Cs | *Cystoseira sauvegeuana* |
| Csp | *Cystoseira spinosa* |
| Fs | *Fucus spiralis* |
| Lb | *Lithophyllum byssoides* |

* *Cystoseira ericaefolia* group – *Cystoseira tamariscifolia*, *C. amentacea* var. *stricta* and *C. mediterranea*.

**Table 2-** Scale of relative abundance according the degree of population development for each considered specie.

| Relative abundance | Value |
|---|---|
| Continuous belt or meadow | 5 |
| Almost continuous belts or meadows | 4 |
| Abundant patches or stands | 3 |
| Abundant scattered specimens | 2 |
| Rare scattered specimens or stands | 1 |
| Absence | 0 |

*Species composition.*

In each site, a stratified sampling, registering all subhabitats, was carried out to obtain a species list of marine macrophytes. Each sampling was carried out along 50–60 m width of the whole rocky intertidal shore. When identification of specimens *in situ* was impossible, they were taken to the laboratory for a detailed observation. To identify small seaweeds (e.g. small Ceramiales and epiphytes), three replicates of quadrats of 17x17 $cm^2$ were taken, scraping off all organisms from the surface. To decrease community variability due to environmental factors, quadrats were taken in horizontal intertidal (between 0 and 30º) in the upper-most level of sub-littoral zone, avoiding very sheltered zones. The samples were kept in 5% formalin.

Subsequently, samples were carefully sorted in the laboratory, and algae were identified to the genus/species level. The taxonomic algal nomenclature used followed AlgaeBase (Guiry & Guiry, 2013).

**Sea surface temperature (SST)**

The SST was obtained from images taken by the Advanced Very High Resolution Radiometer on board National Oceanic and Atmospheric Administration series satellites. Particularly, 8 days-averaged images with 4x4 km resolution were extracted using the PO.DAAC Ocean ESIP Tool (http://poet.jpl.nasa.gov/). Temporal data set covered from 2003 to 2009, using 314 images (45 images per year). The coastal pixels close to land may give erroneous SST values; thus, SST data were taken in ocean pixels contiguous to the coastal site studied.

**Tidal range**

The tidal range in each site was estimated based on the data provided by "Puertos del Estado" (http://www.puertos.es/) for six harbours from the studied area (Tarifa, Algeciras, Málaga, Motril, Almería, and Alicante), and the distance following the coastline between sampling sites and harbours. In this case, only the major four tidal components were considered (M2, S2, N2 and K1). Subsequently, the tidal range for each site was calculated based on a linear interpolation considering the distance between the sampling point and the adjacent harbours.

**Data analyses**

To examine geographical patterns of community similarity, the Vegan package for R and PRIMER 6 (Plymouth Routines in Multivariate Ecological Research) software were used. In all statistical analysis significance was set at 5% probability.

*Identifying oceanographic regions*

Empirical Orthogonal Function (EOF) analysis was applied aiming to describe the spatio-temporal variation of SST in the region of interest (Fig. 1). The EOF analysis provides a description of the spatial and temporal variability of a time series in terms of orthogonal functions called empirical modes (Emery & Thompson, 1998). Aiming to search for the variation of temporal patterns, the temporal mean was removed in the EOF analysis, following the “temporal EOF” described by Lagerloef & Bernstein (1988). This ‘‘temporal EOF’’ is suggested more appropriate when analyzing structures associated with the temporal variability in a particular area. A Singular Values Decomposition method was used following Björnsson &

Venegas (1997). To assess if the calculated modes were statistically significant, the distance-error concept (Equation 3; North et al., 1982) was used:

$$\delta\lambda = \lambda \times (2/N)^{0.5} \tag{2}$$

where λ is the eigenvalue and N is the number of images used in the EOF analysis (N=314). A mode is considered significant only if the difference between its associated eigenvalue and the next one is bigger than δλ.

*Identifying biogeographical subregions*

To identify biogeographic subregions, two cluster analysis using group average linking were carried out: i) one based on landscape data using Bray-Curtis similarity index (Bray & Curtis, 1957) among 27 sites; and ii) one based on species composition data using Dice index (Dice, 1945) among 21 sites. In both cases, a SIMPROF test using 10,000 permutations was run for the dendrogram to indicate significant group structure.

*Agreement between biogeographic and oceanographic subregions*

To test the concordance between biogeographic and oceanographic subregions, a weighted kappa analysis (Cohen, 1960) following the scale proposed by Monserud & Leemans (1992) was applied.

*Variation in landscape and species composition*

Analyses of Similarity (ANOSIM; Clarke & Warwick, 2001) were performed to test for differences in landscape and in species composition among oceanographic areas. Pairwise ANOSIM comparisons were made between areas, using 10,000 simulations in each case. Moreover, to detect what species most contributed to landscape dissimilarity among the oceanographic areas, an analysis of species contribution to similarity (SIMPER; Clarke & Gorley, 2006) was carried out. On the other hand, a corological analysis was performed based on the species composition for each identified area. For this purpose, taxa were classified in four groups according to the geographic distribution and climatic affinities based on previous studies (González & Conde, 1991; Bárbara et al., 2005; Serio et al., 2006) and distribution data from Algaebase (Guiry & Guiry, 2013). The considered groups were: Mediterranean, Warm-Temperate, Cold-Temperate, and Wide-Distributed species. The floristic similarity between areas was calculated using the Dice index (Dice, 1945).

*Assessing the influence of environmental factors in littoral and upper sublittoral landscape*

In addition to the influence of oceanographic conditions, the natural geomorphological variability of the coastal environment may also play an important role in the development of littoral and upper sublittoral communities (Ballesteros et al., 2007). Thus, to assess the relative importance of these factors in the landscape patterns, a multivariable analysis was performed. In this case, environmental variables related to the morphology of the coastline (% of coastline constituted by blocks; % of high coast; % of low coast; % of wave exposed coast) and oceanographic conditions (average SST and tidal range) were considered (Appendix S1). A non-metric multidimensional scaling (MDS) analysis (Clarke & Warwick, 2001) was performed based on landscape data for each site, and subsequently, the environmental variables considered were fitted onto the MDS using the function "envfit" of the Vegan package for R (Oksanen et al., 2012). The significance of fitted variables was assessed based on 10,000 permutations.

## RESULTS

### Oceanographic regions

The climatologic distribution of the temporal-average SST showed main hydrological structures in the area (Fig. 2). These structures include an upwelling zone along the northwestern coast of Alboran and the WAG. The EAG was not so easily detected. Regarding the northern coast of the Alboran Sea, temporal-averaged SST image of the area showed a longitudinal gradient: Minimum value (17ºC) was found in the upwelling area, from the Strait of Gibraltar to Cape Calaburras; in contrast maximum averaged coastal SST (19.5 ºC) was found close to the Cape of Palos.

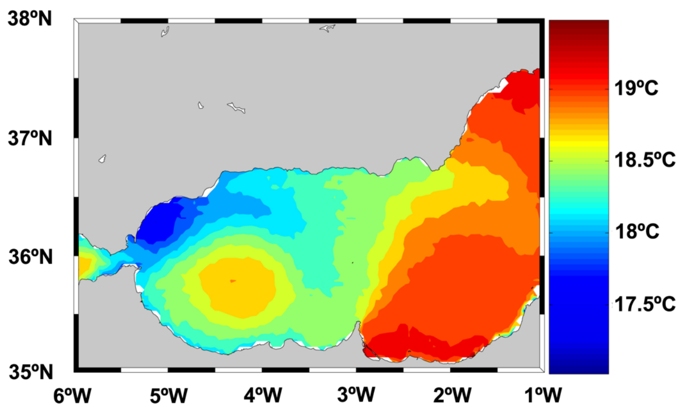


**Fig. 2.-** Temporal averaged Sea Surface Temperature spatial distribution along the Alboran Sea.

Nevertheless, climatological average SST can mask underlying spatio-temporal patterns in the region; thus EOF analysis was carried out to analyze this information. The results of the

EOF analysis are shown in Fig. 3 and Appendix S2. Only the first three modes were statistically significant. The modes explained, respectively, 29%, 6% and 4% of the normalized variance. The spatial coefficients of the different modes represent the extension and the influence of the processes in the studied area. Thus, the value of the spatial coefficient is directly related to the intensity of the phenomenon in the area (Appendix S2). The temporal amplitude of the EOF modes indicates when a phenomenon is relevant and its relative importance (Fig. 3def). To facilitate the interpretation of spatio-temporal patterns of SST in this area, homogeneous areas were defined using a limit of 0.015 for the spatial coefficients (Fig. 3 abc).

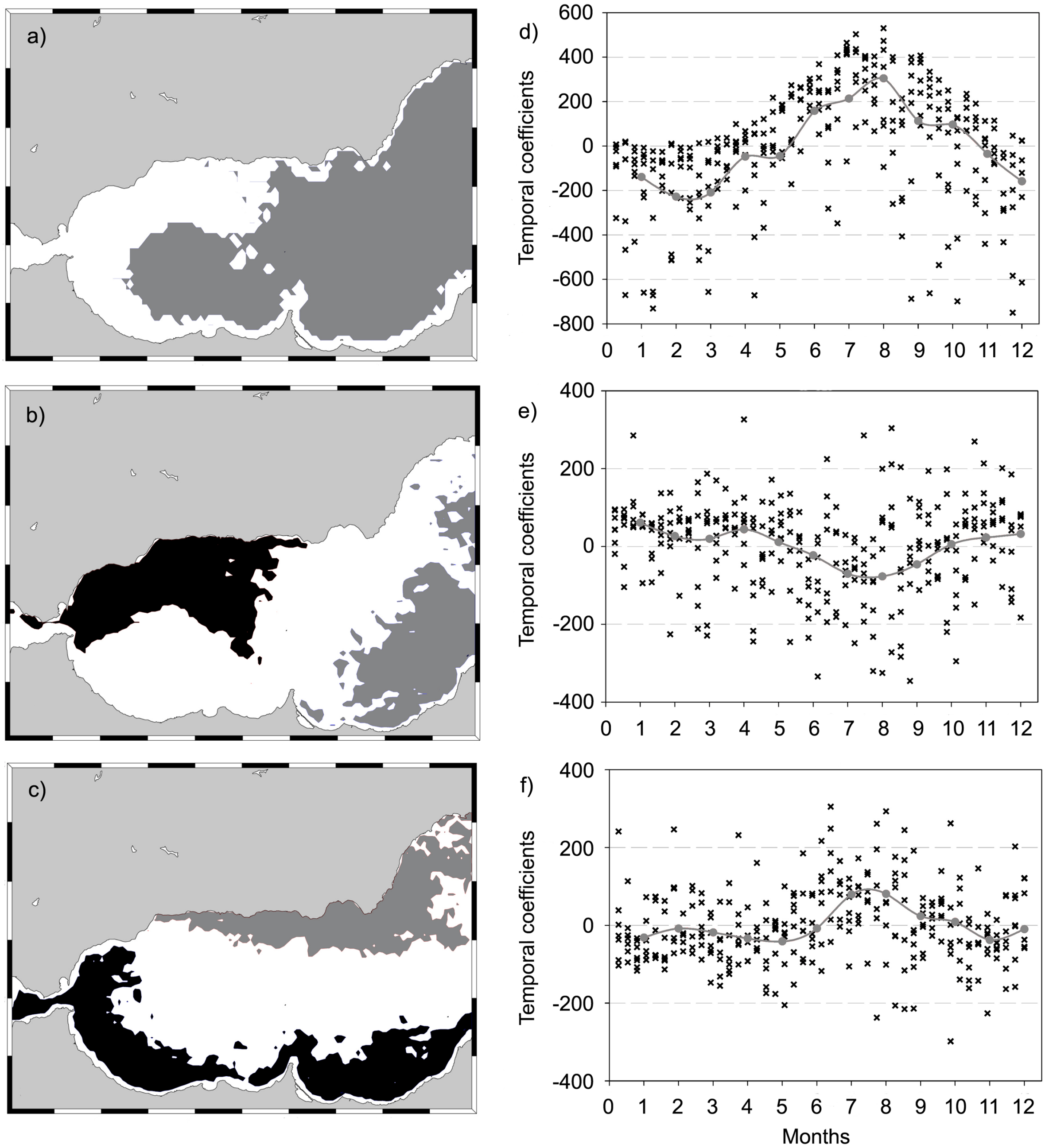


**Fig. 3.- a, b and c -** Homogeneus regions based on the highest absolute values of the spatial coefficient for each mode: a-mode 1; b-mode 2; c-mode-3; **d, e and f –** Temporal coefficients of the three first modes of the EOF and monthly averages series (grey line).

The first mode showed highly positive values in the southeast and easternmost part of the area, mainly from Cape Sacratif to the Cape of Palos (Fig. 3a). In the rest of the northern coast (from the Strait of Gibraltar to Cape Sacratif), values were almost null or slightly negative. The second mode (Fig. 3b) marked a region in a wide band along the Spanish coast, with high positive values from the Strait of Gibraltar to Punta Entinas. The third mode (Fig. 3c) presented two well-differenced areas: positive values in a northern coastal-strip (from Malaga to Cape Palos) and negative values from Calaburras Headland to the Strait of Gibraltar.

Regarding the temporal coefficients of the significant modes, the mode 1 presented the highest absolute values and a clear seasonal signal, being positive during summer and negative in winter (Fig. 3d). On the other hand, modes 2 and 3 presented scattered patterns with similar absolute values; this high variability is especially enhanced during summer period (Fig. 3ef). Nevertheless, some trends could be observed as mode 3 showed the same behaviour than mode 1; the monthly average was slightly positive during summer (Fig. 3df). Temporal coefficients of mode 2 presented the opposite behaviour of modes 1 and 3, presenting negative monthly-averaged values during summer (Fig. 3e).

Therefore, three homogenous areas with different dynamics along the northern coast of Alboran Sea can be defined as a result of the combination of the three modes, as will be discusssed below: i) from the Strait of Gibraltar to Calaburras Headland (western Alboran; sites from 1 to 6), ii) from Calaburras Headland to Punta Entinas (central Alboran; sites from 7 to 17), and iii) from Punta Entinas to the Cape of Palos (eastern Alboran; sites from 18 to 27, Fig. 1).

**Biogeographic subregions**

According to the landscape, three biogeographic subregions were identified by the SIMPROF test corresponding to a slice in the dendrogram arising from the cluster analysis at a Bray–Curtis similarity around 60% (Fig. 4a). Overall, the first cluster corresponded to the sampling stations located in the Strait of Gibraltar (from 1 to 4; see Fig. 1); the second one grouped the sites placed between the Strait of Gibraltar and the nearby Cape Sacratif (from 5 to 14); and the last one and more differentiated corresponded to the stations sited eastwards Cape Sacratif (from 15 to 27).

The second cluster analysis based on species composition (Fig. 4b) differed slightly from the previous one. In this case, the limits between clusters are displaced eastward in comparison with the landscape results. The first cluster (from 1 to 6) showed its eastern limit in Calaburras Headland instead of the Strait of Gibraltar, and the second in Punta Entinas (from 7 to 17) instead of Cape Sacratif. In this case, the eastern subregion was also the most differentiated.

The weighted kappa analysis yielded a "very good" or "excellent" agreement between oceanographic subregions and biogeographic ones identified in the Alboran Sea (Fig. 4) based on landscape (kappa = 0.752) and species composition (kappa = 0.924).

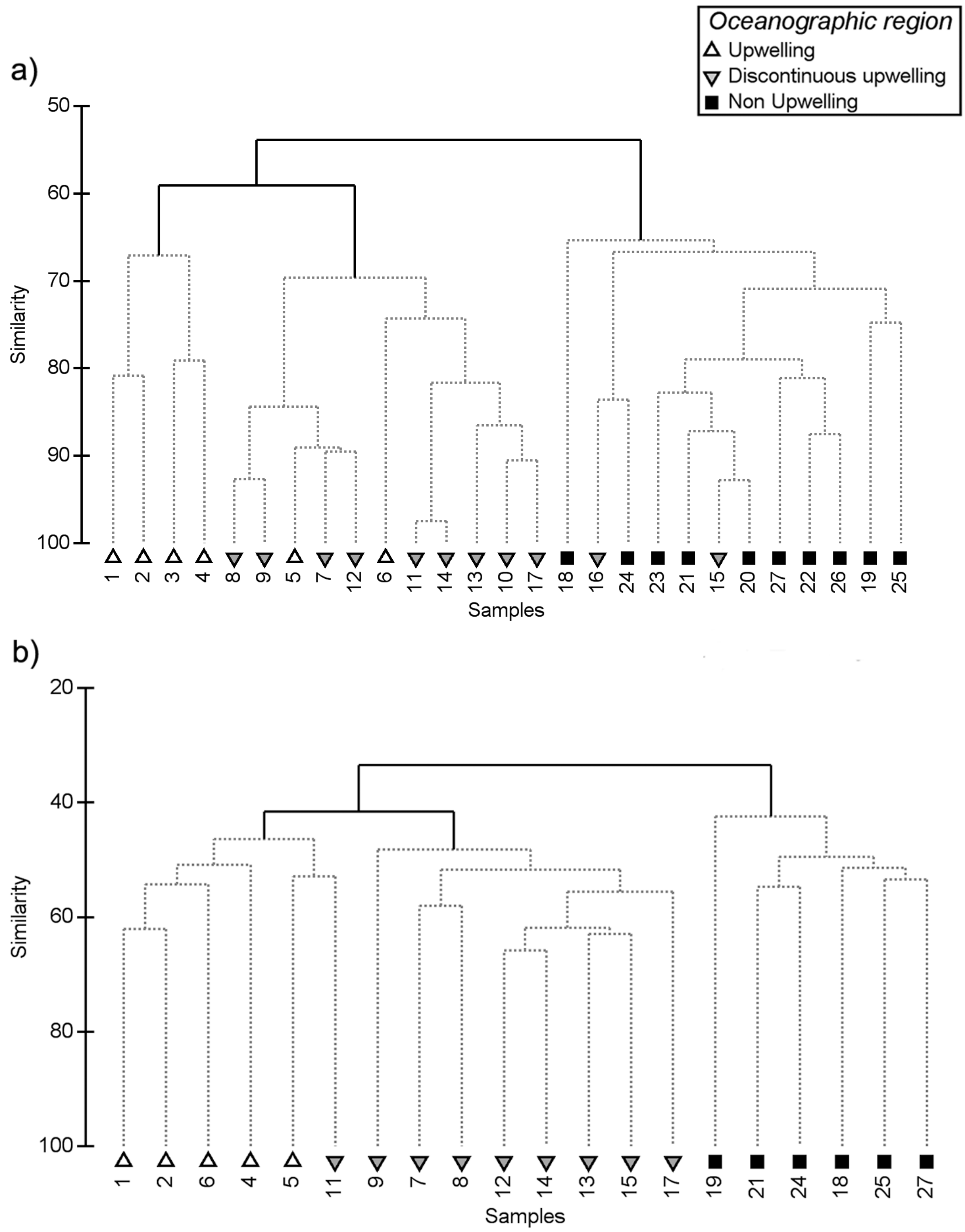


**Fig. 4.-** Dendrogram depicting mutual similarities of littoral and sub-littoral communities of the sampled locations. Solid lines show the different groups formed by the SIMPROF analyses. **a-** Dendrogram based on landscape dataset; **b-** Dendrogram based on species composition.

### Variation in landscape and species composition

The ANOSIM indicated that landscape ($R = 0.564$; p-value $< 0.001$) and species composition ($R = 0.810$; p-value $< 0.01$) differed significantly along the Alboran Sea, being these differences significant for all pairwise comparisons between the different oceanographic subregions identified (table 3).

**Table 3-** Results of ANOSIM pairwise comparisons between oceanographic regions identified in Alboran Sea according to landscape data and species composition. *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001

| Regions | R-landscape | R-species composition |
|---|---|---|
| western-central | 0.387* | 0.705*** |
| western-eastern | 0.767*** | 0.853** |
| central-eastern | 0.689*** | 0.937*** |

*Landscape*

The SIMPER analysis (table 4) based on landscape data showed that *Posidonia oceanica, Cystoseira ericaefolia* and *Mytilus spp.* were the species that most contributed to the dissimilarity between the oceanographic subregions identified (table 4 and Fig. 5). The eastern subregion was the most differentiated area in comparison with the western and central Alboran Sea, (Fig. 5, table 3 and 4). In this subregion, *C. ericaefolia* and *P. oceanica* were present more frequently, usually forming dense continuous belts and meadows, whereas *Mytilus* was rare. In contrast, *Mytilus* was an important component of littoral communities in the western and central Alboran Sea, especially between the Strait of Gibraltar and Cape Sacratif. In these areas, *P. oceanica* was rare and *C. ericaefolia* was less abundant than in eastern Alboran; these species were found forming stands and scattered belts. On the other hand, the absence of notorious elements of the typical community from the Strait of Gibraltar, as *Lithophyllum byssoides*, *Fucus spiralis* or *Cystoseira mauritanica*, which were absent eastward Calaburras Headland, and the importance of *Mytilus* beds in littoral assemblages of central Alboran, were the most important elements to explain the dissimilarity between western and central Alboran.

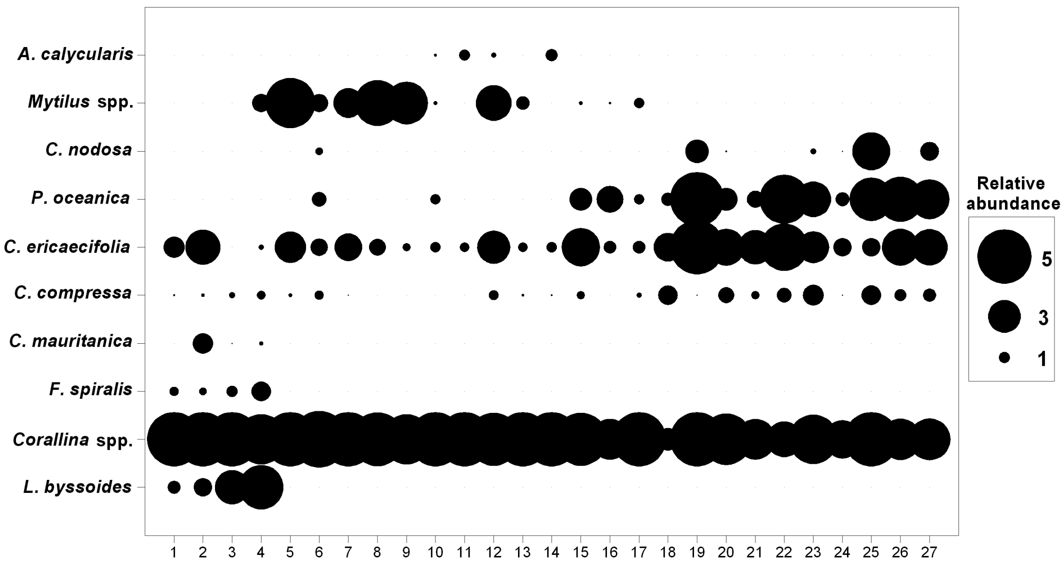


**Fig. 5.-** Geographical pattern of distribution and abundance for the species that contributed most to the dissimilarity between biogeographical subregions. Numbered study sites are referenced and located in Fig. 1.

**Table 4-** Results of SIMPER showing the percentage of contribution to the landscape dissimilarity for each species between the three oceanographic regions identified. Cut off for low contributions at 95%. Average dissimilarities: Western-Central Alboran = 36.82, Central-Eastern Alboran = 43.43, Western-Eastern Alboran = 48.88.

| Species | Western-Central Alboran | Central-Eastern Alboran | Western-Eastern Alboran |
|---|---|---|---|
| Ac | 3.55 | 2.95 | - |
| My | 22.93 | 16.22 | 10.90 |
| Cn | 1.55 | 7.44 | 6.23 |
| Po | 8.86 | 26.59 | 23.03 |
| Zn | - | - | - |
| Cp | - | - | - |
| Co | 3.17 | 11.84 | 9.87 |
| Ce | - | - | - |
| Ct | 16.60 | 19.44 | 14.41 |
| Cc | 5.28 | 10.82 | 7.02 |
| Cf | - | - | - |
| Chh | - | - | - |
| Chm | - | - | - |
| Cm | 4.78 | - | 3.14 |
| Cs | - | - | - |
| Csp | - | - | - |
| Fs | 9.54 | - | 6.21 |
| Lb | 22.16 | - | 14.42 |

*Species composition*

A total of 168 macrophyte taxa (3 seagrasses, 35 Phaeophyceae, 104 Rhodophyta, 26 Chlorophyta) were identified (Appendix S3). Even though the number of sampled sites differed between western (5), central (9) and eastern (6) subregions, the results suggested a decreasing number of species in the central Alboran (Fig. 6). Attending to the chorological differences in specific composition across the Alboran Sea, it must be highlighted the increase in Mediterranean endemisms from the western to the eastern subregion and the decrease in species with cold-temperate affinity, which followed the expected trend. However, the rate of change between subregions was different, being more gradual for Mediterranean species and more abrupt for cold temperate species, especially between the western and central Alboran. The number of species of warm temperate affinity was minimum in central Alboran and maximum in the eastern subregion. In the case of wide distributed species, the number was very similar for all subregions. On the other hand, the similarity between the central and western subregions was the highest (66%), showing eastern Alboran the most differentiated flora (56% of similarity with the central subregion and 54% with the western one).

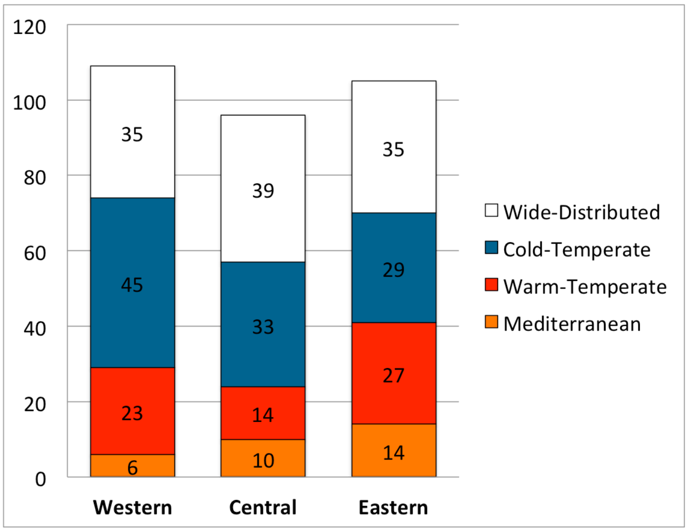


**Fig. 6.-** Total number of marine macrophytes at the three identified subregions along the Alboran Sea.

### Assessing the influence of environmental factors in littoral and upper sublittoral landscape

The MDS based on landscape data (Fig. 7) showed a clear geographical west-east gradient along the Alboran Sea. According to this, sites were distributed on the plot from the western sites on the right to the eastern ones on the left. In this way, the species with the most positive values of axis 1 (NMDS1) were more frequent in eastern Alboran such as *Caulerpa prolifera*, *Cystoseira elegans*, *Cystoseira sauvegeuana*, *Cystoseira spinosa* or *Cymodocea nodosa.* Conversely, the species with the most negative values of NMDS1 were typical western Alboran species such as *Fucus spiralis*, *Cystoseira mauritanica*, or *Lithophyllum byssoides*. In the case of central Alboran, the results showed that the major development of filter-feeders assemblages (*Mytilus* and *Astroides*) was the most characteristic landscape feature. On the other hand, the higher arrow length of the environmental variables related to oceanographic conditions suggested a better correlation with the landscape structure than geomorphological ones (table 5), showing all of them significant correlations. In the case of geomorphological variables, only the percentage of low coast was significantly correlated with the landscape similarity ($r = 0.471$; p-value $< 0.05$). These results suggested that the landscape structure along the Alboran Sea was better explained by regional oceanography patterns than by local geomorphological characteristics.

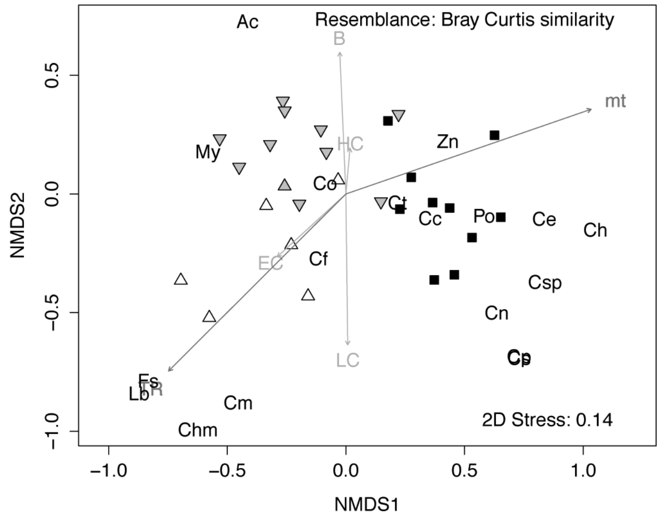


**Fig. 7.-** Non-metric multidimensional ordination plot based on landscape data for each of the 27 sites within the three biogeographical subregions. Open triangle - western subregion; inverse grey triangle - central subregion; black square - eastern subregion. Species are indicated by codes referenced in Table 1. Variables are draw in dark grey when they are related with oceanographic conditions, and in grey when they are related to coastal morphology.

**Table 5-** Results of fit seven environmental variables onto a non Metric Multidimensional Scaling (MDS) based on landscape data along Alboran Sea. NMDS1 and NMDS2 corresponded with direction cosines of the vectors, and R2 is the squared correlation coefficient. *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001

| Variable | NMDS1 | NMDS2 | $R^2$ |
|---|---|---|---|
| Distance | 0.947 | 0.322 | 0.674*** |
| mean SST | 0.945 | 0.326 | 0.655*** |
| Tidal Range | -0.707 | -0.707 | 0.611*** |
| % Blocks | -0.053 | 0.999 | 0.199 |
| % Low Coast | 0.011 | -0.999 | 0.222* |
| % High Coast | 0.093 | 0.996 | 0.020 |
| % Exposed Coast | -0.734 | -0.675 | 0.084 |

## DISCUSSION

Beyond among-site variability, the environmental conditions related to oceanographic patterns are the most important factors to explain the species distribution and the community structure along Alboran Sea (Fig. 7). The results obtained support the existence of three biogeographical subregions matching with oceanographic patterns (Fig. 4): western, central and eastern Alboran. This relationship suggests a strong link between the littoral and upper sublittoral communities, and the oceanic processes. The coincidence between marine species distribution and the main oceanographic features was suggested previously in this area (Conde, 1989; Cebrián & Ballesteros, 2004; Bermejo et al., 2013; González-Duarte et al., 2013), but the mechanism underlying these spatial patterns were not studied.

### Oceanographic processes effects on the coastal conditions

At the light of the EOF analysis, the coastal conditions in the three subregions identified can be briefly described according to the oceanographic patters. In eastern Alboran, which comprises mostly the extension area of mode 1 (Fig. 3a). The prevailing coastal conditions are determined by the dynamics of the Mediterranean Surface Waters (MSW). These waters are oligotrophic and show the broadest seasonal thermal amplitude in the Alboran Sea, oscillating from 25º (summer) to 14º C (winter) (Parada and Canton, 1998; Baldacci et al., 2001).

Westward from Punta Entinas (Fig. 3a), the influence of MSW in the coastal environment declines significantly. In this area, dominant processes (collected in mode 2 and 3) present a scattered pattern with scales of variation far from seasonal cycles, more related to short-term atmospheric processes as wind conditions (Fig. 3ef).

The coastal environment in western Alboran is influenced by the existence of a quasi-permanent upwelling area associated to the WAG (Vargas-Yáñez et al., 2002; Renault et al., 2012), being the environmental conditions relatively stable.

By contrast, in central Alboran the coastal conditions are the most variable. This subregion can be affected by strong upwelling processes due to strong westerlies or an enhanced Atlantic Inflow (Macías et al., 2008; Renault et al., 2012) (Fig. 3b, mode 2); or can be filled with MSW (Fig. 3d, mode 3), especially during summer (Fig. 3ef), due to the occurrence of strong easterlies or southward displacements of the Atlantic Jet (Sarhan et al., 2000; Macías et al., 2007).

### Biogeographical subregions

The western Alboran presents the most Atlantic character (Conde, 1989). In this subregion, it can be found species with very different climatic affinities (Fig. 6), reflecting the importance of the Strait of Gibraltar as a geographical pivotal point at the junction of three ecoregions (Spalding et al., 2007): i) Alboran Sea, within the Mediterranean province; ii) the South European Atlantic Shelf; and iii) the Saharan upwelling, both within the Lusitanian province. In this sense, some characteristic Atlantic (e.g. *C. mauritanica* or *Fucus spiralis*) and Mediterranean species (e.g. *P. oceanica*) have their distribution limits here (Conde, 1989; Barceló-Martí et al., 2000). Regarding the landscape, it is remarkable the presence of shallow stands or forest of highly productive macrophytes such as *Saccorhiza polyschides* (Flores-Moya, 2012) or *Cystoseira usneoides* (Barceló-Martí et al., 2000). In contrast, from site 4 to Calaburras Headland, *Mytilus* beds became an important component of the benthic communities; while, *S. polyschides* or *C. usneoides* populations were less frequent and deeper, practically disappearing eastward Calaburras (Conde, 1989; Cebrián & Ballesteros, 2004; Flores-Moya, 2012).

In central Alboran, benthic communities are neither typically Atlantic nor typically Mediterranean, since most genus and species defining Atlantic or Mediterranean communities are lacking (Cebrián & Ballesteros, 2004) or present residual biomass. This subregion showed the lowest species richness and the less differentiated flora. In fact, only 13 macrophytes were exclusive from this subregion, whereas 28 were exclusive from the western subregion and 30 from the eastern one. In addition, considering previous studies (Flores-Moya et al., 1995a, 1995b; Conde et al., 1996) these differences were even greater, being nine, two and eight the number of exclusive macroalgal taxa in the western, central and eastern Alboran, respectively. These facts indicate that central Alboran is a divergent boundary between the Atlantic Ocean and the Mediterranean Sea, at least for marine macrophytes. In this sense, it is remarkable that the reduction in the species number is mainly consequence of the absence of several cold-temperate and warm-temperate species (Fig. 6), while wide distributed species remain. On the other hand, filter feeders as *Mytilus* spp., *Astroides calycularis* (Fig. 5) or *Anemonia sulcata/viridis* (pers. obs.) are important for the configuration of the landscape of littoral and upper sublittoral communities, while slow-growing (e.g. *P. oceanica*) or highly productive (e.g. *S. polyschides*) macrophytes are scarce or absent. This dominance of filter feeders suggests the existence of subsidies (phytoplankton and detritus as food for filter feeders) related to the presence of upwelling episodes in this subregion. In this sense, the highest surface chlorophyll concentrations have been reported in this area (nearby Malaga) (Macías et al., 2007).

Eastern Alboran showed the most differentiated littoral and upper sublittoral communities (Fig. 4). Benthic communities found in this subregion were typically Mediterranean (Ballesteros & Pinedo, 2004), being the landscape dominated by slow-growing macrophytes as *C. ericaefolia*, *C. nodosa* and *P. oceanica* (Fig. 5); and showing a flora with a more warm-temperate affinity and a major number of Mediterranean endemisms (Fig. 6). Several Mediterranean endemisms (e.g. *Cystoseira elegans* or *C. spinosa*) and vicariated populations of warm-temperate species, absent in adjacent Atlantic areas (e.g. *Digenea simplex* or *Acetabularia acetabulum*), present their distribution limits in this subregion (González, 1994). Accordingly, some authors have placed the limit between Atlantic and Mediterranean regions in this area (van den Hoek, 1975; Álvarez-Cobelas et al., 1989).

### Coupling regional oceanography and benthic community patterns

The analysis of the results suggests that the oceanographical processes must be the main driver determining spatial patterns of the community structure and composition along the northern coast of Alboran Sea. Despite further comparative and experimental approaches are needed to evaluate potential underlying mechanisms, two preliminary and complementary hypotheses are proposed based on the analysis of community features and oceanographic conditions.

*Functional ecological differences*

One major mechanistic hypothesis linking nearshore oceanography to community patterns is related to the spatial and temporal variability in coastal upwelling (Blanchette et al., 2006). Upwelling intensity has been suggested to play an important role in the balance between dominance by filter feeders or highly productive macrophytes (Menge et al., 2003; Schiel, 2004; Blanchette et al., 2006). Variable upwelling with frequent relaxation may be correlated with high rates of larval arrival to coast for barnacles and mussels, besides phytoplankton and detritus, favouring the dominance of sessile invertebrates (Menge et al., 2003). In contrast, consistently strong upwelling has been proposed to limit invertebrate recruitment (Bustamante & Branch, 1996; Broitman et al., 2001) because they prevent the accumulation along coastal fronts and the shore arrival of planktonic larvae and phytoplankton, while allow the maintenance of high nutrient rich waters in the near shore, favouring the dominance of highly productive macrophytes. In the Alboran Sea upwelling episodes are not permanent (Fig. 3) and this hypothesis support the filter-feeders dominance in central Alboran, especially where upwelling processes are more discontinous. However, in the Strait of Gibraltar intertidal stretch of coast dominated by *Mytilus* beds are absent and the sublittoral is dominated by high productive macrophytes. This fact could be consequence of the particular dynamic of the Strait of Gibraltar, with very intense inflowing currents towards the Alboran Sea. This flow of nutrient rich waters, however, must prevent the accumulation of planktonic larvae and phytoplankton along coastal fronts.

In eastern Alboran the landscape is dominated by slow-growing species, which are more competitive in oligotrophic conditions (Duarte, 1995; Cloern, 2001), such as the MSW prevalent in this subregion (Parada & Canton, 1998; Baldacci et al., 2001). In this sense, the capability of *P. oceanica* or *C. nodosa* to store nutrients (Delgado, 1985), the possibility of *Cystoseira* to remain in a latent stage during autumn and winter (Barceló-Martí et al., 2000), the presence of anti-hervibory defences (McMillan, 1984; Amico, 1995) and the relatively low nutrients requirements of these species (Duarte, 1995) must suppose an advantage for their dominance.

*Central Alboran as divergent boundary*

The alternative episodes of upwelling and MSW in the central region, favour short-time oscillations in temperature and nutrient availability during summer, which should cause an acute stress for benthic organisms. In this case, species with a narrower distribution range (i.e. cold-temperate and warm-temperate species) seems to be the most affected in comparison with wide-distributed species that even increased their numbers. This suggests that temperature stress play a relevant role in the functioning of this divergent boundary. Furthermore, considering that many taxa show the maximum fertility during spring-summer in the Alboran Sea (González, 1994; Barceló-Martí et al., 2000) and the vulnerability of many species during the first life stages (Brawley & Johnson, 1991; Steen & Rueness, 2004), the oscillations in temperature and nutrient availability during summer in the central subregion could hamper the success in seedling settlement of several macroalgal species, specially if they are in their distribution limits.

### Conclusions and future outlook

This study is a first step for a better understanding of the functioning of the Alboran Sea as a biogeographical border between the Atlantic and the Mediterranean. The use of EOF analysis instead of averaged value of SST to define dynamic oceanographic conditions and the combination of landscape and species composition datasets at large scales to analyse biogeographical patterns, have supposed a good approximation for the understanding of the influence of oceanic conditions in littoral and sublittoral communities along this sea.

The main conclusion is the division of Alboran Sea into three subregions. While the western and eastern subregions showed a marked Atlantic and Mediterranean character, respectively, the central subregion acts as a divergent boundary showing a poorer and less differentiated flora, with its landscape dominated by filter-feeders. This special character of central Alboran must be consequence of the alternative occurrence of upwelling episodes and the arrival of MSW, causing short-time variations in physico-chemical conditions, and its influence in the arrival of food and filter-feeders larvae.

The knowledge about the ecological functioning of biogeographical boundaries and the underlying mechanisms is essential to forecast past and future changes in the distribution and composition of benthic communities. This fact is especially important considering the actual context of climate change, and the transitional character of southern Iberia as a biogeographical limit for many taxa (Conde, 1989; González, 1994; Boaventura et al., 2002). In this sense, this study provides a database useful to measure the effects of climate change, to monitor further habitat destruction, and to identify community features and relevant areas for management and conservation purposes.

### *Author Contributions*

Conceived and designed the experiments: RBL, ERR, IH, JJV. Analyzed the data: RBL, ERR. Wrote the manuscript: RBL, ERR, IH, JJV.

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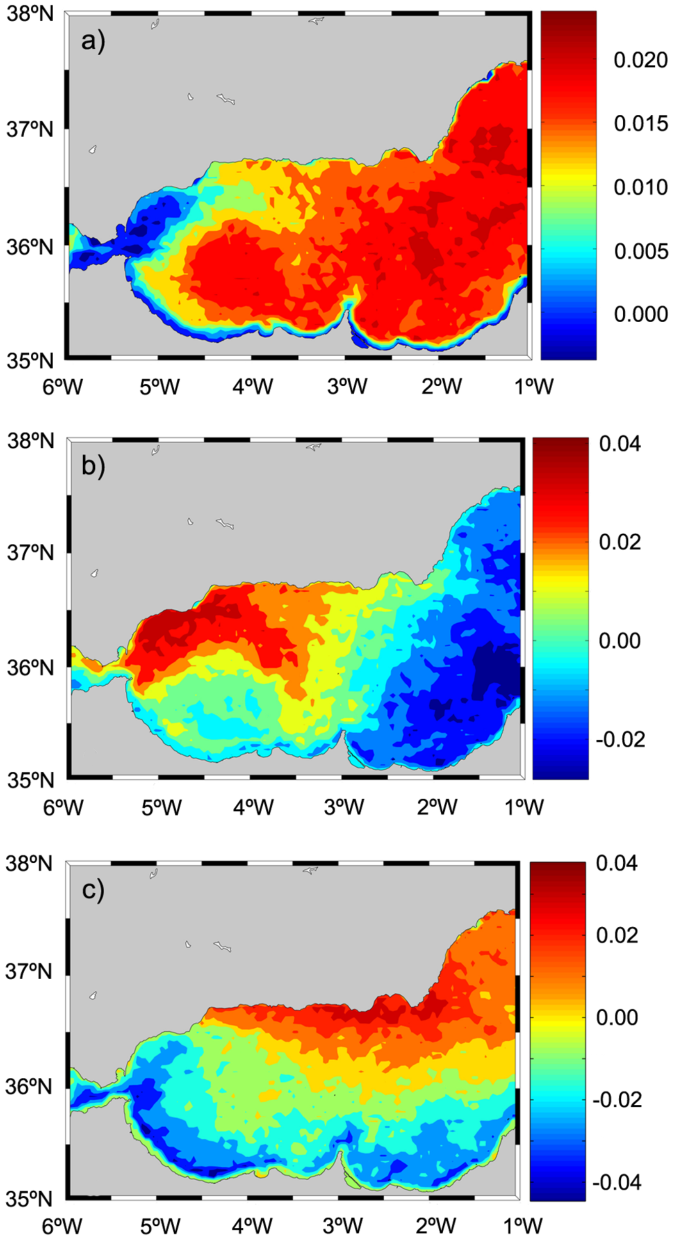
**Appendix S1-** Values of surveyed longitude (SL), percentage of high coast (%HC), percentage of low coast (%LC), percentage of coast constituted by blocks (%B), percentage of wave exposed coast (%EC), tidal range (TR), mean Sea Surface Temperature (mean SST) and distance in kilometres from site 1, for the 27 sites studied.

| Code | Site | SL (m) | %HC | %LC | %B | %EC | TR (cm) | mean SST (ºC) | Distance (km) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Camarinal | 1500 | 27.33 | 15.33 | 57.33 | 100.00 | 162.52 | 18.40 | 0 |
| 2 | Punta Paloma | 1500 | 0.00 | 100.00 | 0.00 | 100.00 | 152.61 | 17.98 | 8 |
| 3 | Guadalmesí | 1500 | 0.00 | 100.00 | 0.00 | 100.00 | 129.07 | 16.94 | 27 |
| 4 | Punta Carnero | 1500 | 0.00 | 84.00 | 16.00 | 100.00 | 114.20 | 17.10 | 39 |
| 5 | Torreguadiaro | 1220 | 0.00 | 90.16 | 9.84 | 100.00 | 98.84 | 17.68 | 69 |
| 6 | Calaburras | 1370 | 0.00 | 64.96 | 35.04 | 100.00 | 79.05 | 17.58 | 129 |
| 7 | Torrequebrada | 600 | 0.00 | 65.00 | 35.00 | 100.00 | 73.12 | 17.96 | 147 |
| 8 | Araña-Rincón | 1090 | 77.98 | 12.84 | 9.17 | 77.06 | 66.07 | 18.36 | 174 |
| 9 | Nerja | 900 | 31.11 | 16.67 | 52.22 | 100.00 | 60.01 | 18.51 | 216 |
| 10 | Maro | 1500 | 23.33 | 0.00 | 76.67 | 100.00 | 59.29 | 18.40 | 221 |
| 11 | Cerro Gordo | 1500 | 64.00 | 0.00 | 36.00 | 88.00 | 58.57 | 18.40 | 226 |
| 12 | Almuñecar | 1110 | 73.87 | 13.51 | 12.61 | 83.78 | 57.56 | 18.38 | 233 |
| 13 | Salobreña | 1200 | 27.50 | 0.00 | 72.50 | 94.17 | 56.55 | 18.49 | 240 |
| 14 | Rijana | 1030 | 59.22 | 0.00 | 40.78 | 53.40 | 53.35 | 18.39 | 260 |
| 15 | Castel de Ferro | 970 | 0.00 | 14.43 | 85.57 | 100.00 | 52.17 | 18.64 | 267 |
| 16 | Cala del Ruso | 710 | 12.68 | 0.00 | 87.32 | 100.00 | 50.16 | 18.46 | 279 |
| 17 | La Alcazaba | 880 | 0.00 | 0.00 | 100.00 | 86.36 | 48.81 | 18.57 | 287 |
| 18 | Guardias Viejas | 1500 | 0.00 | 96.00 | 4.00 | 96.00 | 44.77 | 18.96 | 311 |
| 19 | Almería | 450 | 84.44 | 0.00 | 15.56 | 100.00 | 37.71 | 19.33 | 353 |
| 20 | Cabo Gata | 1310 | 71.76 | 28.24 | 0.00 | 47.33 | 35.59 | 18.98 | 386 |
| 21 | San José | 1320 | 38.64 | 37.12 | 24.24 | 73.48 | 34.97 | 19.10 | 396 |
| 22 | Isleta Moro | 1170 | 39.32 | 18.80 | 41.88 | 72.65 | 34.46 | 19.44 | 404 |
| 23 | El Playazo | 930 | 50.54 | 49.46 | 0.00 | 59.14 | 34.15 | 19.41 | 409 |
| 24 | El Algarrobico | 900 | 2.22 | 87.78 | 10.00 | 100.00 | 32.77 | 20.13 | 431 |
| 25 | Terreros | 1490 | 0.00 | 97.32 | 2.68 | 100.00 | 30.19 | 19.82 | 472 |
| 26 | Cala Salitrona | 780 | 11.54 | 14.1 | 74.36 | 97.44 | 25.92 | 19.98 | 540 |
| 27 | Cabo de Palos | 700 | 20.00 | 55.71 | 24.29 | 70.00 | 22.97 | 19.15 | 587 |




**Appendix S2-** Spatial coefficient for each representative mode: **a-** mode 1; **b-** mode 2; **c-** mode-3

**Appendix S3-** Species list of macrophytes observed at each subregion identified along Alboran Sea: western, central and eastern. Absence: 0, Presence: 1. Climatic affinity is indicated as warm-temperate (WT), cold-temperate (CT), Mediterranean (Med) and Wide Distributed (WB).

| Distribution | Species | Western | Central | Eastern |
|---|---|---|---|---|
| | SEAGRASSES | | | |
| WT | *Cymodocea nodosa* (Ucria) Ascherson | 1 | 0 | 1 |
| Med | *Posidonia oceanica* (L.) Delile | 1 | 1 | 1 |
| CT | *Zostera noltei* Hornemann | 0 | 0 | 1 |
| | PHAEOPHYCEAE | | | |
| CT | *Cladostephus spongiosus* (Hudson) C.Agardh | 1 | 1 | 1 |
| WT | *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier | 1 | 1 | 1 |
| WT | *Cutleria adspersa* (Mertens ex Roth) De Notaris | 1 | 0 | 0 |
| Med | *Cystoseira amentacea* var. *stricta* Montagne | 0 | 0 | 1 |
| Med | *Cystoseira compressa* (Esper) Gerloff&Nizamuddin | 1 | 1 | 1 |
| Med | *Cystoseira elegans* Sauvageau | 0 | 0 | 1 |
| Med | *Cystoseira foeniculacea* (L.) Greville | 1 | 0 | 1 |
| Med | *Cystoseira humilis* Schousboe ex Kützinng | 0 | 0 | 1 |
| CT | *Cystoseira humilis var. myriophylloides* (Sauvegeau) J.H.Price&D.M.John | 1 | 0 | 0 |
| WT | *Cystoseira mauritanica* Sauvageau | 1 | 0 | 0 |
| Med | *Cystoseira mediterranea* Sauvageau | 0 | 1 | 1 |
| Med | *Cystoseria sauvageuana* Hamel | 0 | 0 | 1 |
| Med | *Cystoseira spinosa* Sauvageau | 0 | 0 | 1 |
| CT | *Cystoseira tamariscifolia* (Hudson) Papenfuss | 1 | 1 | 0 |
| CT | *Cystoseira usneoides* (L.) M.Roberts | 1 | 0 | 0 |
| WD | *Dictyota dichotoma (Hudson)* J.V.Lamouroux | 1 | 1 | 1 |
| WT | *Dictyopteris polypodioides* (A.P.De Candolle) J.V.Lamouroux | 1 | 0 | 1 |
| WD | *Ectocarpales* Bessey | 1 | 1 | 0 |
| CT | *Fucus spiralis* Linnaeus | 1 | 0 | 0 |
| CT | *Halopteris filicina* (Grateloup) Kützing | 1 | 1 | 1 |
| WD | *Halopteris scoparia* (Linnaeus) Sauvageau | 1 | 1 | 1 |
| CT | *Leathesia marina* (Lyngbye) Decaisne | 1 | 0 | 0 |
| CT | *Laminaria ochroleuca* Bachelot de la Pylaie | 1 | 0 | 0 |
| WT | *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C.Oliveira | 0 | 0 | 1 |
| WT | *Padina pavonica* (L.) Thivy | 1 | 0 | 1 |
| WD | *Ralfsia verrucosa* (Areschoug) Areschoug | 1 | 1 | 1 |
| CT | *Saccorhiza polyschides* (Lightfoot) Batters | 1 | 0 | 0 |
| WT | *Sargassum vulgare* C.Agardh | 1 | 1 | 1 |
| WD | *Scytosiphon lomentaria* (Lyngbye) Link | 0 | 1 | 0 |
| CT | *Scytosiphon dotyi* M.J.Wynne | 1 | 0 | 0 |
| WD | *Sphacelaria cirrosa* (Roth) C.Agardh | 1 | 1 | 1 |
| CT | *Sphacelaria fusca* (Hudson) S.F.Gray | 0 | 0 | 1 |
| WD | *Sphacelaria tribuloides* Meneghini | 0 | 0 | 1 |
| WD | *Taonia atomaria* (Woodward) J.Agardh | 1 | 0 | 0 |
| WT | *Zonaria tournefortii* (J.V.Lamouroux) Montagne | 1 | 0 | 0 |
| | RHODOPHYTA | | | |
| WT | *Acrosorium ciliolatum* (Harvey) Kylin | 1 | 1 | 0 |
| CT | *Aglaothamnion hookeri* (Dillwyn) Maggs&Hommersand | 1 | 1 | 0 |
| CT | *Ahnfeltiopsis devoniensis* (Greville) P.C.Silva&DeCew | 1 | 1 | 0 |
| Med | *Alsidium corallinum* C.Agardh | 0 | 0 | 1 |
| WT | *Amphiroa beauvoisii* J.V.Lamouroux | 1 | 1 | 0 |
| Med | *Amphiroa cryptarthrodia* Zanardini | 0 | 1 | 1 |
| WD | *Amphiroa rigida* J.V.Lamouroux | 1 | 1 | 1 |
| WD | *Anotrichium tenue* (C Agardh) Nägeli | 0 | 0 | 1 |
| Med | *Anthithamniella elegans* (Berthold) J.H. Price&D.M. John | 0 | 1 | 0 |
| WD | *Antithamnion cruciatum* (C.Agardh) Nägeli | 1 | 1 | 1 |
| CT | *Apoglossum ruscifolium* (Turner) J.Agardh | 1 | 0 | 0 |
| CT | *Asparagopsis armata* Harvey | 1 | 1 | 0 |
| WT | *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon | 1 | 1 | 1 |
| CT | *Bangia atropurpurea* (Mertens ex Roth) C.Agardh | 0 | 1 | 0 |
| CT | *Boergeseniella fruticulosa* (Wulfen) Kylin | 1 | 1 | 1 |
| CT | *Bonnemaisonia asparagoides* (Woodward) C.Agardh | 0 | 0 | 1 |
| WT | *Botryocladia botryoides* (Wulfen) Feldmann | 0 | 0 | 1 |
| WD | *Callithamnion corymbosum* (Smith) Lyngbye | 1 | 1 | 1 |
| CT | *Callithamnion granulatum* (Ducluzeau) C.Agardh | 1 | 1 | 1 |
| WD | *Caulacanthus ustulatus* (Mertens ex Turner) Kützing | 1 | 1 | 0 |
| WT | *Centroceras clavulatum* (C.Agardh) Montagne | 1 | 0 | 0 |
| WD | *Ceramium ciliatum* (J. Ellis) Ducluzeau | 1 | 1 | 1 |

**Appendix S3 (cont.)-** Species list of macrophytes observed at each subregion identified along Alboran Sea: western, central and eastern. Absence: 0, Presence: 1. Climatic affinity is indicated as warm-temperate (WT), cold-temperate (CT), Mediterranean (Med) and Wide Distributed (WB).

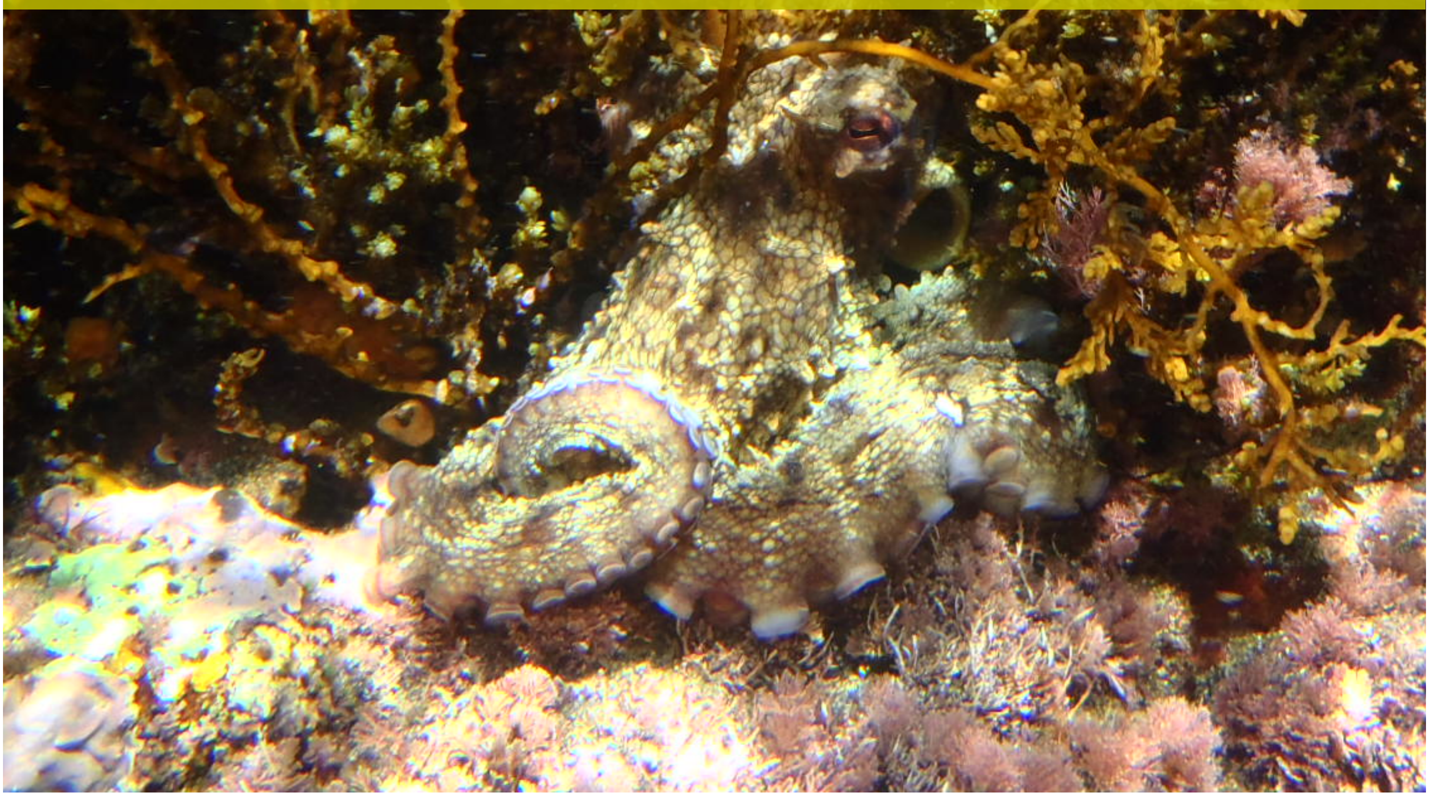
| Distribution | Species | Western | Central | Eastern |
|---|---|---|---|---|
| | RHODOPHYTA | | | |
| WD | *Ceramium diaphanum* (Lightfoot) Roth | 1 | 0 | 0 |
| Med | *Ceramium echionotum* J.Agardh | 1 | 1 | 0 |
| CT | *Ceramium cf secundatum* Lyngbye | 1 | 1 | 1 |
| WD | *Ceramium cf tenerrimum* (G.Martens) Okamura | 0 | 1 | 1 |
| WD | *Champia parvula* (C. Agardh) Harvey | 1 | 1 | 1 |
| WD | *Chondracanthus acicularis* (Roth) Fredericq | 1 | 1 | 1 |
| CT | *Chondracanthus teedei* (Mertens ex Roth) Kützing | 0 | 1 | 0 |
| CT | *Chondria capillaris* (Hudson) M.J.Wynne | 1 | 0 | 1 |
| CT | *Chondria coerulescens* (J.Agardh) Falkenberg | 0 | 1 | 0 |
| WD | *Chondria dasyphylla* (Woodward) C.Agardh | 0 | 1 | 1 |
| CT | *Chylocladia verticillata* (Lightfoot) Bliding | 1 | 0 | 0 |
| WD | *Corallina* Linnaeus | 1 | 1 | 1 |
| WT | *Cottoniella filamentosa* (M.A.Howe) Børgesen | 0 | 0 | 1 |
| WD | *Crouania attenuata* (C.Agardh) J.Agardh | 0 | 1 | 1 |
| WT | *Cryptonemia seminervis* (C.Agardh) J.Agardh | 0 | 0 | 1 |
| WD | *Dasya* C. Agardh | 1 | 1 | 1 |
| WT | *Digenea simplex* (Wulfen) C.Agardh | 0 | 0 | 1 |
| CT | *Drachiella spectabilis* J. Ernst & Feldmann. | 1 | 1 | 1 |
| CT | *Dudresnaya verticillata* (Withering) Le Jolis | 0 | 0 | 1 |
| CT | *Falkenbergia rufolanosa* (Harvey) F.Schmitz | 1 | 1 | 1 |
| Med | *Gastroclonium clavatum* (Roth) Ardissone | 1 | 1 | 1 |
| WD | *Gastroclonium reflexum* (Chauvin) Kützing | 1 | 1 | 0 |
| CT | *Gastroclonium ovatum* (Hudson) Papenfuss | 0 | 1 | 1 |
| WD | *Gayliella flaccida* (Harvey ex Kützing) T.O.Cho & L.J.McIvor | 1 | 1 | 1 |
| CT | *Gelidium corneum* (Hudson) J.V.Lamouroux | 1 | 0 | 0 |
| WT | *Gelidium crinale* (Hare ex Turner) Gaillon | 1 | 1 | 1 |
| CT | *Gelidium microdon* Kützing | 1 | 0 | 0 |
| CT | *Gelidium pusillum* (Stackhouse) Le Jolis | 1 | 1 | 1 |
| WD | *Gelidium spinosum* (S.G. Gmelin) P.C. Silva in Silva, Basson & Moe | 1 | 1 | 1 |
| WT | *Gracilaria cf armata* (C.Agardh) Greville | 0 | 1 | 1 |
| CT | *Gracilaria multipartita* (Clemente) Harvey | 1 | 1 | 0 |
| CT | *Grateloupia lanceola* (J.Agardh) J.Agardh | 0 | 1 | 0 |
| WT | *Griffithsia opuntioides* J.Agardh | 1 | 1 | 1 |
| CT | *Gymnogongrus crenulatus* (Turner) J.Agardh | 1 | 1 | 0 |
| CT | *Gymnogongrus griffithsiae* (Turner) Martius | 0 | 1 | 1 |
| CT | *Haliptilon squamatum* (L.) H.W.Johansen et al. | 0 | 0 | 1 |
| WT | *Halopithys incurva* (Hudson) Batters | 1 | 0 | 1 |
| Med | *Haraldia lenormandii* (Derbès & Solier) Feldmann | 0 | 1 | 0 |
| WT | *Herposiphonia secunda* (C.Agardh) Ambronn | 1 | 1 | 1 |
| WD | *Hildenbrandia rubra* (Sommerfelt) Meneghini | 1 | 0 | 1 |
| WT | *Hypnea musciformis* (Wulfen) J.V.Lamouroux | 1 | 0 | 1 |
| CT | *Hypoglossum hypoglossoides* (Stackhouse) F.S.Collins & Hervey | 1 | 0 | 1 |
| CT | *Jania longifurca* Zanardini | 1 | 1 | 1 |
| WD | *Jania rubens* (L.) J.V.Lamouroux | 1 | 1 | 1 |
| Med | *Jania virgata* (Zanardini) Montagne | 0 | 0 | 1 |
| WD | *Laurencia obtusa* (Hudson) J.V.Lamouroux | 1 | 1 | 1 |
| WT | *Liagora distenta* (Mertens ex Roth) J.V.Lamouroux | 1 | 1 | 0 |
| CT | *Lithophyllum byssoides* (Lamarck) Foslie | 1 | 0 | 0 |
| WD | *Lithophyllum corallinae* (P.L. Crouan & H.M. Crouan) Heydrich | 1 | 1 | 1 |
| CT | *Lithophyllum incrustans* Philippi | 1 | 1 | 1 |
| WD | *Lithophyllum pustulatum* (J.V. Lamouroux) Foslie | 0 | 1 | 1 |
| CT | *Lomentaria articulata* (Hudson) Lyngbye | 1 | 0 | 1 |
| CT | *Lomentaria catenata* Harvey | 1 | 0 | 0 |
| CT | *Lomentaria orcadensis* (Harvey) F.S.Collins | 1 | 1 | 0 |
| WD | *Lophosiphonia reptabunda* (Suhr) Kylin | 1 | 0 | 0 |
| WD | *Mesophyllum lichenoides* (J. Ellis) Marie Lemoine | 1 | 1 | 1 |
| WD | *Nemalion helminthoides* (Velley) Batters | 0 | 1 | 1 |
| WT | *Neogoniolithon brassica-florida* (Harvey) Setchell & L.R.Mason | 0 | 1 | 1 |
| CT | *Osmundea hybrida* (A.P.de Candolle) K.W.Nam | 1 | 1 | 1 |
| WT | *Osmundea pinnatifida* (Hudson) Stackhouse | 1 | 1 | 0 |
| WT | *Palisada perforata* (Bory de Saint-Vincent) K.W.Nam | 0 | 0 | 1 |
| WT | *Peyssonnelia* Decaisne | 1 | 1 | 1 |

**Appendix S3 (cont.)-** Species list of macrophytes observed at each subregion identified along Alboran Sea: western, central and eastern. Absence: 0, Presence: 1. Climatic affinity is indicated as warm-temperate (WT), cold-temperate (CT), Mediterranean (Med) and Wide Distributed (WB).

| Distribution | Species | Western | Central | Eastern |
|---|---|---|---|---|
| | RHODOPHYTA | | | |
| CT | *Peyssonnelia dubyi* P.L.Crouan & H.M.Crouan | 0 | 0 | 1 |
| WD | *Plocamium cartilagineum* (Linnaeus) P.S.Dixon | 1 | 1 | 0 |
| CT | *Plocamium raphelisianum* P.J.L.Dangeard | 0 | 1 | 0 |
| WD | *Polysiphonia* Greville | 0 | 1 | 0 |
| CT | *Porphyra cf leucosticta* Thuret | 1 | 1 | 0 |
| WT | *Pterocladiella capillacea* (S.G. Gmelin) Santelices & Hommersand | 1 | 1 | 0 |
| CT | *Pterosiphonia complanata* (Clemente) Falkenberg | 1 | 1 | 0 |
| CT | *Pterosiphonia parasitica (Hudson) Falkenberg* | 0 | 1 | 0 |
| WD | *Pterosiphonia pennata* (C.Agardh) Sauvageau | 1 | 1 | 1 |
| Med | *Pterothamnion crispum* (Ducluzeau) Nägeli | 1 | 0 | 0 |
| CT | *Rhodophyllis divaricata* (Stackhouse) Papenfuss | 0 | 1 | 1 |
| Med | *Rhodymenia ardissonei* (Kuntze) Feldmann | 0 | 0 | 1 |
| WD | *Rhodymenia pseudopalmata* (J.V.Lamouroux) P.C.Silva | 1 | 1 | 1 |
| Med | *Rissoella verruculosa* (Bertoloni) J. Agardh | 0 | 1 | 0 |
| WT | *Rytiphlaea tinctoria* (Clemente) C.Agardh | 1 | 0 | 0 |
| CT | *Schottera nicaeensis* (J.V.Lamouroux ex Duby) Guiry & Hollenberg | 1 | 0 | 1 |
| CT | *Scinaia furcellata* (Turner) J.Agardh | 0 | 0 | 1 |
| CT | *Sphaerococcus coronopifolius* Stackhouse | 1 | 0 | 1 |
| CT | *Taenioma nanum* (Kützing) Papenfuss | 0 | 1 | 1 |
| CT | *Trailliella intricata* Batters* | 1 | 0 | 0 |
| | CHLOROPHYTA | | | |
| WT | *Acetabularia acetabulum* (Linnaeus) P.C. Silva | 0 | 0 | 1 |
| WT | *Anadyomene stellata* (Wulfen) C.Agardh | 0 | 0 | 1 |
| Med | *Bryopsis cupressina* J.V.Lamouroux | 0 | 1 | 0 |
| WD | *Bryopsis hypnoides* J.V.Lamouroux | 1 | 1 | 0 |
| WT | *Caulerpa prolifera* (Forsskål) J.V.Lamouroux | 0 | 0 | 1 |
| WT | *Caulerpa racemosa* (Forsskål) J.Agardh | 0 | 0 | 1 |
| WD | *Chaetomorpha aerea* (Dillwyn) Kützing | 1 | 0 | 1 |
| WD | *Chaetomorpha ligustica* (Kützing) Boergesen | 0 | 1 | 0 |
| WD | *Cladophora cf albida* (Nees) Kutzing | 1 | 1 | 1 |
| CT | *Cladophora cf hutchinsiae* (Dillwyn) Kützing | 1 | 1 | 1 |
| CT | *Cladophora laetevirens* (Dillwyn) Kützing | 1 | 1 | 1 |
| WD | *Cladophora prolifera* (Roth) Kützing | 1 | 1 | 1 |
| CT | *Codium adhaerens* C.Agardh | 1 | 1 | 0 |
| CT | *Codium fragile* (Suringar) Hariot | 1 | 0 | 0 |
| WD | *Codium vermilara* (Olivi) Delle Chiaje | 0 | 1 | 1 |
| WT | *Dasycladus vermicularis* (Scopoli) Krasser in Beck & Zahlbruckner | 0 | 0 | 1 |
| CT | *Derbesia marina* (Lyngbye) Solier | 1 | 0 | 0 |
| WT | *Flabellia petiolata* (Turra) Nizamuddin | 0 | 0 | 1 |
| WT | *Halimeda tuna* (J.Ellis & Solander) J.V.Lamouroux | 0 | 0 | 1 |
| CT | *Pedobesia simplex* (Meneghini ex Kützing) M.J.Wynne & Leliaert | 1 | 0 | 0 |
| WT | *Ulva clathrata* (Roth) C.Agardh | 1 | 0 | 0 |
| WD | *Ulva compressa* Linnaeus | 1 | 1 | 1 |
| WD | *Ulva flexuosa* Wulfen | 1 | 0 | 0 |
| WD | *Ulva linza Linnaeus* | 0 | 1 | 1 |
| WD | *Ulva rigida* C.Agardh | 1 | 1 | 1 |
| WT | *Valonia utricularis* (Roth) C.Agardh | 1 | 0 | 1 |

# Chapter 5

## Preliminary assessment of the identity and genetic population structure of *Cystoseira ericaefolia* group in southern Iberian Peninsula.



*"The extent of genetic divergence between refugial genomes varies among species and is a measure of the time of their separation. Populations in regions where lineages persist through several climatic cycles can accumulate genetic differences and possibly speciate. They are not affected by their relatives that colonize other parts, but are eliminated by climatic reversals."*

Godfrey M Hewitt

(Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London (2004) 359: 183-195)

*Octopus vulgaris* in a *Cystoseira amentacea* stand. Cabo de Gata. August 2012.

Photograph by Ricardo Bermejo Lacida

# Preliminary assessment of the identity and genetic population structure of *Cystoseira ericaefolia* group in southern Iberian Peninsula.

**Authors:** Ricardo Bermejo, Joana Ferreira-Costa, Aschwin Engelen, Juan J. Vergara, Ignacio Hernández, Nuria Marbà, Carlos Duarte, Ester Serrão.

**In preparation**

## ABSTRACT

The *Cystoseira ericaefolia* group is conformed for three closely related species: *C. amentacea*, *C. mediterranea* and *C. tamariscifolia*. The former and the latter overlap their distribution area in eastern Alboran Sea, where they can occur in sympatry or parapatry. However, serious concerns arise due to the morphological plasticity of these species and the possible existence of hybrids. To clarify the taxonomic situation of *C. ericaefolia* group in southern Iberian Peninsula and the possible existence of hybrids, nuclear microsatellites and mitochondrial markers were used. Nine sites were sampled along this area. In eight sites only one of the two species was found, and in one locality both species were found in parapatry. Based on sequences of the mitochondrial 23S obtained for several specimens from the studied area and others sequences of these species retrieved from GeneBank from distant Mediterranean and Atlantic localities, a phylogenetic analysis was developed. Genetic structure analyses based on microsatellites were performed to determine the levels of gene flow between the putative taxa. The phylogenetic analysis based on the mitochondrial 23S suggested that only one genetic entity was present in Alboran Sea. The analysis of the genetic population structure in the locality where supposedly both species were present did not show significant differences between the genetic entities identified. Moreover, when this parapatric population was compared with other populations of these species along southern Iberian Peninsula, *C. tamariscifolia* and *C. amentacea* from the site were more similar to each other than to the other populations. On the other hand, the genetic patterns along southern Iberian Peninsula suggested a relevant genetic flux between Atlantic and Mediterranean populations in Western and Central Alboran. In spite of the preliminary research, the results suggested that all specimens of *C. ericaefolia* found along Alboran Sea can be considered one specific entity, probably *C. tamariscifolia*.

## INTRODUCTION

The *Cystoseira ericaefolia* group is conformed for three closely related species: *C. amentacea*, *C. mediterranea* and *C. tamariscifolia* (Amico et al., 1985; Barceló et al., 1994; Amico, 1995). The latter is an Atlantic species distributed from the British Isles to Mauritania and the Cape Verde archipielago, being also present in the Mediterranean Sea in areas of Atlantic influence (southern Spain, Sicily, Malta, Morocco, Algeria and Tunisia) (Barceló-Martí et al., 2000; Guiry and Guiry, 2013; Rodríguez-Prieto et al., 2013). The other two species are considered Mediterranean neo-endemisms, being their distribution restricted to the western Mediterranean, Adriatic Sea and Aegean Sea. Bearing in mind the geological history of the Mediterranean Sea, *C. tamariscifolia* has been considered the ancestor of the other two species, which must be appeared independently (Amico et al., 1985; Gómez-Garreta et al., 1994).

These species are perennial, monoecious algae, producing sperm and large, non-motile eggs in hermaphroditic conceptacles (Barceló-Martí et al., 2000; Susini, Thibaut, et al., 2007). Fertilization is external, occurring within hours of gamete release (Guern, 1962). Zygotes are large and negatively buoyant; they rapidly sink, secrete adhesive wall polymers and adhere to surfaces at 12 h postfertilization (Guern, 1962). Thus, zygote dispersal is hypothesized to be very limited, as in other species of the Fucales (Clayton, 1990; Brawley et al., 1999). The *C. ericaefolia* species inhabit in wave exposed or moderately exposed places, in the littoral and upper sublittoral zone always near the surface (Barceló-Martí et al., 2000; Rodríguez-Prieto et al., 2013), forming sympatric or parapatric populations in areas where the distribution of the species is overlapped. In the Mediterranean Sea they constitute dense meadows, which are an important element of the littoral landscape, playing an essential role in the maintaining of the biodiversity and ecosystem functioning (Giaccone et al., 1994; Airoldi and Beck, 2007). Due to this ecological importance and the declined of their populations within the past few decades as consequence of anthropogenic disturbances (Borowitzka, 1972; Ballesteros et al., 1984; Thibaut et al., 2005), these species have been recently protected in the Mediterranean Sea (Annex II of the Barcelona Convention, COM/2009/0585 FIN).

Between two (Barceló-Martí et al., 2000; Ballesteros & Pinedo, 2004) and three (Flores-Moya et al., 1995) species of the *C. ericaefolia* group have been cited in Alboran Sea. However, the morphological plasticity of these species, their resemblance, and the seasonal variability in their appearance hamper their accurate identification (Gómez-Garreta et al., 1994). It is not uncommon to find individuals with intermediate morphologies that are impossible to assign unambiguously on the basis of morphology (Ballesteros and Catalán, 1981; Gómez-Garreta et al., 1994; Ballesteros and Pinedo, 2004). The observation of these morphologically intermediate plants in the field has been considered as evidence of natural hybridization (Sauvageau, 1912; Gómez-Garreta et al., 1982; Amico et al., 1985), which have been demonstrated in other species of the Fucales (e.g. Engel et al., 2005; Neiva et al., 2010; Zardi et al., 2011). Ballesteros & Catalán (1981) and Gómez-Garreta (com. pers.) pointed out the necessity to assess this group of species, a difficult task specially in places where the three entities have been reported. In this sense, Ballesteros & Pinedo, 2004 suggested that molecular tools would be helpful to complement conventional morphological and taxonomic approaches to clarify the identity of the specimens from south-eastern Iberian Peninsula.

Microsatellite markers have been successfully used to distinct taxon units (Bergstrom et al., 2005; Zardi et al., 2011) and/or admixture between close genetic entities (Wallace et al., 2004; Engel et al., 2005) in *Fucus* species, as well in others studies ranging from the population genetic and ecology (Engelen et al., 2001) to the phylogeography of Fucales (Olsen et al., 2010).

Therefore, the main objectives of the present study were: i) to assess the identity of putative *C. tamariscifolia* and *C. amentacea* var. *stricta* in the Alboran Sea; and ii) to characterize the genetic structure of *C. ericaefolia* along southern Spain using six microsatellite markers developed by MAREE (MARine Ecology and Evolution) Research group.

## MATERIAL AND METHODS

### Sampling sites

Specimens of *C. tamariscifolia* and *C. amentacea* var. *stricta* (hereafter referred to as *C. amentacea*) were collected in nine localities along the southern Iberian Peninsula (Fig. 1). These species overlap their distribution (Fig. 1, contact zone; Barceló-Martí et al., 2000) in the easternmost part of Alboran Sea, and they can be found forming sympatric and parapatric populations. In this case, only in one site (El Playazo, Almería) these species where found together in parapatry. Individuals of *C. amentacea* were found close to the surface forming a dense meadow, while individuals morphologically attributable to *C. tamariscifolia* were found scattered between 1 and 2 meters of deep. In the other eight sites, in two locations *C. amentacea* occurred alone, and in six *C. tamaricifolia* was the only species found.

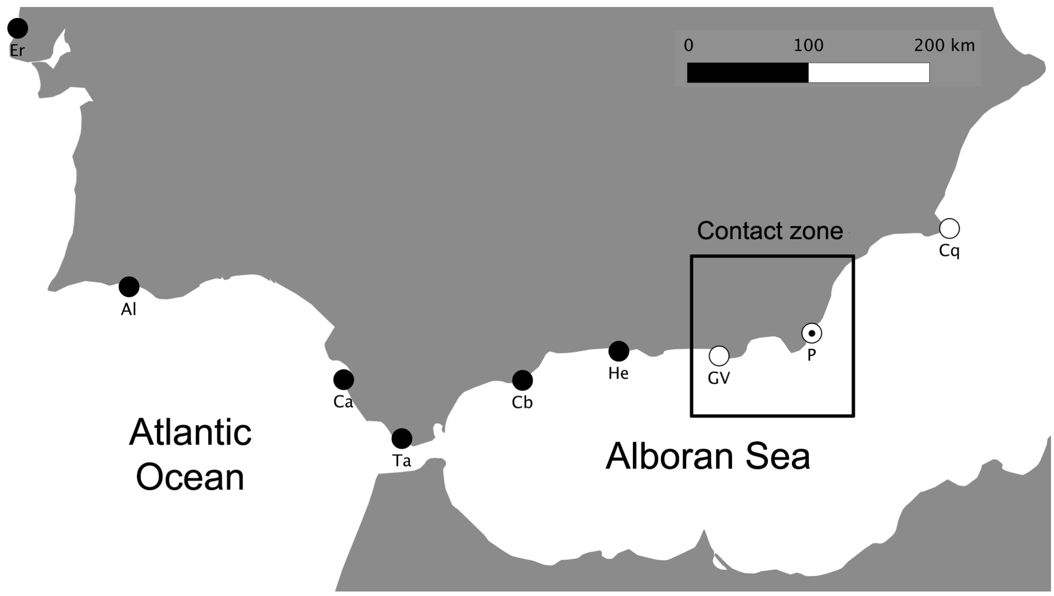



**Figure 1-** Geographical distribution of the different sampling points along the European Coast of the Gibraltar Strait and Western Alboran Sea. The geographical border proposed by the WFD between Atlantic and Mediterranean ecoregions is indicated with the black line. Er- Ericeia; Al- Albufeira; Ca- Cadiz; Ta- Tarifa island; Cb- Calaburras; He- Herradura; GV- Guardias Viejas; P- Playazo (i- intertidal; s- subtidal); Cq- Calblanque. Black dots- Populations of *C. tamariscifolia*; White dots- Populations of *C amentacea*; White dot with small black dot in the middle- Parapatric population of *C. tamariscifolia* and *C. amentacea*.

At each locality, between 27 and 32 individuals of *C. tamariscifolia* and/or *C. amentacea* were sampled. To minimize the damage to the sampled meadows of these endangered species, only a piece of the apical branch was collected per individual. The minimum distance between sampled individuals was 5 metres. After collection, samples were dried and stored in silica gel at room temperature.

Identification of *C. tamariscifolia* and *C. amentacea* was based on general overall cauloid morphology (Barceló-Martí et al., 2000; Cormaci et al., 2012). In this case, plants formed by a creeping axis from which several erect axes arise (caespitose plants) were considered as *C. amentacea* (Fig. 2a). On the other hand, plants with a single axis attached to the substratum by a disk or by thick-branched haptera, which may be fused (non caespitose plants), were assigned to *C. tamariscifolia* (Fig. 2b).


**Figure 2-** Habit of putative specimens of *C. tamariscifolia* (a) and *C. amentacea* (b) from El Playazo.

### DNA extraction genotyping and sequencing

DNA was isolated from 5–10 mg of dried tissue of the apical tips with the CTAB method, but a silica filter plate (Milipore MultiScreen HTS, FB Cat. # MSFBN6B10) was used instead of the silica fines step. Six microsatellite loci that were polymorphic in *C. tamariscifolia* (MAREE group, unpublished) were used to genotype each individual. The amount of template DNA used was 5 µL of diluted 1:10. Polymerase Chain Reactions (PCRs) were perform in 10 µL volumes containing 25 mM $MgCl_2$, 4 mM dNTPs, 10 µM forward primer (labeled), 10 µM reverse primer

(Operon), 0.5 U GOTaq (Promega), 5x GOTag polymerase buffer, and water to adjust volume to 10 µL per reaction. Amplifications were carried out on a Thermal Cycler 2720 (Applied Biosystems) using the following profile: initial denaturation at 94ºC for 5 minutes; 35 cycles of 94ºC for 30 seconds, followed by 30 seconds at specific anelling temperature for each primer, and 72ºC for 40 seconds; and a final extension at 72ºC for 20 minutes. PCR products were determined using an ABI 3730 automated sequencer (Applied Biosystems). Alleles were scored using STRand Analysis Software (Locke et al., 2000).

On the other hand, the partial mitochondrial 23S (mt 23S; c. 380-410-bp) was determined for eight individuals of *C. tamariscifolia*, three of *C. amentacea*, two of *C. mediterranea* and one of *C. spinosa* var. *tenuior*. Forward and reverse primers developed by Draisma et al. (2010) were used to amplify this gene region. The PCR reagents were the same that for microsatellite amplification, but the dNTPs concentration was lower, 1 mM instead 4mM. PCRs were perform with a Thermal Cycler 2720 (Applied Biosystems) using the following profile: initial denaturation at 94ºC for 2 minutes; 35 cycles of 94ºC for 30 seconds, followed by 30 seconds at 50º C, and 72ºC for 40 seconds; and a final extension at 72ºC for 5 minutes. Finally, forward, or forward and reverse strands were sequenced using an ABI 3730 automated sequencer (Applied Biosystems).

**Data analyses**

*Preliminary phylogenetic analysis based on partial mitochondrial 23S*

To check the identity and the affinity of *C. tamariscifolia* and *C. amentacea* from southern Iberian Peninsula, a preliminary phylogenetic analysis based on the mt 23S was developed. In addition to the fourteen obtained sequences for mt 23S, other five sequences of this gene were retrieved from GeneBank: one sequence of *C. spinosa* var. *tenuior* from Menorca, two of *C. tamariscifolia* from Galicia (North-western Iberian Peninsula), and two of *C. amentacea* from Sicily. These nineteen sequences of four *Cystoseira* species (*C. tamariscifolia*, *C.amentacea*, *C. mediterranea* and *C. spinosa*) were aligned using MEGA 4.2 and ClustalW (Larkin et al., 2007). *C. spinosa* specimens were considered as outgroup sequences. The software Mr. Model Test 1.1 (Posada and Crandall, 1998; Nylander, 2004) was run in PAUP* (Swofford, 2002) to select the best model of DNA substitution. Based on Akaike information criterion scores, the selected model was General Time Reversal model with substitution rates varying between among sites according to a gamma distribution (GTR+G). Bayesian analyses were perform using MrBayes (Ronquist and Huelsenbeck, 2003) according to the substitution model selected, leaving the remaining options as default (nst = 6; rate=gamma; statefreqpr = Dirichlet (1,1,1,1)). Two parallel Metropolis-coupled Markov chain Monte Carlo searches, each with four chains, were run for 2,000,000 generations, sampling trees and parameters every 100 generations

*Concordance between morphological and genetic entities in "El Playazo"*

Departures from Hardy-Weinberg Equilibrium can be due to biological factors such as population structure, non-random mating and selection against hybrids. Inbreeding and selfing is hypothesized be frequent considering reproductive traits of these species, beside the occurrence of hybrids is also probable. These facts may induce linkage and Hardy-Weinberg disequilibrium, which may not be suitable for assignment tests in Structure software. For this reason, InStruct software (Gao et al., 2007) was used instead Structure (Pritchard et al., 2000) to assign individuals to species and to detect putative hybrids from microsatellites in "El Playazo". This software applies a Bayesian clustering approach similar than Structure, but InStruct takes into account the possibility of selfing or inbreeding. In this case, a model considering individual selfing rates was assumed, where there were from 1 to 5 populations (K clusters). Each K was replicated 4 times for 200,000 iterations after a burn-in period of 100,000, without any prior information on the population of origin of each sampled individual. The height of the modal value of ΔK distribution for the posterior probability of the data for a given K was used as an indicator of the strength of the signal detected by Instruct and considered as the real number of K cluster (Evanno et al., 2005). In this case were two the number of clusters considered (Fig. S1a). Subsequently, to assess if genetic and morphological entities identified are related, or in contrast phenotype and genotype are independent, a chi-square ($\chi^2$) test was carried out.

Analysis of molecular variance (AMOVA) was used to reveal the relationships between individuals and putative species. The individuals were divided into three putative taxa based on the Bayesian assignment analysis: *C. tamariscifolia*, *C. amentacea* and hybrids. The AMOVA (n = 999 permutations) was computed using GenAlex 6.5 (Peakall and Smouse, 2006). Furthermore, the mean number of alleles, the average expected heterozygosity ($H_e$, sensu Nei 1978), the observed heterozygosity ($H_o$), the number of privative alleles and departures from Hardy-Weinberg equilibrium were calculated for each group using GenAlex 6.5 (Peakall and Smouse, 2006).

*Genetic structure of "C. ericaefolia" along Alboran Sea*

Finally, to elucidate the relationships among clusters and populations, a neighbour-joining (NJ) tree and a Bayesian assignment analyses from microsatellites allele frequency data were developed. The NJ tree was based on Cavalli-Sforza and Edwards (1967) chord distances, and it was constructed using GenePop software. Confidence levels on tree topology were estimated by the percentage of 5,000 bootstraps perform from resampling allele frequencies. For the assignment test using Instruct, previous parameters and criteria were considered. In this sense, the best K values according to Evanno et al. (2005) were two and four (Fig S1b). For the AMOVA, localities were divided in three and five groups based on the results of the Bayesian assignment test: i) Atlantic (Er, Al, Ca and Ta), Western and Central Alboran (Cb, He, GV) and

Eastern Alboran (Pi, Ps and Cq); and ii) Portugal (Er and Al), Gulf of Cadiz (Ca and Ta), Western and Central Alboran (Cb, He and GV), Eastern Alboran (Pi and Ps), and Cape of Palos (Cq). On the other hand, a Mantel test was developed to assess the influence of geographic distance on population genetic differentiation measured as $F_{ST}$.

## RESULTS

*Phylogenetic analysis based on partial mt23S gene*

The partial mt23S gene data set consisted in 383 nt, four being parsimony-informative within the ingroup (*C. ericaefolia*; Table 1). The phylogeny based on the partial mitochondrial 23S gene did not support the morphological delimitation of *C. amentacea* and *C. tamariscifolia* (Fig. 3). The analysis revealed that *C. amentacea* and *C. tamariscifolia* from the Atlantic coast of the Iberian Peninsula and the Alboran Sea are divided in two groups without geographical or taxonomic support. From one of these groups, a specimen of *C. amentacea* and putative *C. mediterranea* from the north-western Mediterranean localities emerged as a supported clade (posterior bayesian probability > 0.9).

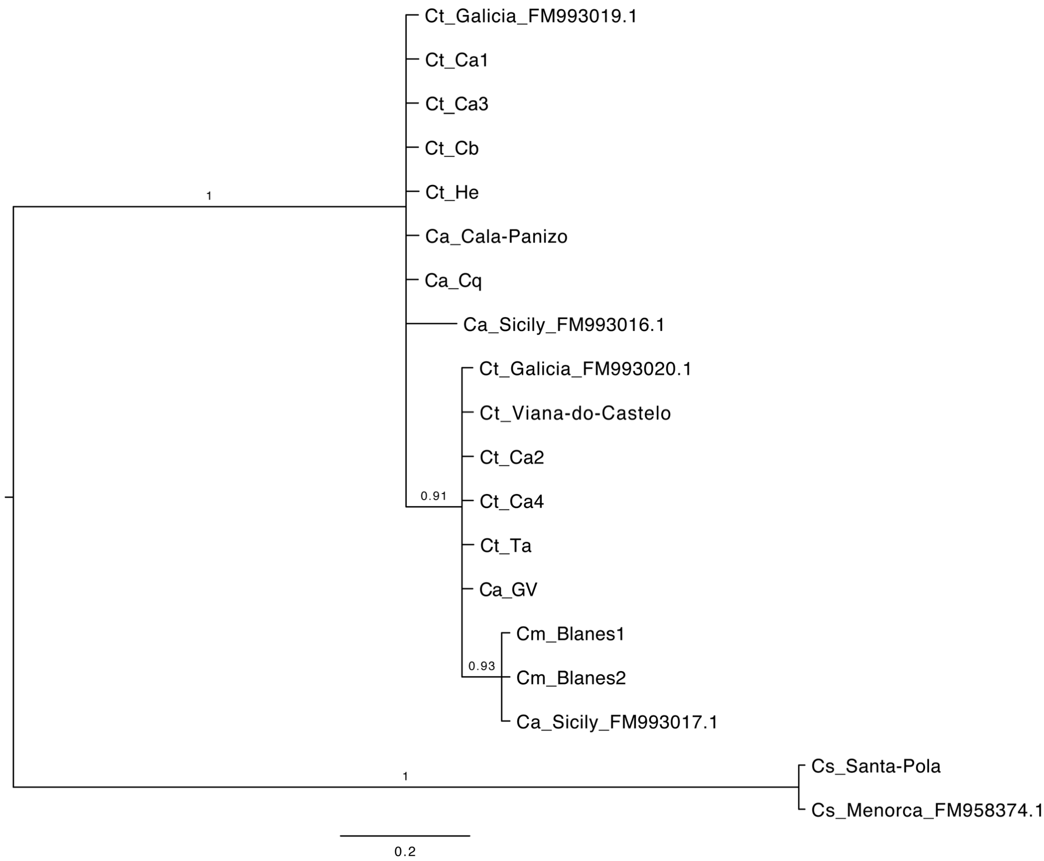



**Figure 3-** Phylogenetic tree of fragments of a mt 23 S gene sequences of *Cystoseira ericaefolia* denoting the phylogenetic position of *C. tamariscifolia* (Ct) and *C. amentacea* (Ca) specimens from southern Iberian Peninsula. *C. spinosa* (C. sp) was used as outgroup. Individuals are labelled as Specie_Locality_GeneBank ascension number. Numbers above the branches are Bayesian posterior probabilities (>50%).

| | All taxa | *C ericaefolia* |
|---|---|---|
| Length of alignment | 383 | 383 |
| Number of constant positions | 346 | 377 |
| Number of variable positions | 37 | 5 |
| Number of parsimony-informative positions | 37 | 4 |
| Number of gapped positions | 4 | 0 |

**Table 1-** Summary of alignment properties.

*Definition of genetic entities based on microsatellites, and their concordance with morphological traits*

The analysis showed a strong association of individuals identified as *C. amentacea* with one cluster ($q_1$ = 0.838) and a weaker association of individuals identified as *C. tamariscifolia* with the other cluster ($q_2$ = 0.673) (Fig. 4). In this case, individuals showing a $q_1^{(i)}$ equal or greater than 0.90 were assigned to *C. amentacea* and those with a $q_2^{(i)}$ equal or higher than 0.90 were assigned to *C. tamariscifolia*. Individuals that showed a $q_1^{(i)}$ between 0.10 and 0.90 were considered genetically intermediate between these species. Although the value of this criterion was somewhat arbitrary, it did not greatly affect pattern of assignements of individuals to clusters (Engel et al., 2005).


**Figure 4-** Proportion of ancestry of each sampled individual (columns) as inferred with InStruct for six microsatellite loci in "El Playazo", assuming the admixture model considering individual selfing rates.

The genetic cluster and cauloid number were significantly associated ($\chi^2$ = 26.23; $p < 0.0001$) as can be observed in Table 2. However, the percentage of genetically intermediate individuals was high (approximately 26%) and no significant differences were observed between intertidal and subtidal ($\chi^2$ = 1.023; $p > 0.05$).

| Genetic cluster | OC | MC | N | A | *He* | *Ho* | $F_{IS}$ | PA | SSA (> 0.05) |
|---|---|---|---|---|---|---|---|---|---|
| *C. amentacea* | 4 | 22 | 26 | 8.67 ± 0.92 | 0.69 ± 0.06 | 0.58 ± 0.05 | 0.15 ± 0.05 | 13 | 3 |
| *C. tamariscifolia* | 18 | 2 | 20 | 8.17 ± 0.60 | 0.79 ± 0.02 | 0.66 ± 0.03 | 0.16 ± 0.05 | 12 | 6 |
| Intermediate | 10 | 6 | 16 | 8.83 ± 0.75 | 0.80 ± 0.01 | 0.68 ± 0.06 | 0.14 ± 0.09 | 8 | 0 |

**Table 2-** Classification of "El Playazo" individuals by number of cauloids (OC - One cauloid; MC - Multiple cauloids) and genetic clustering results with a summary of genetic diversity statistic for each cluster. N, total number of assigned individuals; A, mean number of alleles per locus; *He* total expected heterozygosity; *Ho* observed heterozygosity; $F_{IS}$ Fixation index; PA, number of privative alleles; SSA (> 0.05), number of privative alleles at a frequency > 0.05.

Among the three groups, the allele frequency distributions was similar across loci, with the exception of individuals identified as *C. amentacea*, which showed relatively low polymorphism at Ct 2.7 (allele 159; frequency > 0.6) and Ct 2.8 (allele 205; frequency > 0.7) loci (Fig. 5). Genetic diversity in terms of mean number of alleles and expected heterozygosity was similar among the three clusters (ANOVA, p-values > 0.05) (Table 3). The number of alleles ranged from six at locus Ct 2.4 in *C. amentacea* to twelve at locus Ct 3.3 in intermediate group. In general terms, the three groups showed deficits in heterozygotes for all loci (average $F_{IS}$ ranged from 0.14 to 0.16). Departures from Hardy-Weinberg equilibrium were significant in seven out of the eighteen comparisons.

The AMOVA analysis revealed that most genetic variation occurred within taxa (92%), being the variation among putative taxa only 8%. The fixation index $F_{ST}$ yielded a significant, but low variation being the average value of 0.047, indicating little differentiation.

| **Specie** | | **Ct2.4** | **Ct2.7** | **Ct2.8** | **Ct2.9** | **Ct3.3** | **Ct4.3** |
|---|---|---|---|---|---|---|---|
| ***C. amenatacea*** | **N** | 26 | 24 | 26 | 24 | 26 | 25 |
| | **Na** | 6 | 9 | 6 | 9 | 11 | 11 |
| | **Ho** | 0.58 | 0.42 | 0.46 | 0.63 | 0.77 | 0.64 |
| | **uHe** | 0.66 | 0.60 | 0.46 | 0.86 | 0.82 | 0.84 |
| | **$F_{IS}$** | 0.13 | 0.30 | -0.01 | 0.28* | 0.06 | 0.23* |
| | **PA** | 0 | 2 | 1 | 4 | 2 | 4 |
| | **SSA (>0.05)** | 0 | 0 | 1 | 1 | 1 | 0 |
| ***C. tamariscifolia*** | **N** | 20 | 19 | 19 | 20 | 20 | 20 |
| | **Na** | 9 | 9 | 6 | 8 | 7 | 10 |
| | **Ho** | 0.75 | 0.58 | 0.63 | 0.70 | 0.75 | 0.55 |
| | **uHe** | 0.81 | 0.81 | 0.72 | 0.81 | 0.82 | 0.88 |
| | **$F_{IS}$** | 0.08 | 0.28* | 0.12 | 0.13 | 0.09 | 0.38* |
| | **PA** | 1 | 2 | 0 | 3 | 2 | 4 |
| | **SSA (>0.05)** | 0 | 2 | 0 | 1 | 1 | 2 |
| **Intermediate** | **N** | 16 | 13 | 16 | 15 | 16 | 16 |
| | **Na** | 8 | 8 | 7 | 8 | 12 | 10 |
| | **Ho** | 0.94 | 0.69 | 0.75 | 0.47 | 0.63 | 0.63 |
| | **uHe** | 0.77 | 0.82 | 0.81 | 0.84 | 0.85 | 0.88 |
| | **$F_{IS}$** | -0.21 | 0.15 | 0.08 | 0.44* | 0.27* | 0.29* |
| | **PA** | 1 | 1 | 0 | 2 | 3 | 3 |
| | **SSA (>0.05)** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Overall** | **$F_{IT}$** | 0.27 | 0.23 | 0.23 | 0.22 | 0.26 | 0.22 |
| | **$F_{ST}$** | 0.05 | 0.05 | 0.03 | 0.05 | 0.06 | 0.05 |

**Table 3-** Summary of genetic diversity statistic for each cluster and locus. N, number of individuals; Na, number of alleles; Ne, efective number of alleles; *H*o, observed heterozygosity; u*He*, unbiased expected heterozygosity; $F_{IS}$ Fixation index (* Significant deviation from Hardy-Weinberg equilibrium, p-value < 0.05); PA, number of privative alleles; SSA (> 0.05), number of privative alleles at a frequency > 0.05.

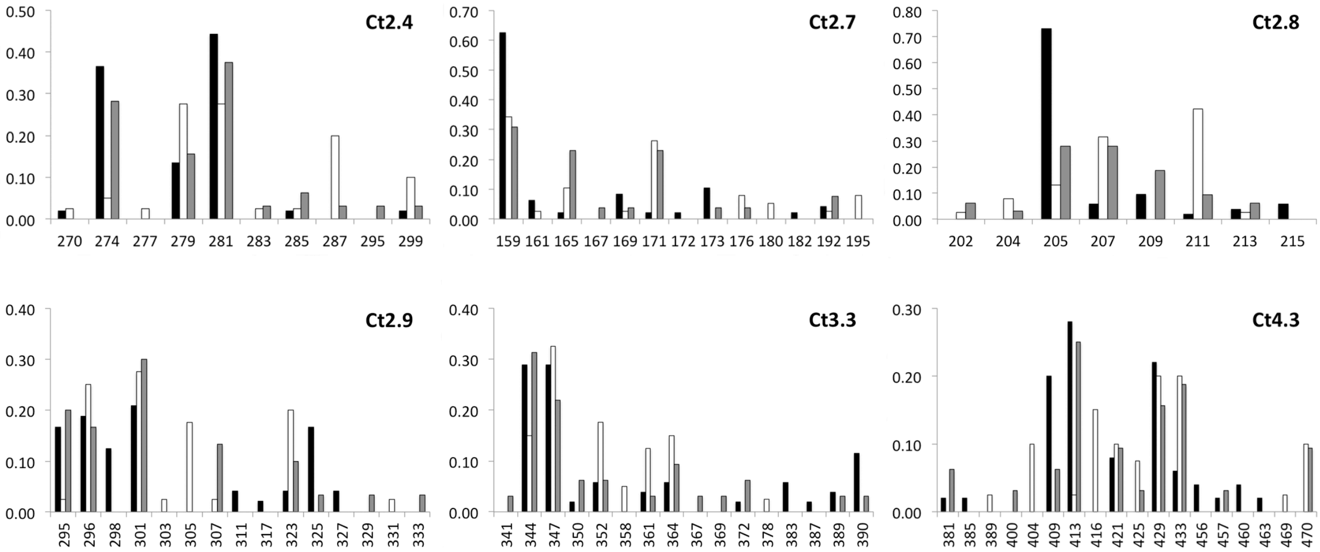



**Figure 5-** Allele frequencies at the six microsatellite loci for the three taxa identified in "El Playazo": *C amentacea* (black), *C tamariscifolia* (white) and hybrids (grey)

*Genetic structure of "C. ericaefolia" along Alboran Sea*

The NJ tree and the assignment analysis do not support the existence of two genetic entities in southern Iberian Peninsula. These results revealed two distinct clades separating population from the Atlantic Ocean and the Alboran Sea (Fig. 6 and 7). In the case of the NJ tree, this separation is less clear and more complex. Although populations are grouped by geographical location, the highest distances occur between sites instead between groups of populations (Fig. 6). This fact suggests that genetic variation can be related to geographical isolation. In this sense, the results of Mantel test (Fig. 8) showed a significant correlation between geographical distance and $F_{ST}$ (Mantel r = 0.733; Fig S2). The results of $F_{ST}$ yielded a moderate variation among sites, being the average value for all loci 0.128, ranging from 0.026 (Cb-He distant 80 km) to 0.271 (Er-Cq distant 1087 km; Table S1).

The assignment analysis for K=2 (Fig. 7) clearly revealed an Atlantic (Er, Al, Ca, Ta) and a Mediterranean (Pi, Ps, Cq) cluster, with a contact area in Western and Central Alboran (Cb, He, GV), where the two clusters are mixed. Considering K=4, the genetic structure patterns are similar, but Atlantic and eastern Alboran groups are divided in two subgroups, which are all represented in admixture in western and central Alboran.

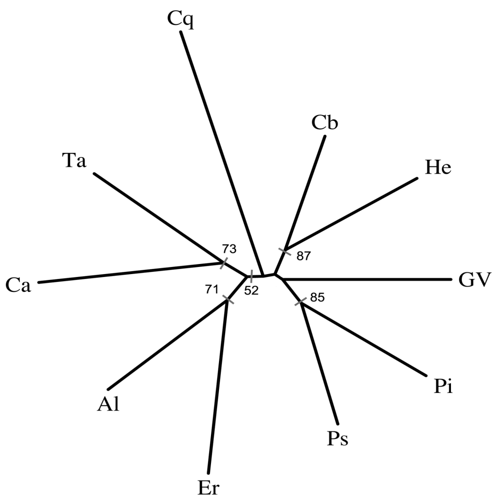



**Figure 6-** Neighbour-joining tree based on Cavalli-Sforza and Edwards's (1967) chord distances. Numbers (%) indicate robustness of given branch out of 5000 bootstrap permutations of allele frequencies. Code of localities as in Fig 1.

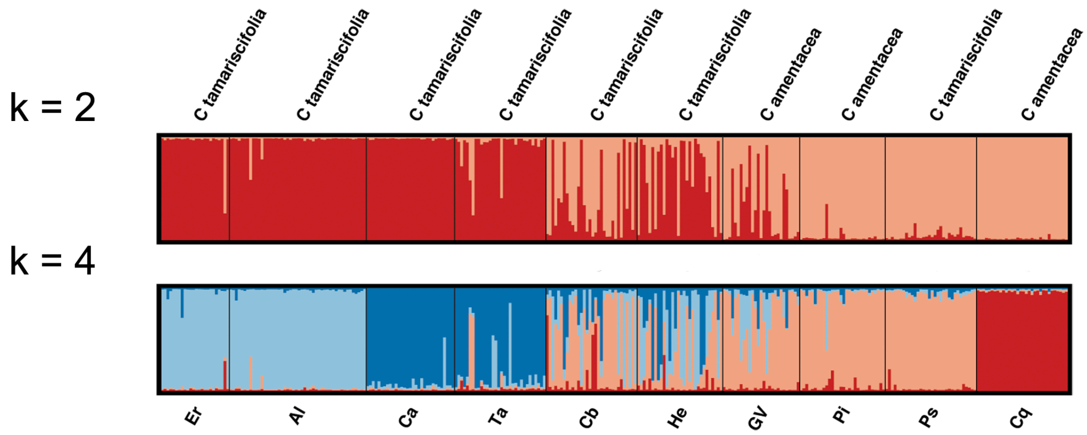



**Figure 7-** Proportion of ancestry of each sampled individual (columns) as inferred with InStruct for six microsatellite loci, assuming the admixture model considering individual selfing rates. Code of localities as in Fig 1.

The AMOVA results (Table 4) considering three areas showed that most genetic variation occurred within individuals (80%), being 17% the variation among population and only 3% among areas. Nevertheless, if five areas are considered a higher percentage of the variance is explained among areas (10%) and the variation among populations is reduced at 11%. In any case, this fact suggests a strong differentiation within populations at the spatial scale considered.

All localities exhibited high positive $F_{IS}$ values (heterozygote deficit) (Table 5), being these values lower in Cadiz (Ca) and Tarifa (Ta). There was no geographical pattern of heterozygote deficiency. Average number of alleles per locus ranged from 4.8 in Er to 17 in Cb. Private allelic richness ranged from 1 in Er and Ca to 11 in Cb. Localities from western and central Alboran yielded the maximum values of genetic diversity in terms of mean number of alleles, and also were the most singular showing the maximum number of privative alleles (tables 5 and 6). By contrast, Atlantic localities presented the lowest values of genetic diversity and the less differentiated allelic composition, despite a major number of specimens were considered (Table 6). In eastern Alboran, the relation between privative alleles and number of alleles was maxima, and privative alleles had a major quantitative importance for the genetic structure of populations (i.e., these localities SSA showed the highest values).

| a) | Source of variation | d.f. | Variance | P-value |
|---|---|---|---|---|
| | Among areas | 2 | 3% | < 0.001 |
| | Among localities | 7 | 17% | < 0.001 |
| | Within localities | 308 | 80% | < 0.001 |
| b) | Among areas | 4 | 10% | < 0.001 |
| | Among localities | 5 | 11% | < 0.001 |
| | Within localities | 308 | 79% | < 0.001 |

**Table 4-** Analysis of molecular variance (AMOVA) of spatial genetic variation between the three areas identified, for six microsatellite markers. a) AMOVA results considering three groups of populations: Atlantic (Er, Al, Ca and Ta), western and central Alboran (Cb, He and GV), eastern Alboran (Pi, Ps and Cq); b) AMOVA results considering five groups of populations: Atlantic (Er and Al), Gulf of Cadiz (Ca and Ta), western and central Alboran (Cb and He), eastern Alboran (GV, Pi and Ps) and Cape of Palos (Cq).

| Region | Code | Specie | LONG | LAT | N | A | PA | SSA (>0.05) | $F_{IS}$ |
|---|---|---|---|---|---|---|---|---|---|
| Atlantic | Er | Ct | 39.002ºN | 9.364ºW | 24 | 4.8 ± 1.0 | 1 | 0 | 0.25 ± 0.15 |
| Atlantic | Al | Ct | 37.082ºN | 8.257ºW | 48 | 6.7 ± 0.9 | 2 | 1 | 0.14 ± 0.11 |
| Atlantic | Ca | Ct | 36.478ºN | 6.264ºW | 31 | 7.7 ± 0.8 | 1 | 1 | 0.07 ± 0.08 |
| Atlantic | Ta | Ct | 36.058ºN | 5.710ºW | 32 | 11.7 ± 0.9 | 4 | 1 | 0.05 ± 0.07 |
| Alboran | Cb | Ct | 36.506ºN | 4.642ºW | 32 | 17.0 ± 2.0 | 11 | 1 | 0.11 ± 0.05 |
| Alboran | He | Ct | 36.737ºN | 3.755ºW | 30 | 11.6 ± 1.7 | 3 | 1 | 0.20 ± 0.02 |
| Alboran | GV | Ca | 36.696ºN | 2.852ºW | 27 | 10.7 ± 0.6 | 2 | 1 | 0.17 ± 0.05 |
| Alboran | Pi | Ca | 36.858ºN | 2.004ºW | 30 | 9.8 ± 1.2 | 4 | 2 | 0.20 ± 0.06 |
| Alboran | Ps | Ct | " | " | 32 | 9.8 ± 1.1 | 4 | 1 | 0.17 ± 0.05 |
| Alboran | Cq | Ca | 37.606ºN | 0.725ºW | 32 | 7.7 ± 1.3 | 8 | 6 | 0.11 ± 0.07 |

**Table 5-** Sampling locations and diversity measures for microsatellites. Code of localities can be found in Fig 1; Species can be *C tamariscifolia* (Ct) or *C amentacea* (Ca); LONG and LAT, Longitude and Latitude; N, number of individuals sampled; A, mean number of alleles per locus; PA, number of privative alleles; SSA (> 0.05), number of privative alleles at a frequency > 0.05; $F_{IS}$, Fixation index.

## DISCUSION

The obtained results do not support the existence of two genetic entities of *C. ericaefolia* in Alboran Sea. According to the phylogeny infer from the mt 23S (Fig. 3), individuals identified as *C. amentacea* in Alboran Sea would be closer related to *C. tamaricifolia* from the Atlantic Ocean than to Mediterranean specimens of *C. mediterranea* or *C. amentacea* (Fig 3). The analysis of the genetic population structure infer from nuclear microsatellites in "El Playazo", where supposedly both species were present, did not show important differences between the genetic entities identified. Moreover, when this parapatric population of *C. tamariscifolia* and *C.*

*amentacea* is compared with other populations of these species along southern Iberian Peninsula, *C. tamariscifolia* and *C. amentacea* from "El Playazo" are more similar to each other than to the other populations (Fig 6 and 7). Furthermore, the genetic patterns along southern Iberian Peninsula suggest an important genetic flux between Atlantic and Mediterranean populations in western and central Alboran (Fig 7). Therefore, in spite of the preliminary research, the results suggest that all specimens of *C. ericaefolia* found along Alboran Sea can be considered one specific entity, probably *C. tamariscifolia*.

From the analysis of the mt 23S gene fragment, the morphological differences observed between *C. tamariscifolia* and *C. amentacea* from southern Iberian Peninsula lack a genetic basis. This analysis showed little differences between the three species belong to *C. ericaecifolia* group. In spite of this statement, some considerations must be taken in account. For instance, considering that incomplete reproductive isolation and hybridization can allow organelle exchange across species boundaries, the use of organellar DNA is not the most suitable to resolve the phylogeny of very close species (Coyer et al., 2006; Neiva et al., 2010). Moreover, the mt 23S provide few informative characters and it is more suitable to infer intergeneric than interspecific relationships in the family Sargaceae (Draisma et al., 2010). On the other hand, non type localities have been considered in the analysis for *C. tamariscifolia* (Cornwall, UK) and *C. amentacea* (Alger, Algeria). Furthermore, in the case of *C. amentacea* the sequences retrieved from GeneBank are specimens from Sicily, which singularities in their reproductive structures were pointed out by Amico et al. (1985). Thus, these results must be taken with caution and further studies considering nuclear markers and more locations along the all distribution range of these species must be developed to assess the phylogeny of this group of species.

Despite the previous concerns for the phylogenetic analysis based on the mt 23S gene, the study of nuclear microsatellites also support the idea that only one genetic entity is found in southern Iberian Peninsula. Although a significant concordance between genotype and phenotype was found in "El Playazo", the degree of genetic differentiation was low (average $F_{ST}$ = 0.047) and *C. tamariscifolia* and *C. amentacea* from "El Playazo" were more similar to each other than to the other populations. These facts suggest an important genetic flux between the two morphological entities. Furthermore, as these morphotypes occurs in parapatry in different environmental conditions (littoral vs. sublittoral) and canopy features (dense meadow vs. scarce individuals), the number of cauloids could be a phenological adaptation to the environment. In this sense, previous studies on others fucoids identified important morphological variations (e.g. branch length, number of main axes, or holdfast size) along depths, population density or wave exposure gradients (Arenas et al., 2002; Engelen et al., 2005; Prathep et al., 2007; Endo et al., 2013). Thus, this trait seems to have an uncertain taxonomic value for the definition of these putative *Cystoseira* species.

The NJ tree (Fig 6) revealed that the highest distances occur between sites instead between groups of populations. This fact suggests that most genetic variation must be found within populations, as the AMOVA results confirmed. Similar results were obtained by Susini et al.

(2007) comparing four populations of *C. amentacea* in the Ligurian Sea (NW Mediterranean). These authors found most genetic variation within populations (71 %). These results and the significant correlation between genetic ($F_{ST}$) and geographical distance, suggested an important role of distance in the genetic structure of populations along the studied area as it was expected attending to the reproductive traits of these species.

The Bayesian assignment analysis identified an Atlantic and a Mediterranean clusters (Fig 7), existing a contact area in Central Alboran. Regarding to allelic diversity, this putative contact area showed the highest values of allelic richness. In this sense, previous works have found in terrestrial animals and plants the highest diversity levels in secondary contact areas recolonized after glacial periods (Taberlet et al., 1998). This high genetic diversity has been attributed to the admixture of different linages coming from the different refuges areas. However, in this case is also in this area where can be found a major number of privative alleles. This fact suggests that this area could be a possible refuge during glacial periods instead a contact area. Nevertheless, this statement requires further investigation with expanded sampling area.

Based on the obtained results so far, it can be concluded that only one specific entity probably *C. tamariscifolia*, is present in Alboran Sea. Furthermore, the obtained results provide a relevant source of information for the management of these threatened species in southern Iberian Peninsula. The study of the genetic structure of threatened species with reduce dispersion such as *C. ericaecifolia* group, which play an important role in the maintaining of the biodiversity and ecosystem functioning (Giaccone et al., 1994; Airoldi and Beck, 2007) in littoral communities of the Mediterranean and the proximate coast of the Lusitanian provinces (Spalding et al., 2007), could yield important information to favour the resilience of littoral communities or to develop a suitable restoration. For instance, the understanding of genetic structure and connectivity patterns of these species can help to design a net of Marine Protected Areas (MPAs) that cover its protection needs favouring natural recolonization; or to identify the most suitable donor population in case of necessary ecosystem reforestation (Susini et al., 2007; Sales et al., 2011; Gianni et al., 2013) avoiding problems of genetic contamination or homogenization. In this sense, beside the deleterious effects in the genetic diversity and population fitness of genetic contamination and homogenization (e.g. lost of specific adaptations), an inadequate transplantation could destroy biogeographic genetic patterns useful to reconstruct the natural history of the region, which must be considered an important natural heritage.

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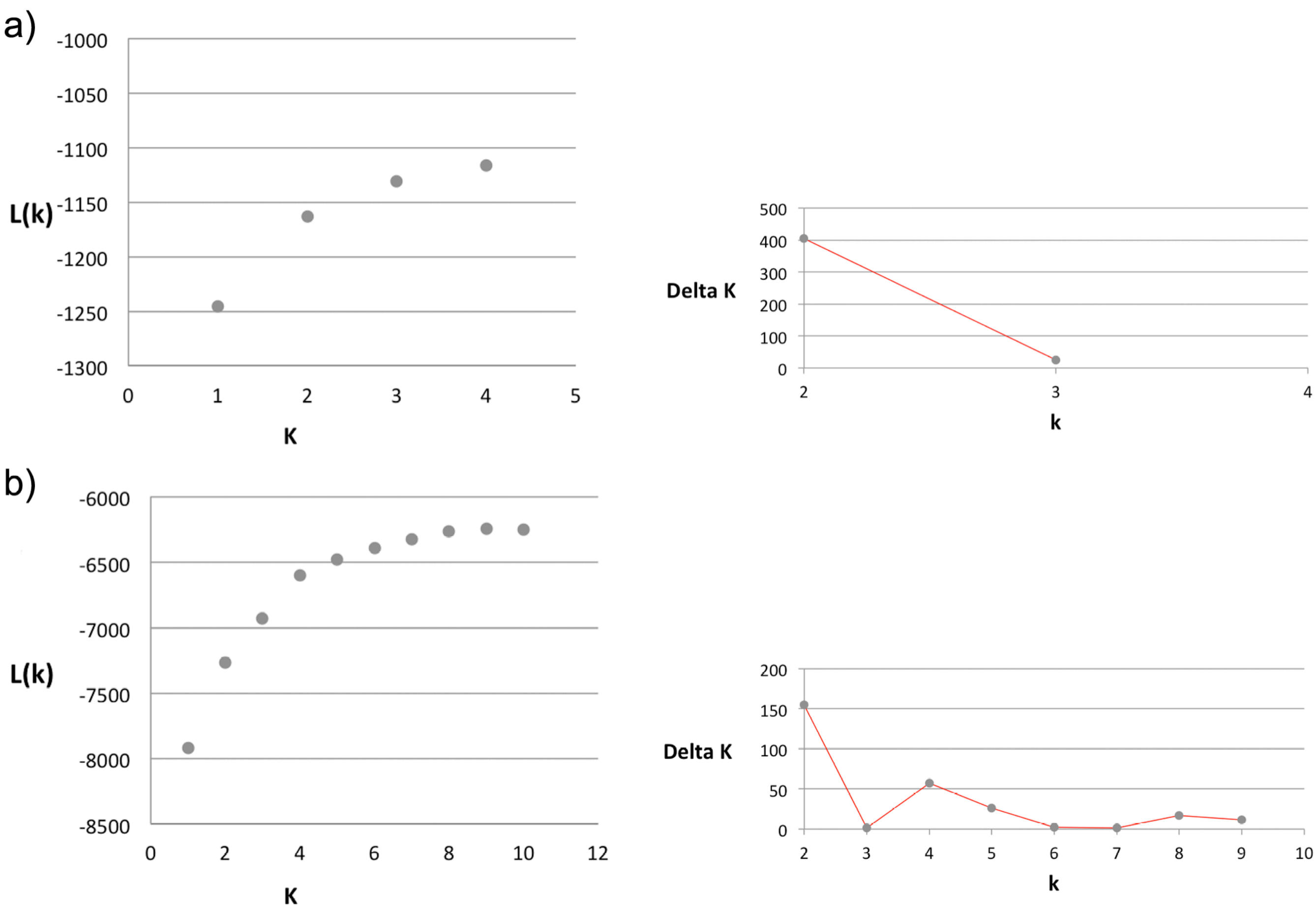


**Figure S1-** Plot of L(K) and ΔK as a function of the number of clusters (K) across the 20 runs. a) First InStruct run considering only "El Playazo". b) Second InStruct run along the whole data set.

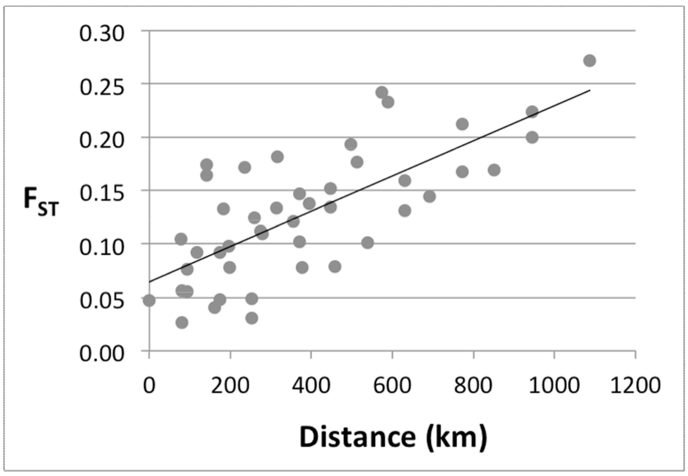


**Figure S2-** Relationship between $F_{ST}$ and geographical distance.

# Discussion

*“Truth is the shattered mirror strown in myriad bits; while each believes his little bit the whole to own.”*

Richard Francis Burton
(The Kasîdah of Hâjî El-Yerdî, 1870).

Intertidal pool dominated by *Cystoseira compressa* assemblage in Getares Bay. Strait of Gibraltar. Julio 2011.
Photograph by Antonio Bermejo Lacida

## Discussion

This study has focused on the application and reassessment of indices based on macroalgae to assess the ecological status in Andalusian coasts (Southern Spain) considering their biogeographic singularities and relevant issues for management. The three first chapters dealt with the adaptation and comparison of indices based on macroalgae for the assessment of the ecological status in Andalusia. Chapter four and five dealt with ecological and biogeographic issues needed for a better understanding of the indices and the proper management of littoral communities in southern Iberian Peninsula.

Overall, the results presented in this thesis suggested that the RSL (Bermejo et al., 2012 - chapter 1) and the CARLIT (Bermejo et al., 2013 - chapter 2) indices can be suitable indicators to assess the ES of Atlantic and Mediterranean coastal waters of southern Spain, respectively. In addition, when these indices were compared in the Strait of Gibraltar and the western Alboran Sea, they yielded very similar outcomes, reflecting the consistency and comparability of the obtained results despite the marked methodological differences (Bermejo et al., 2014 - chapter 3). Beyond the reassessment and comparison of indices, the analysis of the information provided by these studies revealed the influence of the regional oceanography in benthic community patterns along the Alboran Sea, being remarkable the existence of three different subregions coinciding with regional oceanographic features (Chapter 4). On the other hand, the preliminary study of the genetic structure of *Cystoseira ericaefolia* group (Chapter 5) offered a relevant issue for management, considering the important ecological role of these species and their sensibility to anthropogenic pressures, which usually dominate littoral assemblages in undisturbed or pristine ecosystems. The results suggested that there is only a genetic entity in the southern Iberian Peninsula, with a moderate differentiation between populations at a spatial scale of tens of kilometres.

From the results obtained in this study, some questions emerge to obtain a broader vision of the use of indices based on macroalgae in Andalusia: Are indices based on intertidal rocky shore assemblages suitable to assess the ES in the coastal waters of Andalusia? Is it possible to use the same index based on rocky shore assemblages in the Atlantic and the Mediterranean coast of Andalusia? What useful information derives for the assessment of the overall ES of Andalusian WBs using macroalgae as a BQE? And finally, what are the implications for management?

**Are indices based on intertidal rocky shore assemblages suitable to assess the ES in the coastal waters of Andalusia?**

The major limitation of indices based on intertidal rocky shores assemblages is that they cannot be used in sandy shorelines (Ballesteros et al., 2007). Furthermore, in coastlines with low percentages of rocky shores the development of well developed macroalgal assemblages and the presence of sensitive species will be more difficult due to the high natural risk of local extinction and the low probability of recolonization. Thus, the sensitivity of these fragile assemblages to anthropogenic pressures will be lower in comparison to natural risks.

The coast of Andalusia is divided into 49 coastal water bodies defined according to their typology and their anthropogenic pressures and impacts according to the WFD (Fig. 1). 21 coastal WBs belongs to the Atlantic eco-region and 28 to the Mediterranean.

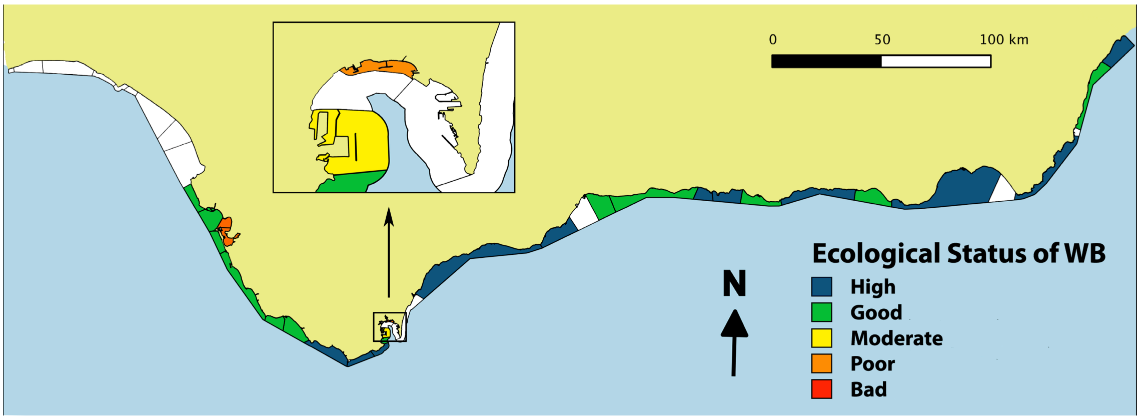


**Fig. 1.-** Cartographical representation of the Ecological Status of the different coastal water bodies of Andalusia between 2010 and 2012.

In the Atlantic eco-region (Table 1), nine of 21 coastal WBs are completely sandy or devoid of natural rocky shores. Three of these coastal WBs without natural rocky shores were small and highly modified, and they do not represent the ecological quality of open waters (Ballesteros et al., 2007). Nevertheless, these modified WBs could be assessed using indices based on rocky shore assemblages because artificial rocky shores and man-made structures, where some littoral and upper-sublittoral communities can be found, are present (Blanfuné et al., unpublished). Thus, 12 (or 15 if harbours and marinas are considered) of 21 coastal WBs could be assessed in the Atlantic area. However, the western Atlantic coasts of Andalusia are essentially sandy (Fig. 1); thus a large coastal area (the entire province of Huelva) cannot be assessed properly with this BQE, which suppose an important limitation for indices based on rocky shore assemblages, especially considering the important industrial activity in this area (Fernández-Caliani et al., 1997; Grande et al., 1999; Blasco et al., 2010).

On the other hand, in the Mediterranean eco-region (Table 2), nine of 28 coastal WBs are completely devoid of natural rocky shores. It is remarkable that six out of nine WBs without natural rocky shore, but with artificial rocky shore, were very small and highly modified -mainly inner parts of harbours and marinas-. Thus, 19 (or 25 if harbours and marinas are considered) of 28 coastal WBs could be assessed in the Mediterranean area, comprising most of the areas of the European coasts of the Alboran Sea.

**Table 1-** Ecological status corresponding to every Atlantic coastal water body of Andalusia, and ecological quality ratio (EQR) for each site sampled. NA - Non-Assessed; NRSA - Natural Rocky Shore Absent.

| Site | EQR (RSL/CARLIT) | Water body name | Nature | ES (RSL/CARLIT) |
|---|---|---|---|---|
| - | - / - | Pluma del Guadiana | Natural | NRSA |
| - | - / - | Isla Cirstina | Natural | NRSA |
| - | - / - | Isla Cristina-Punta Umbría | Natural | NRSA |
| - | - / - | Punta Umbría-Espigón de Huelva | HMWB | NRSA |
| Espigón de Huelva | - / - | Espigón de Huelva-Mazagón | HMWB | NRSA |
| - | - / - | Mazagón-Matalascañas | Natural | NRSA |
| - | - / - | Matalascañas-PN Doñana | Natural | NRSA |
| - | - / - | PN Doñana | Natural | NRSA |
| Corrales-Faro Chipiona | - / - | Pluma del Guadalquivir | Natural | NA |
| Punta Pegina | 0.74 / - | Pluma del Gudalquivir-Punta de Rota | Natural | Good / - |
| Base de Rota | 0.65 / - | Base Naval de Rota | HMWB | Good / - |
| Caleta derecha<br>Caleta izquierda | 0.49 / -<br>0.56 / - | Bahía externa de Cádiz | Natural | Good / - |
| Acualdero<br>La Puntilla | 0.32 / -<br>0.30 / - | Desembocadura del Guadalete | HMWB | Poor / - |
| Polvorines Fadrica | 0.25 / - | Bahía interna de Cádiz | HMWB | Poor / - |
| El Chato | 0.82 / - | Punta de San Sebastián-San Fernando | Natural | Good / - |
| Sancti Petri<br>Roche<br>Cala del Aceite | 0.69 / -<br>0.74 / -<br>0.56 / - | San Fernando-Trafalgar | Natural | Good / - |
| Caños de Meca<br>Hierbabuena | 0.66 / -<br>0.74 / - | Marismas del Barbate | Natural | Good / - |
| Barra de Barbate | 0.64 / - | Marismas del Barbate-Cabo de Gracia | Natural | Good / - |
| Camarinal<br>Piscinas de Bolonia<br>El Mirlo | 0.80 / 0.94<br>0.86 / 0.93<br>0.84 / 0.93 | Cabo de Gracia-Punta de Tarifa | Natural | High / High |
| Puerto de Tarifa | - / - | Puerto de Tarifa | HMWB | NRSA |
| Isla de Tarifa<br>E Puerto de Tarifa<br>Punta Camorro | 0.80 / 0.88<br>0.62 / 0.88<br>0.83 / 0.88 | Punta de Tarifa-Cala Arenillas | Natural | High / High |

**Table 2-** Ecological status corresponding to every Atlantic coastal water body of Andalusia, and ecological quality ratio (EQR) for each site sampled. NE - Non-Assessed; NRSA - Natural Rocky Shore Absent.

| Site | EQR (RSL/CARLIT) | Water body name | Nature | ES (RSL/CARLIT) |
|---|---|---|---|---|
| Punta Carnero | 0.69 / 0.94 | Cala Arenillas-Punta Carnero | Natural | High / High |
| Punta San García | 0.57 / 0.93 | Punta Carnero-Desembocadura del Getares | Natural | Good / Good |
| Bahía de Algeciras | 0.40 / 0.49 | Desembocadura del Getares-PN Alcornocales | HMWB | Moderate / Moderate |
| Puerto Algeciras | 0.11 / 0.41 | Puerto de Algeciras | HMWB | Bad / Moderate |
| - | - / - | PN Alcornocales-Muelle Campamento | Natural | NRSA |
| Refineria CEPSA | 0.52 / 0.37 | Desembocadura del Guadarranque | HMWB | Good / Poor |
| - | - / - | Muelle Campamento-Aeropuerto Gibraltar | Natural | NRSA |
| - | - / - | Puerto de La Línea de la Concepción | HMWB | NRSA |
| - | - / - | Gibraltar-Desembocadura del Guadiaro | Natural | NRSA |
| Torreguadiaro | 0.63 / 0.88 | Desembocadura del Guadiaro-Punta Calaburras | Natural | Good / High |
| Cala de Mijas | 0.65 / 0.74 | | | |
| Calaburras | 0.75 / 0.98 | | | |
| Torrequebrada | - / 0.78 | Punta Calaburras-Torremolinos | Natural | - / High |
| - | - / - | Torremolinos-Puerto de Málaga | Natural | NRSA |
| - | - / - | Puerto de Málaga | HMWB | NRSA |
| Araña-Acantilados de Rincón | - / 0.70 | Puerto de Málaga-Rincón de la Victoria | Natural | - / Good |
| Faro de Torrox | - / 0.44 | Rincón de la Victoria-Acantilados de Maro | Natural | - / Good |
| Playa Burriana | - / 0.69 | | | |
| Acantilados Maro | - / 0.80 | Acantilados de Maro-Cerro Gordo | Natural | - / High |
| Peñon del Santo<br>Playa del Tesorillo | - / 0.80 | Cerrogordo-Salobreña | Natural | - / High |
| Caleta | - / 0.67 | Salobreña-Calahonda | Natural | - / Good |
| - | - / - | Puerto de Motril | HMWB | NRSA |
| Rijana | - / 0.89 | Calahonda-Puerto de Adra | Natural | - / High |
| Castel de Ferro | - / 0.82 | | | |
| Cala del Ruso | - / 0.70 | | | |
| Alcazaba | - / 0.63 | | | |
| Adra | - / 0.74 | Puerto de Adra-Guardias Viejas | Natural | - / Good |
| Guardias Viejas | - / 0.66 | | | |
| Roquetas | - / 0.68 | Guardias Viejas-Rambla de Morales | Natural | - / High |
| Playa de las Olas | - / 1.00 | | | |
| - | - / - | Puerto de Almería | HMWB | NRSA |
| - | - / - | Rambla de Morales-Cabo de Gata | Natural | NRSA |
| Cabo de Gata | - / 0.90 | Cabo de Gata | Natural | - / High |
| San José | - / 0.90 | | | |
| Isleta del Moro | - / 0.99 | | | |
| Cala Carnaje | - / 1.00 | | | |
| El Playazo | - / 0.79 | | | |
| - | - / - | Puerto de Carboneras | MWB | NRSA |
| El Algarrobico | - / 0.68 | Puerto de Carboneras-Villaricos | Natural | - / Good |
| Mojácar | - / 0.95 | | | |
| Villaricos | - / 0.45 | | | |
| Cala Panizo | - / 1.00 | Villaricos-Límite Región de Murcia | Natural | - / High |
| San Juan de los Terreros | - / 0.92 | | | |

**Is it possible to use the same index based on rocky shore assemblages in the Atlantic and the Mediterranean coast of Andalusia?**

As some WBs in coastal waters of Andalusia belong to the Atlantic and others to the Mediterranean eco-region, up to seven indices based on the BQE macroalgae could be applied: five in the Atlantic ecoregion: CFR (Guinda et al., 2008; Juanes et al., 2008), RSL (Wells et al., 2007), CCO (NEA GIG, 2011), RICQI (Díez et al., 2012), MarMAT (Neto et al., 2012); and two in the Mediterranean: EEIc (Orfanidis et al., 2001; Orfanidis et al., 2011) and CARLIT (Ballesteros et al., 2007). This diversity of indices based on macroalgal assemblages reflects the suitability of this BQE as bioindicator and the importance of biogeographical and ecological differences in the assessment of the ES. This ES must be understood as the degree of similarity between hypothetic undisturbed situations or reference conditions and the study site. The differences between the methodologies proposed in the context of the WFD (Table 3) do not respond only to environmental, ecological or biogeographical differences between areas (wave exposure, tidal range, littoral communities...). Also personal criteria and choices (Hering et al., 2010) hamper the comparison between areas and the interpretation of the results, introducing more variability in the assessment of the ES. Thus, considering the objectives of the WFD and the natural complexity of rocky intertidal assemblages, the *pros* and *cons* of the different methodological characteristics are analysed:

*Spatial scale and replication*

It is remarkable the great variability in the spatial scale between methodologies developed to assess the ES (Table 3), from hundreds squared centimetres (e.g. EEIc) to large cartographies of several kilometres (e.g. CARLIT). In this sense, if a small sampling size is considered, the effort and time of fieldwork can be reduced, which is important in places where the tidal period determines time available for sampling. The use of standardised size of quadrats makes easier the comparison between them and gives an accurate measure of the algal cover or biomass of the species present in each sample. However, the representativeness of these measures is limited due to the high natural spatial variability of littoral communities. The zonation, patchiness and the hierarchical nature of spatial variability in seaweed assemblages is very important, occurring at scale of dozens centimetres to several metres (Levin, 1992; Benedetti-Cecchi et al., 2001; Bulleri et al., 2002). This vertical and horizontal spatial variability (Fig. 2) is produced by the effects of physical disturbances (wave exposure, sand scour or desiccation), microtopography, and differences in patterns of recolonisation of disturbed patches due to the interactive effects of a variable recruitment, grazing and pre-emption of the substratum (Benedetti-Cecchi et al., 2001). Therefore, to reduce the influence of this natural variability in the ecological assessment, some authors propose to take the samples in specific conditions (e.g. Pinedo et al., 2007; Díez et al., 2012), stratifying the sampling design in different levels according to the tidal height and/or increasing the number of replicates (e.g. Juanes et al., 2008;

Neto et al., 2012). However, a question arises: is the obtained information enough and representative to assess the ES of WBs which comprise several tens of kilometres?

**Table 3-** Spatial scale, number of replicates, multimetric character, destructive sampling and concepts considered in the methodological design for the assessment of the ecological status in Atlantic and Mediterranean indices based on intertidal assemblages of macroalgae. CS - Community Structure; SR - Species Richness.

| Index | Spatial scale | Replicates | Multimetric | Destructive | Concepts |
|---|---|---|---|---|---|
| EEIc | 400 $cm^2$ | 1 | No | Yes | CS |
| MarMAT | 400 $cm^2$ | 21 | Yes | No | CS+SR |
| CCO | 1000 $cm^2$ | 9 | Yes | No | CS+SR |
| RICQI | 2500 $cm^2$ | 10 | Yes | No | CS+SR |
| CFR | 5 - 20 m | 3 | Yes | No | CS+SR |
| RSL | 50 - 75 m | 1 | Yes | No | SR |
| CARLIT | 100s of meters | 1 | No | No | CS |

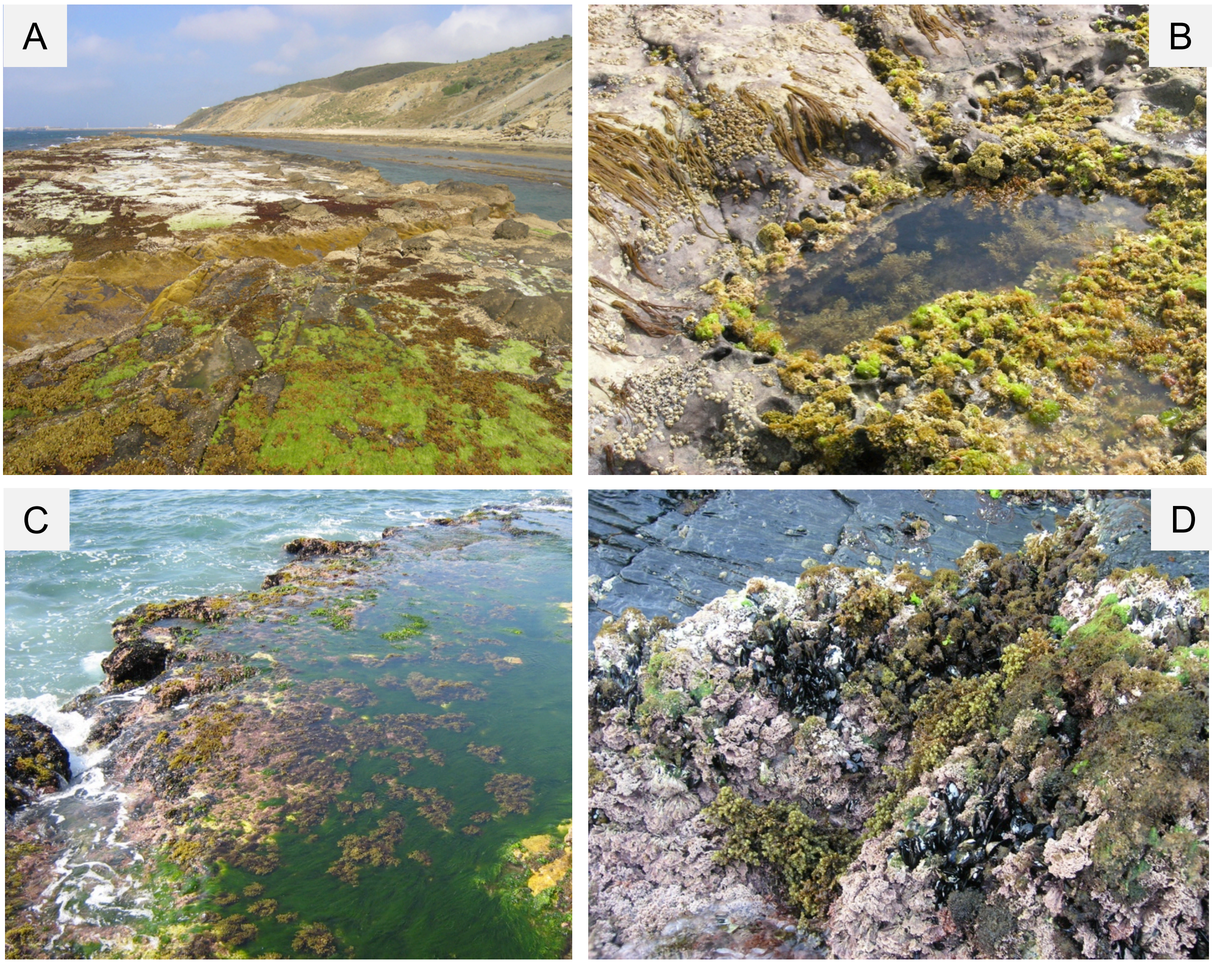


**Fig. 2.-** Examples of spatial variability in littoral rocky communities at different scales. A: upper intertidal codominated by *Fucus spiralis* and *Ulva compressa* in Punta Camorro (Tarifa); B: patchiness in upper intertidal due to the microtopography of the rocky shore in Punta Camorro (Tarifa); C: Intertidal platform codominated by *Ulva compressa*, *Corallina* spp., *Cystoseira ericaefolia* and *Mytilus* spp. (La Araña, Malaga); D: patchiness in upper intertidal codominated by *Corallina* spp., *Mytilus* spp. and *Cystoseira ericaefolia* (Almuñecar, Granada).

Although the effects of sewages, outfalls or other punctual disturbances can be monitored using punctual measures of intertidal assemblages along a gradient from the source of disturbance (e.g. Diez et al., 1999; Soltan et al., 2001; Seridi et al., 2007; O'Connor, 2013). This kind of experimental design is less appropriate than cartographies to assess the ES of WBs, considering the natural spatial and temporal variability of intertidal assemblages, the size of these WBs, and the spatial scale and gradual character of punctual anthropogenic disturbances. For these reasons, cartographies of littoral and upper-sublittoral communities may be more suitable and representative to assess the ES of WBs of tens of kilometres and monitor long-term changes related to anthropogenic disturbances (Ballesteros et al., 2007). Furthermore, these cartographies let to identify punctual sources of pollution and to estimate the extension of the disturbance at a landscape level. In this sense, Mascaró et al. (2013) found the spatial scale as the most important source of uncertainty in the assessment of the ES, being this factor more important than time or surveyor, and suggesting that macrophyte-based sampling schemes should prioritize large spatial replication over temporal replication to maximize the effectiveness and reliability of WB classification within the WFD.

*Multimetric v.s. simple indices*

According to Karr (1999), the principle of indices is to detect divergences from biological integrity attributable to human actions. The goal is not to document and understand all the variation that arises in natural systems. Thus, when is necessary to choose between a multimetric and a simple index, the first criteria considered must be the sensitivity to the anthropogenic pressure studied and secondly the simplicity of the index. However, this statement is arguable, because the utility of the information that these indices provided for management should be considered as important as the value of the index. In this sense, the interpretation of the results yielded by biological indices is essential when management actions are proposed (Moss, 2007; Dufour and Piégay, 2009; Lopez y Royo et al., 2011).

*Destructive sampling*

Destructive samplings allow a more precise identification of species and abundance quantification in the laboratory. Moreover, these samples can be stored for further analysis or studies. However, destructive sampling in monitoring programs pose some problems, because they affect the succession in the community, cause a new source of disturbance, and suppose a threat for local populations of some late successional species, with slow rates of natural recolonization (e.g. *Cystoseira*; Thibaut et al., 2005; Mangialajo et al., 2012).

*Assessment concepts*

Macroalgal communities are sensitive to an array of anthropogenic pressures (e.g. Borowitzka, 1972; Diez et al., 1999; Arévalo et al., 2007). These pressures are superimposed on those caused by natural environmental factors (Crowe et al., 2000), which have their own effects in the community structure and species composition (Guinda et al., 2008; chapter 3 - Bermejo et al., 2014). The suitability of indices based on species composition or community structure (i.e. considering abundances) will depend of natural community features, which determine the ability of indices to avoid the influence of natural pressures and their sensitivity to anthropogenic pressures.

Indices considering species abundance are easier to interpret and more informative about ecosystem functioning (e.g. Serio et al., 2006; Tuya et al., 2009; Asnaghi et al., 2013), than indices based on species composition. However, in places where natural pressures determine an extended and homogeneous landscape dominated by tolerant species, and sensitive species are restricted to singular subhabitats, indices based on species composition could be more suitable. For example, *Corallina* assemblages dominate the entire emerged rocky intertidal coast between the Strait of Gibraltar and Guadalquivir River (Seoane-Camba, 1965), being other sensitive habitat-forming species scarce and restricted to intertidal pools or caves (Fig. 3). In this situation, the RSL index was sensitive to anthropogenic pressures in Andalusian Atlantic coasts, but the CFR index, which consider species abundance, was not (Bermejo, 2009).

*The case of Andalusia*

The results obtained in this study demonstrate that the RSL and CARLIT were suitable to the assessment of the ES in the coastal waters of Andalusia. Both indices follow a non-destructive methodology, which is essential for conservation aims, considering that recolonization of rocky bare substrates for some late successional species is very slow (Thibaut et al., 2005; Mangialajo et al., 2012). Furthermore, although the RSL shows some difficulties to discriminate between good and high ES (Bermejo et al., 2012 and 2014 - chapter 1 and 3), both indices were sensitive to anthropogenic pressures (Bermejo et al., 2012, 2013 and in press - chapters 1, 2 and 3) and yielded comparable results (Bermejo et al., 2014 - chapter 3). Nevertheless, regarding the methodological characteristics of these indices, the CARLIT index shows some methodological advantages. For instance, the big spatial scale considered by CARLIT reduces the uncertainty in the ES assessment (Mascaró et al., 2013). The simultaneous use of both flora and fauna makes this index more sensitive, providing better evidences of changes in the community structure (Díez et al., 2012; Underwood, 1996). The use of relative abundance of habitat forming species gives an idea about the assemblage structure and functioning, which is not considered in indices based on species richness (O´Connor, 2013). Thus, the possibility to apply the CARLIT index in the Atlantic coast of Southern Spain could be considered. The use of a unique index would make easier the interpretation of the results by local managers. Furthermore, although this point is not considered in the WFD, the application of this index in Atlantic WBs could be an opportunity to intercalibrate Atlantic and Mediterranean ecoregions (Bermejo et al., 2014 - chapter 3). However, from the legal point of view, the EU already consider RSL index as the authorized one in Andalusian Atlantic coasts.


**Fig. 3.-** Main aspect of different emerged intertidal rocky shores along the Atlantic coast of Cadiz. Caespitose assemblages dominate these intertidal platforms, where *Corallina* is usually the main specie. A: Trafalgar (Barbate); B: El Chato (Cadiz); C: Sancti Petri (Chiclana); D: Punta Pegina (Rota).

**What useful information derives for the assessment of the overall ES of Andalusian WBs using macroalgae as a BQE?**

As Montefalcone (2009) pointed out, the WFD focuses on water quality in coastal and transitional water bodies. However, many disturbances affecting coastal ecosystems do not necessarily compromise directly the water quality (e.g. destructive fishing activities, overfishing, boat anchoring, dragging, siltation, introduction of alien species, etc.). In this sense, the interest on the status of coastal ecosystem must be beyond its relation to water quality, considering the ecological services that coastal ecosystems provide (Costanza et al., 1997) and the unpredictable consequences for human activities and biodiversity if thresholds or non return points are reached. On the other hand, the status of an ecosystem is linked to its historical memory of the environmental situations in which it developed and, consequently, to all disturbances it suffered in the past (Knowlton, 2004; Montefalcone, 2009). The recovery of degraded communities, when it is possible, may take several years after the anthropogenic pressure that produced the disturbance cease (Thibaut et al., 2005; Ugarte, 2011; Mangialajo et al., 2012; Alagna et al., 2013), being not necessarily dependent on the quality water. In contrast, the processes that produce their degradation can act in a shorter time due to the existence of non-linear responses and positive feedbacks loops. These facts support the importance of the precautionary principle, and the need for a profound knowledge about coastal ecosystems, which cannot be summarised in a single value between 0 and 1. The merit of the indices developed in the context of the WFD is not their final value; it relies on the interpretation of this value and the metrics they are based on. Thus, indices should be not only sensitive to anthropogenic pressures but also informative to identify the causes of ecological degradation. This interpretation requires expert knowledge if the final objective is beyond the treatment of symptoms of ecological degradation.

The periodical estimation of these indices will provide an important source of information. In the future, when the temporal dataset will be large enough (provided an adequate funding for that purpose), temporal comparison must be easier to interpret than the present space based comparison. This should facilitate the identification of cause-effect relations between anthropogenic pressures and ecosystem degradation in a long-term record, and the establishment of effective management measures. Furthermore, the obtained data set can be a baseline for measuring the response of the distribution of considered species to global change (Boaventura et al., 2002). This fact is particularly important considering: i) the special condition of the Alboran Sea as the limit between the Atlantic Ocean and the Mediterranean Sea (Alberto et al., 2008; Conde, 1989; chapter 4); ii) that habitat destruction or degradation as the most important threat to the diversity, structure, and functioning of marine coastal ecosystems (Claudet and Fraschetti, 2010; Coll et al., 2010; Lotze et al., 2006); iii) that some of the species are protected by specific national or international legislation.

The studies conducted in this thesis have also an intrinsic value providing new records of biodiversity in Andalusian coasts. A total of ten new species for Andalusian marine flora were recorded, two new records for Almería region, beside ten new records and three confirmations for Cádiz region has been reported (Bermejo et al., 2010; Hernández et al., 2010, 2011; Bárbara et al., 2012; Bermejo et al., 2012); this information will be valuable to define a baseline for management purposes.

**What are the implications for management?**

According to the WFD, Member States should achieve at least a good ES for all WBs and prevent further deterioration by defining and implementing the necessary measures within integrated programmes of measures. Thus, degraded WBs (ES lower than good) should be restored/recovered, reducing anthropogenic pressures causative of ecological degradation and favouring the retrieving of aquatic ecosystems. This restoration can be understood at different levels, reflected in treatment of symptoms, treatment of proximate causes, or treatment of ultimate causes, being the latter the most appropriate approach (Moss, 2007). This approach requires an extensive knowledge of littoral ecosystems to identify causal anthropogenic pressures, degraded areas and proposing effective actions to favour natural resilience.

The definition of homogeneous areas with proper spatial reference conditions is the base for a successful assessment of the ES. The point of regional classification is to group places where living systems are similar at higher taxonomic and ecological levels in the absence of human disturbance, and where the biological responses are similar after human disturbance (Karr, 1999). In this sense, the knowledge of biogeographic and ecological variations (Chapter 4) has been a key issue to define suitable reference conditions. As can be seen in chapter 3 (Bermejo et al., 2014) and others studies (Guinda et al., 2008; Bermejo et al., 2011; López-Royo et al., 2011), indices different in their structure and conception could yield comparable results, if their rationale in the definition of anthropogenic pressures and reference conditions are similar. Furthermore, the biogeographic and ecological analysis of the studied area (Chapter 4) let to identify the mechanisms that determined the distribution of the species. This information will be very useful to understand or forecast the evolution of the ES associated with the climate change, and to distinguish between the effects of natural and anthropogenic pressures on littoral ecosystems.

On the other hand, the study of the genetic structure of threatened species with reduce dispersion rates such as *C. ericaecifolia* group (Chapter 5) could yield valuable information to favour the resilience of littoral communities or to develop a suitable restoration. These species play a relevant role in the conservation of the biodiversity and ecosystem functioning (Giaccone et al., 1994; Airoldi and Beck, 2007) in wave exposed littoral communities of the Mediterranean and the proximate coast of the Lusitanian provinces (Lüning, 1990; Spalding et al., 2007). As *C. ericaefolia* group is very sensitive to anthropogenic pressures, they are suffering a general decline in the Mediterranean Sea remaining in pristine or undisturbed situations (Thibaut et al., 2005; Airoldi and Beck, 2007), where these species usually dominate littoral assemblages (Ballesteros et al., 2007; Orfanidis et al., 2011). Therefore, the conservation and restoration/recovery of *C. ericaefolia* populations is a key issue to reach a high/good ES. Due to the low recovery potential of *Cystoseira* species (Sales et al., 2011; Tsiamis et al., 2013), some authors have proposed transplantation or reforestation actions to accelerate the recovery of littoral ecosystems, in places where populations of these species have been historically recorded and anthropogenic disturbances have been reduced (Susini, et al., 2007; Gianni et al., 2013). For a proper reforestation or transplantation, the knowledge of the genetic similarity between populations let to know which are the best donor populations, avoiding genetic contamination and homogenization of populations. In this sense, besides the deleterious effects in the genetic diversity and population fitness due to a genetic pollution and homogenization (e.g. loss of specific adaptations), an inadequate transplantation could destroy biogeographic genetic patterns that are useful to reconstruct the natural history of the region, which must be considered an important natural heritage. On the other hand, the information about the genetic structure and connectivity patterns of these habitat forming species with reduced dispersion could be an argument for the design of a network of Marine Protected Areas (MPAs) that cover its protection needs (Underwood et al., 2009), favouring the natural recolonization.

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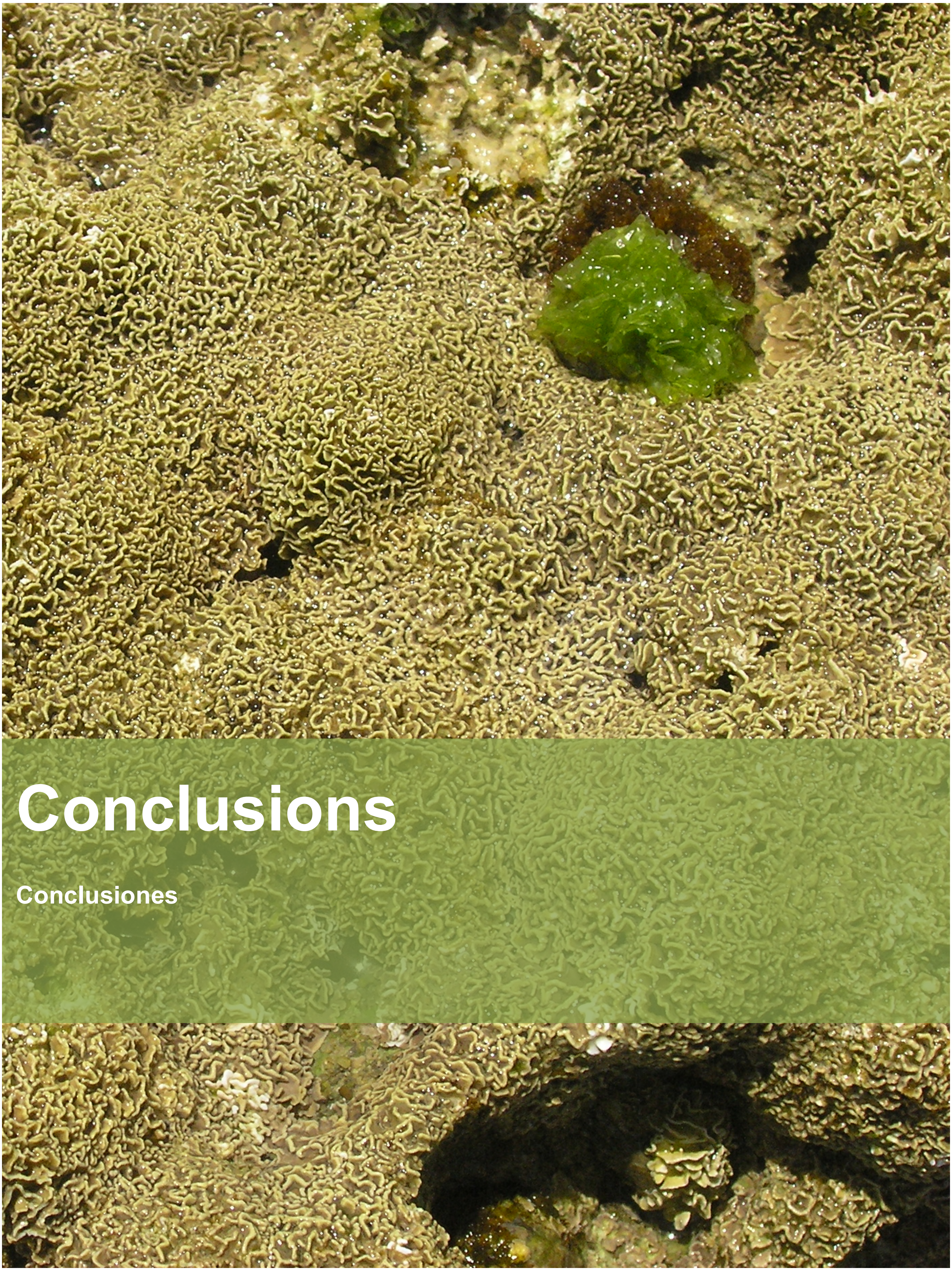


# Conclusions

## Conclusiones

*"Ítaca te ha concedido ya un hermoso viaje.*

*Sin ellas, jamás habrías partido;*

*mas no tiene otra cosa que ofrecerte.*

*Y si la encuentras pobre, Ítaca no te ha engañado.*

*Y siendo ya tan viejo, con tanta experiencia,*

*sin duda sabrás ya qué significan las Ítacas."*

Konstantíno Kaváfis
(Ítaca, 1911)

Intertidal pool dominated by *Lithophyllum byssoides* assemblage. Isla de Tarifa. Julio 2011.
Photograph by Antonio Bermejo Lacida

## Conclusions

1. The RSL index was suitable to assess the ES in the Atlantic coast of southern Spain. This index shows consistent results with those expected from water quality analytical monitoring and anthropogenic pressures. However, the RSL index did not discriminate clearly between good and high status classes.
2. The CARLIT index was suitable to assess the ES in Alboran Sea. The definition of two regions with different reference conditions should be considered to accurately assess the ES of these waters, encompassing the natural variations that occur over this coast. However, the EQR and the final ES can be dependent on the length of the rocky shore in each WB assessed.
3. The CARLIT and RSL indices were equally sensitive to anthropogenic pressures and they could be used to assess the ES in the Strait of Gibraltar and western Alboran Sea. The possibility to apply the RSL and CARLIT indices in the transition zone between the Mediterranean and Atlantic eco-regions opens the possibility of an experimental comparison between the Atlantic and Mediterranean eco-regions at a bigger scale.
4. Overall, Andalusian WBs showed a good or high ES according to the WFD, being Algeciras and Cadiz bay the most problematic areas. The existence of WBs with an ES lower than good in both bays will not have legal consequences as they are modified or highly modified WBs.
5. The major limitation for the application of indices based on intertidal macrophyte assemblages in Andalusia was the existence of a length stretch of coast devoid of natural rocky shores in the westernmost Atlantic coast (the entire province of Huelva).
6. Two fringes with a different intertidal composition of algal species were identified along the Atlantic coast of Cadiz: Atlantic Cadiz and the Strait of Gibraltar.
7. Alboran Sea was a transitional area between Atlantic Ocean and Mediterranean sea. Three different subregions can be identified based on landscape and species composition: western, central and eastern Alboran. The regional oceanography play a key role in determining the structure and composition of benthic communities along these areas.
8. The central subregion of the Alboran Sea acted as a divergent boundary showing a poorer and less differentiated flora, with a the landscape dominated by filter-feeders. This divergence could be a consequence of the alternative occurrence of upwelling episodes and the arrival of Mediterranean oligotrophic waters in this area.
9. The preliminary results about the population genetic structure and taxonomy of *Cystoseira ericaefolia* group did not support the existence of differentiated genetic entities in southern Iberian Peninsula, existing only one species, probably *C. tamariscifolia*.
10. Populations of *C. ericaefolia* showed a moderate differentiation in southern Iberian Peninsula, being the most genetically diverse populations located at western and central Alboran.

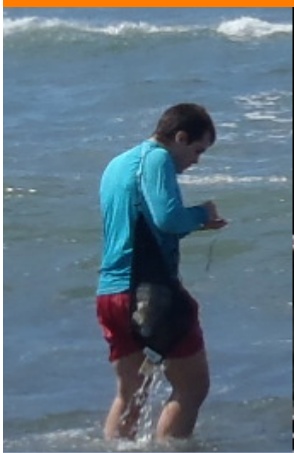
## Conclusiones

1. El índice RSL fue adecuado para evaluar el estado ecológico de las costas atlánticas del sur de España. Este índice mostró resultados similares a los esperados considerando las características físico-químicas del agua y las presiones antrópicas. Sin embargo, el RSL no discriminó entre los estados bueno y alto.
2. El índice CARLIT fue adecuado para la evaluación del estado ecológico en el Mar de Alborán. Fue necesario definir dos regiones con diferentes condiciones de referencia para evitar la influencia de la variabilidad natural de esta costa, permitiendo de este modo una adecuada evaluación de estas aguas. Sin embargo, el valor de EQR y el estado ecológico final puede ser dependiente de la longitud del cuerpo de agua evaluado.
3. Los índices CARLIT y RSL fueron igualmente sensibles a las presiones antrópicas y podrían ser utilizados para evaluar el estado ecológico en el Estrecho de Gibraltar y oeste del Mar de Alborán. La posibilidad de aplicar estos índices en la zona de transición entre las ecorregiones atlántica y mediterránea abre la posibilidad de una comparación entre estas ecorregiones a una mayor escala.
4. En términos generales, los cuerpos de agua andaluces mostraron un estado ecológico bueno o alto, siendo Algeciras y la bahía de Cádiz las áreas más problemáticas. Sin embargo, los cuerpos de agua con un estado inferior a bueno estaban clasificados como altamente modificados, por lo que no existirán consecuencias legales.
5. La mayor limitación para la aplicación de índices basados en macroalgas en las costas de Andalucía fue la existencia de largos tramos de costa sin sustratos rocosos naturales en la costa atlántica occidental (la provincia de Huelva).
6. Dos tramos con una composición de especies de algas diferente fueron identificados a lo largo de la costa atlántica de Cádiz: Cádiz atlántico y estrecho de Gibraltar.
7. El Mar de Alborán es una zona de transición entre el océano Atlántico y el mar Mediterráneo. Tres subregiones pueden ser identificadas a partir de su paisaje y composición específica: Alborán occidental, oriental y central. La oceanografía regional juega un papel clave en la estructura y composición de las comunidades bentónicas.
8. La subregión central del mar de Alborán actúa como una frontera divergente mostrando una flora más pobre y menos diferenciada, con un paisaje dominado por filtradores. Esta divergencia podría ser consecuencia de la sucesión de episodios de afloramiento y llegada de aguas mediterráneas oligotróficas a este área.
9. Los resultados preliminares sobre la estructura genética de las poblaciones del grupo *Cystoseira ericaefolia* no apoyan la existencia de varias entidades genéticas en el sur de la península ibérica, existiendo solo una especie, probablemente *C. tamariscifolia*.
10. Las poblaciones de *C. ericaefolia* mostraron una diferenciación moderada en el Sur de la Península, siendo las poblaciones del oeste y centro del Mar de Alborán las que mostraron una mayor diversidad genética.

## Conclusões

1. O índice RSL foi adequado para a avaliação do estado ecológico das costas atlânticas do sul de Espanha. Este índice mostrou resultados semelhantes aos esperados, considerando as características físico-químicas da água e das pressões antrópicas. No entanto, o RSL não distinguiu entre os estados bons e altos.
2. O índice CARLIT foi adequado para a avaliação do estado ecológico no Mar de Alboran. Foi necessário definir duas regiões com diferentes condições de referência para evitar a influência da variabilidade natural desta costa, permitindo assim, uma avaliação adequada destas águas. No entanto, o valor de EQR e o estado ecológico final pode ser dependente do comprimento do corpo de água avaliado.
3. Os índices CARLIT e RSL foram igualmente sensíveis às pressões antrópicas e poderiam ser utilizados para avaliar o estado ecológico no Estreito de Gibraltar e a oeste do Mar de Alborán. A possibilidade de aplicar estes índices na zona de transição entre as ecorregiões atlântica e mediterrânea abre a possibilidade de uma comparação entre estas ecorregiões a uma escala maior.
4. No geral, os corpos de água andaluzes mostraram um estado ecológico bom ou alto, sendo Algeciras e a baía de Cádiz as áreas mais problemáticas. No entanto, os corpos de água com um estado inferior a bom foram classificados como altamente modificados, de modo que não haverão consequências legais.
5. A principal limitação para a aplicação de índices com base em macroalgas na costa da Andaluzia, foi a existência de longos segmentos de costa sem substratos rochosos naturais na costa atlântica ocidental (a província de Huelva).
6. Dois segmentos com uma composição de espécies de algas diferentes foram identificados ao longo da costa atlântica de Cádiz: Cádiz Atlântico e Estreito de Gibraltar.
7. O Mar de Alborán é uma zona de transição entre o Oceano Atlântico e o Mar Mediterrâneo. Três sub-regiões podem ser identificadas a partir da sua paisagem e composição específica: Alboran ocidental, central e oriental. A oceanografia regional desempenha um papel fundamental na estrutura e composição das comunidades bentónicas.
8 . A sub-região central do Mar de Alborán actua como uma fronteira divergente demonstrando uma flora mais pobre e menos diferenciada, com uma paisagem dominada por filtradores. Esta divergência poderia ser consequência da sucessão de episódios de afloramento e de entrada de águas mediterrâneas oligotróficas nesta área.
9. Os resultados preliminares sobre a estrutura genética das populações do grupo *Cystoseira ericaefolia* não suportam a existência de várias entidades genéticas no sul da Península Ibérica, existindo apenas uma espécie, provavelmente *C. tamariscifolia*.
10. As populações de *C. ericaefolia* mostraram uma diferenciação moderada no sul da Península, sendo as populações do oeste e centro do Mar de Alborán, as que mostraram maior diversidade genética.


# Agradecimientos



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A mi familia y a Sara.

## Abstract

According to the Water Framework Directive (WFD), the ecological status of European coastal waters must be assessed using different biological quality elements (BQE). One of the four proposed BQE is based on the composition and abundance of the marine macroalgae. Because of the biogegraphical differences along the European coasts, six ecoregions have been considered for biological indices development (Atlantic, Baltic, North Sea, Barents Sea, Norway Sea and Mediterranean Sea). The geographical position of Andalusia (Southern Spain), as a transition zone between the Atlantic and the Mediterranean Sea implies some technical and theoretical difficulties. Coastal waters of Andalusia belong to two different ecoregions, and their evaluation can be carried out with up to seven different macroalgal based indices. Moreover, the existence of a natural gradient along this coast interferes in the final value of the indices.

The main objectives of this thesis were: i) the adaptation and comparison of indices based on macroalgae for the assessment of the ecological status in coastal waters of Andalusia; and ii) the provision of useful information for management about the ecology and the biogeography of littoral communities in southern Iberian Peninsula.

The first objective is addressed in three chapters. In chapters 1 and 2, the Reduced Species List (RSL) and CARtography of LITtoral communities (CARLIT) indices were adapted to the particularities of Andalusian coasts. Afterwards, both indices were compared in the Strait of Gibraltar and the western Alboran Sea (chapter 3). The results showed that these indices were suitable to assess the ecological status in Andalusian coastal waters, and they yielded similar results. Overall, the ecological status of Andalusian water bodies (WBs) was good or high, excepting some highly modified WBs.

The second block is focused on the ecology and biogeography of macroalgal communities in southern Iberian Peninsula. In chapter 4 the biogeographical patterns of the Alboran Sea were studied based on the landscape and the species composition of littoral and upper-sublitoral communities, and compared to regional oceanographic patterns. The results pointed out the influence of regional oceanographic patterns in the littoral communities, and the existence of three different subregions: western, central and eastern Alboran. In chapter 5, considering the ecological importance of Cystoseira mediterranea, C. amentacea and C. tamariscifolia, a genetic approach based on microsatellites was developed to assess the taxonomic identity and the genetic structure of these populations along the southern Iberian Peninsula. The preliminary results suggest that only a genetic entity, probably C. tamariscifolia, is present in the Alboran Sea. Furthermore, these populations showed a moderate differentiation among them, being the most genetically diverse populations those in western and central Alboran. The knowledge of these ecological and biogeographic patterns will be essential for a proper management (e.g. design a network of marine protected areas) and to interpret the results yielded by indices based on macroalgae.