Rui Miguel Martins Gaspar

USING MACROALGAE TO ASSESS
COASTAL AND ESTUARINE ENVIRONMENTS

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UNIVERSIDADE DE COIMBRA

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This PhD thesis comprehends different sections: a general introduction, four different chapters and a concluding remarks section. Both general introduction and concluding remarks integrate the topics address in the four chapters. The chapters are based on the following scientific manuscripts:

Chapter I

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Abstract

Macroalgae are a diverse group of organisms, being important biological elements to the structure and function of both coastal and estuarine environments. While these ecosystems integrity is at stake, the development of ecological indicators (including macroalgal-based approaches) to assess ecosystems health is critical to the successful management and protection of biodiversity, the assessment of anthropogenic impacts and the restoration of altered ecosystems. Critically, in order to assess environmental quality using macroalgae, their assemblages should be described under naturally varying conditions (e.g. ecological reference conditions), so that the assemblages departures from those ecological standards can be recognized as changes due to anthropogenic impacts. This relies largely on the primarily quantification of macroalgal assemblages natural variability patterns across a range of relevant scales, and on understanding the processes and factors that structure the assemblages patterns. This work firstly addresses different macroalgae biodiversity measures that can be taken into account in order to describe macrolagal assemblages under ecological reference conditions (chapter I). Time-consumption and representativeness are central issues in monitoring designs. One problem is that many macroalgae species can be found under natural varying conditions and across a range of spatial and temporal scales, and this implies a high sampling and laboratory processing effort, taxonomical expertise, as well as the need for more knowledge about the structural and functional characteristics of the ecosystems they live in. The use of macroalgal biodiversity surrogates such as by grouping species-level approaches into morphological-functional groups or into trait-based "thallus morphology" approaches may improve ecological syntheses and cost-effectiveness monitorings. However, such surrogacy must be spatial-explicitly tested. Particularly, this work also studied above mentioned macroalgal biodiversity surrogacy approaches, by comparing the variability of macroalgal assemblages across intertidal zonation patterns and along large-spatial scales, namely along the latitudinal gradient of continental Portugal (Chapter II). Furthermore, the trait-based "thallus morphology" approach was tested at intertidal vertical zonation patterns at small-spatial scales, namely across gradients of different wave-exposures associated with different sedimentation regimes (Chapter III). Both studies suggest that trait-based "thallus morphology" approach may be helpful as a complementary surrogate measure of macroalgae biodiversity under macroalgal-based monitoring and assessing needs. Under the naturally varying conditions and gradients considered in both studies, the assemblages structure (composition and abundance) varied deeply. Particularly, changes in the identity of species and its relative abundance along continental Portuguese latitudes is very pronounced, and this was captured in a certain distributional fashion by both morphological-functional groups and trait-based "thallus morphology" approaches, were some groups emerge at the expense of others along latitude. The relative abundance of species also changed in a very strong manner between wave-exposures and respective sedimentation regimes, where the trait-based

"thallus morphology" approach was also highlighted in its ability to detect these small-scale environmental changes, and thus may be useful to define assemblages range of values under naturally varying conditions, so that assemblages changes due to anthropogenic impacts may be detected. Another problem, this work also highlights the need for considering estuarine output effects on adjacent coastal areas, which can be detected through the use of contemporary analytical techniques such as stable isotopic analysis on macroalgae (Chapter IV). The approach may be particularly useful because it allows tracking impacts from the source, such as nutrients pollution from inland estuarine areas, until the final area of impact, where macroalgae, as primary producers, may use those for growth (e.g. originating opportunistic macroalgal blooms). The information provided by this approach, together with the use of ecological/hydrodynamic models, can be helpful to define vulnerable areas and to follow the results of the implementation of mitigation programmes on the adjacent coastal zones.

Resumo

As macroalgas são um grupo diverso de organismos, sendo elementos biológicos importantes para a estrutura e função dos ambientes costeiros e estuarinos. Contribuem para a integridade dos ecossistemas, pelo que o desenvolvimento de indicadores ecológicos, baseados nas macroalgas, para avaliar a saúde dos ecossistemas é fundamental para o êxito da gestão e proteção da biodiversidade, da avaliação dos impactos antropogénicos e da recuperação dos ecossistemas alterados. De forma a avaliar a qualidade ambiental através das macroalgas, as suas populações devem ser descritas sob variações naturais das condições ambientais (condições ecológicas de referência), de modo a que o desvio relativo a esses padrões ecológicos possa ser reconhecido como uma alteração resultante de pressões antropogénicas. Isto depende, em grande parte, da identificação prévia da variabilidade natural dos padrões das populações de macroalgas, numa gama de escalas relevantes, e da compreensão dos processos e fatores que estruturam os seus padrões. Em primeiro lugar, este trabalho aborda diferentes medidas usadas no estudo na biodiversidade das macroalgas, que podem ser usadas na descrição das suas populações sob condições ecológicas de referência (capítulo I). O consumo de tempo e a representatividade são questões centrais nos projetos de monitorização. O problema é que se podem encontrar muitas espécies diferentes de macroalgas sob diversas condições naturais e numa vasta gama de escalas espaciais e temporais; isso implica um elevado esforço de amostragem e de processamento laboratorial, que depende de um conhecimento taxonómico especializado, bem como da necessidade de um conhecimento aprofundado sobre as características estruturais e funcionais dos ecossistemas em que as macroalgas vivem. O uso de medidas alternativas da biodiversidade macroalgal (normalmente baseada nas diferentes espécies encontradas), como por exemplo o agrupamento das espécies em diferentes grupos morfológicos-funcionais ou em grupos baseados na característica "morfologia do talo", podem melhorar a síntese ecológica e o custo/eficácia das monitorações. No entanto, essas medidas alternativas da biodiversidade devem ser testadas espacialmente de uma forma explícita. Em particular, este trabalho também estudou as medidas substitutas/alternativas da biodiversidade macroalgal, acima mencionadas, comparando a variabilidade das populações de macroalgas nos seus padrões de zonação intertidal ao longo de uma escala espacial ampla, nomeadamente ao longo do gradiente latitudinal de Portugal continental (Capítulo II). Além disso, a abordagem baseada na característica "morfologia do talo" foi testada numa escala espacial local, para padrões de zonação intertidal, nomeadamente num gradiente de exposição à ondulação associado a diferentes regimes de sedimentação (Capítulo III). Ambos os estudos sugerem que a abordagem "morfologia do talo" poderá ser útil como uma medida substituta e complementar da avaliação e monitorização da biodiversidade macroalgal. Em ambos os estudos, a estrutura das populações (composição e abundância) variou profundamente de acordo com os gradientes

considerados. Ao longo da latitude de Portugal continental, as mudanças na identidade das espécies e na sua abundância relativa revelou ser particularmente pronunciada, e tal foi também evidenciado pela forma de distribuição das diferentes medidas alternativas de quantificação da biodiversidade estudadas (grupos morfológicos-funcionais e "morfologia do talo"). Ao longo da latitude, alguns grupos de espécies surgem enquanto outros desaparecem. A abundância relativa das espécies também mudou de forma muito pronunciada face à exposição à ondulação e respetivos regimes de sedimentação. Aqui, a abordagem "morfologia do talo" destacou-se pela sua capacidade em detetar mudanças ambientais a uma pequena escala, o que poderá torna-la útil, depois de definidos os intervalos de variação das condições naturais, na deteção de alterações nas populações devidas a pressões antropogénicas. Por outro lado, este trabalho também destaca a necessidade de considerar o efeito da poluição estuarina nas áreas costeiras adjacentes, que pode ser detetado através do uso de técnicas analíticas modernas, tais como a análise dos isótopos estáveis em macroalgas (Capítulo IV). A abordagem pode ser particularmente útil porque permite seguir os impactos a partir da fonte, tais como a poluição por nutrientes provenientes de áreas a montante do estuário, até à derradeira área de impacto, onde as macroalgas, como produtores primários, poderão utilizar esses nutrientes no seu crescimento (podendo originar "blooms" de macroalgas oportunistas). A informação fornecida por esta abordagem, juntamente com o uso de modelos ecológicos/hidrodinâmicos, pode ser útil para definir áreas vulneráveis e acompanhar o resultado de programas de mitigação implementados nas zonas costeiras adjacentes.

Palavras-chave: Macroalgas, Áreas costeiras e estuarinas, Avaliação ambiental, Condições ecológicas de referência, Variabilidade espacial, Grupos funcionais, Características/traços, Morfologia do talo, Medidas substitutas/alternativas da biodiversidade, Zonação intertidal, Gradiente latitudinal, Exposição à ondulação, Sedimentação, Pressões antropogénicas

General introduction

Aiming to protect and manage aquatic ecosystems, the systematic assessment of biological communities is required, namely under contemporary European Union legislations such as the Habitats Directive (HD, 1992), the Water Framework Directive (WFD, 2000) and the Marine Strategy Framework Directive (MSFD, 2008). In this context, benthic communities, including macroalgae, are obligatory biological elements when monitoring European coastal and transitional (estuarine) waters and evaluating their ecological and conservation status.

The successful management and protection of biodiversity, the assessment of anthropogenic impacts and the restoration of altered ecosystems rely largely on quantifying assemblages variability patterns across a range of relevant scales, and on understanding the processes and factors that structure these assemblages (Underwood et al., 2000; Benedetti-Cecchi, L., 2001; Díaz-Tapia, et al 2013 and references therein). However, the inherent spatial and temporal complexity and variability of coastal ecosystems will always present problems for meaningful biomonitoring (de Jonge et al., 2006). Difficulties related with the assessment of benthic communities such as marine macroalgae are well known, not only due to their natural variability across a range of spatial and temporal scales, but also due to their high sampling and laboratory processing effort, as well as the insufficient knowledge about the structural and functional characteristics of coastal ecosystems (Puente and Juanes, 2008).

Since our understanding of different factors driving ecological patterns and processes is spatial scale-dependent (Wiens, 1989; Levin, 1992; Willig et al., 2003), the analysis of spatial patterns of assemblages contributes to identify the major ecological processes that may determine these patterns. Different ecological processes generate variability at different scales (where some processes can only act at small scales and some only at large scales), and therefore the examination of patterns across a range of spatial scales is a fundamental step before ecological explanations for these patterns can be proposed (Underwood & Chapman 1996; Hewitt et al. 2007; Burrows et al. 2009).

Particularly, such variable community patterns may raise somehow problems for monitoring programs and for environmental impact studies purposes. Because macroalgae species naturally vary across space and time, the need to have a spatial-explicit approach has been underline (e.g. Menge et al., 2005). If small-scale variation goes undetected, differences due to impacts may be confused with differences due to natural spatial variability (Coleman, 2002). To quantify assemblages' natural variability is thus imperative in order to identify relevant scales for investigating either natural processes or anthropogenic impacts on ecological systems (Anderson et al., 2005). In order to comprehend and compare monitored values and to evaluate macroalgal assemblages departures resultant from anthropogenic impacts, the need to establish ecological reference conditions, targets or starting point

descriptions of assemblages is therefore of utmost importance for conservation and management issues (e.g. Borja et al., 2012; Gaspar et al., 2012).

Macroalgae thriving in rocky shores are known to vary naturally across space and time in response to local or small- (10s to 100s of centimetres) to middle-scales (10s to 100s of meters) environmental factors and processes. Different species tend to occur in a distinct fashion at specific levels along a height axis, from the lower to the upper shore, giving the idea of species zonation patterns, particularly visible at the intertidal rocky shore landscapes (e.g. Araújo et al., 2005; Martins et al., 2008). The vertical variability of macroalgal assemblages is known to be resultant from the effects of biological and physical factors such as competition, grazing, recruitment, substrate slope, wave action, aerial exposure, irradiance, temperature ranges or time available for nutrient exchange (e.g. Benedetti-Cecchi et al. 1999, 2001; Coleman, 2003; Lobban & Harrison, 1994; Choi & Kim, 2004). However, assemblages vertical zonation patterns may vary along the coastline (horizontal variations) due to processes unrelated to vertical gradients (Chapius et al., 2014). In larger geographical scales such as along latitudinal gradients, most species can vary in their ecology in response to large-scale environmental variability (Brown, 1984). Potential processes responsible for variability at these spatial scales (differences among sites, each one commonly meant to be between 100s and 1000s of m in width) include wave-exposure and ice-scour, recruitment, sea surface temperature, photosynthetically active radiation and nutrients variability (Valdivia et al, 2011 and references therein; Tuya et al., 2012; Ramos et al., 2012).

Critically, broad-scale processes may add extra variability to the patchiness commonly observed at fine scales on rocky shores (Martins et al., 2008). Thus, it has been highlighted the need of more knowledge on how vertical variation (intertidal zonation) compares with horizontal variation measured at increasing spatial scales (in terms of sampling interval along the shoreline) (Araújo et al., 2005; Fraschetti et al., 2005; Martins et al., 2008; Burrows et al., 2009; Cruz-Motta et al., 2010; Valdivia et al., 2011; Veiga et al., 2012; Chapius et al., 2014).

On the other hand, representativeness and time consumption are central issues in monitoring designs (Ballesteros et al., 2007). Species-level approaches to detecting change are costly because are labor-intensive and need high taxonomic expertise. Alternatives to macroalgal species-level approaches can be the use of other taxonomic resolutions, morphologies, functional groups and traits (e.g., Steneck and Dethier, 1994; Konar and Iken, 2009; Orfanidis et al., 2011), which may be useful as surrogate measurses of macroalgae (species-level) biodiversity, depending on the monitoring and assessment needs. Biological traits, in the sense of well-defined, measurable properties of organisms, usually measured at the individual level and used comparatively across species (McGill et al., 2006), may be seen as well as complementary surrogate measure of the (species-level measured) biodiversity. However, the use of surrogates' measures implies that the relationship between the assemblage structure considering species and the surrogate is consistent in space (Colwell and Coddington, 1994). These assumptions have, however, rarely been examined explicitly (Smale, 2010; Rubal et al., 2011; Veiga et al., 2012).

Also through the use of macroalgae, contemporary analytical techniques such as stable isotopic analysis and modeling can be helpful for monitoring and assessing human impacts of coastal environments. Particularly, to detect the origin, fate and distribution of anthropogenic discharges in the sea is of critical importance for the conservation and management of coastal zones (Orlandi et al., 2014). One problem, when trying to identify the spatial footprint of land-derived nutrient estuarine plumes at the adjacent coastal area, is linked to monitoring difficulties because of their inherent high-frequency of temporal and spatial variability (Fernandes et al., 2012). In this context, analytical techniques such as stable isotopic analysis and modeling applied on macroalgae may allow to measure the effect of polluted estuarine waters discharges on adjacent coastal areas (e.g., Costanzo et al., 2001, 2005; Gartner et al., 2002; Savage and Elmgren, 2004).

Under above mention scopes, this PhD thesis was driven by the following specific questions:

A) how do different approaches can be used to investigate marine macroalgae under ecological reference conditions? (Chapter 1);

B) how do the vertical (intertidal zonation) and horizontal (latitudinal gradient) patterns of variability of macroalgae assemblages are structured across continental Portugal, as well as how some macroalgal biodiversity surrogates respond to such large-scale variability patterns? (Chapter 2);

C) how do some macroalgal biodiversity surrogates respond to small-scale naturally varying conditions, such as between different wave-exposures associated with different sedimentation regimes? (Chapter 3);

D) how do estuarine polluted water discharges influence adjacent coastal waters quality? (Chapter 4).

Chapter I

Searching for ecological reference conditions

of marine macroalgae[1]

I.1. Introduction

Worldwide increase of human pressures and consequent degradation of the ecological quality of aquatic systems have been contributing to the awareness that the integrity of marine ecosystems is under threat (e.g., Crain et al., 2009) and hence, in decline from earlier natural or pristine conditions. In this context, the idea of a 'natural' or 'pristine' ecological condition that can be linked to a historic past without significant human pressures, leads to the idea of ecological reference conditions, against which the current degraded ecological conditions can be seen in perspective and compared.

The ecological degradation of marine environments can be caused by multiple stressors, such as the water pollution resultant from urban, industrial and agricultural waste, causing problems like toxic chemical pollution, suspended sediments and excessive nutrients in the water (e.g., UNEP/GPA, 2006). The anthropogenic impacts induce changes on the coastal assemblages, namely on the spatial and temporal patterns of the organisms' distribution, decreasing the species diversity and affecting the physical and biological structure of natural habitats (Benedetti-Cecchi et al., 2001 and references therein). It affects as well the ecosystem goods and services, such as food provision, disturbance prevention, nutrients recycling or leisure and recreation (e.g., Beaumont et al., 2007).

Macroalgae species, being sessile and aquatic organisms, can integrate and reflect along time the water related environmental characteristics of the shores they occupy (Ballesteros et al., 2007). In this sense, contemporary anthropogenic pressures may shift the macroalgae communities from pristine conditions to degraded quality states. These communities show similar patterns when exposed to disturbance: decrease in the species richness, reduction in the complexity of the community structure and changes in the patterns of variability; the decrease or disappearance of the most sensitive species, like large canopy-forming and slow-growing perennial species, tend to be replaced by opportunistic, short-lived and fast-growing species, with lower structural complexity, such turf-forming, filamentous, leaf-like or other annual macroalgae (e.g., Murray and Littler, 1978; Diez et al., 1999; Tewari and Joshi, 1988; Benedetti-Cecchi et al., 2001; Thibaut et al., 2005; Mangialajo et al., 2008; Gorman and Connell, 2009; Perkol-Finkel and Airoldi, 2010).

As a matter of fact, macroalgae have been widely used to evaluate the effects of anthropogenic disturbances, such as climate change (e.g., Lima et al., 2007; Hawkins et al., 2008; Fernandez, 2011; Harley et al., 2012), habitat loss (e.g., Airoldi, 2003; Airoldi and Beck, 2007) or, more commonly, changes resultant from human-polluted waters (e.g., Tewari and Joshi, 1988; Gorostiaga and Diez, 1996; Rodriguez-Prieto and Polo; 1996; Roberts et al., 1998; Soltan et al., 2001; Panayotidis et al.,

2004; Melville and Pulkownik, 2006; Yuksek et al., 2006; Arévalo et al., 2007; Scanlan et al., 2007; Krause-Jensen et al., 2008; Juanes et al., 2008; Orfanidis et al., 2011; Neto et al., 2012).

For example, human activities have been closely linked with the increase of nutrients (e.g., nitrogen based nutrients) in marine waters towards eutrophication, which represents an important issue for ecology and environmental management (e.g., Vitousek et al., 1997; de Jong, 2006; Kelly, 2008). Eutrophication leads to the proliferation of opportunist and tolerant macroalgae (e.g., Morand and Merceron, 2005) featured by fast-growing and high nutrient uptake rates (e.g., Wallentinus, 1984), at the expense of seagrasses and perennial macroalgae (e.g., Duarte, 1995; Schramm, 1996). The opportunistic turf-forming species (genera examples include green macroalgae *Ulva, Chaetomorpha, Cladophora* and *Monostroma*, and red macroalgae *Ceramium*, *Gracilaria* and *Porphyra/Pyropia* or brown macroalgae *Ectocarpus* and *Pilayella*) can bloom into nuisance proportions (Morand and Merceron, 2005; Scanlan et al., 2007). Contrarily, canopy macroalgae, such as the perennial genus *Cystoseira*, are highly sensitive to anthropogenic disturbances and tend to disappear from sites nearby urban areas and with higher levels of nutrient concentration (e.g., Benedetti-Cecchi et al., 2001; Sales and Ballesteros, 2009).

Therefore, as macroalgae communities can reflect ecological degradation, their study can be useful to quantify the level of marine ecosystem integrity or to what extent the conditions have changed from their former pristine environment. It is for such reasons that for water policies such as the European Water Framework Directive (WFD, 2000/60/EC), these organisms are key biological elements when assessing the ecological quality condition of coastal waters.

Yet difficulties may arise when trying to effectively describe ecological reference conditions, given the limited knowledge about coastal environments, of the dynamism of their natural processes, and the linkage between different levels of anthropogenic pressure and the varying conditions of ecological degradation. This work aims to briefly review some investigative approaches concerning the challenge of describing marine macroalgae under ecological reference conditions.

I.2. The spatial and temporal uncertainty of macroalgae natural patterns

Despite their benthic sessile feature, the macroalgae species exhibit populations whose distribution along coastal rocky shores is not uniform, either in space or time, but rather, in a non-random and dynamic way (e.g., Lobban and Harrison, 1994). This results from complex ecological processes, such as succession patterns, where different species have different recruitment, growth and mortality rates (e.g., Foster et al., 2003; Cervin et al., 2005). Naturally, a variety of different 'mosaics', 'patches' or 'assemblages' of macroalgae species can be observed. These assemblages tend to differ in shores, both spatially and temporally, according to the presence or absence (composition) of different species and accordingly to the relative abundance of each species. For example, different

macroalgae species tend to exhibit vertical patterns of distribution, from the uppermost to the lowermost tide levels, giving the idea of different zones of species or zonation patterns. This is because different species have different adaptive responses to several physical (e.g., emersion or exposure to the atmosphere), chemical (e.g., salinity) and biotic (e.g., competition, grazing) factors (e.g., Lobban and Harrison, 1994; Dawes, 1998), which can influence unevenly the different locations on the shore. Being aquatic species, the complexity of the macroalgae communities' structure (composition and abundance) tends to increase from land to sea, as the aquatic environments' characteristics become more effective at lower levels on the shore; the environmental factors variability tends to be higher at upper shore levels (e.g., higher emersion times tend to create more desiccation problems), causing to the species, several vertical stress gradients (Raffaelli and Hawkins, 1999).

Ultimately, the distributional area of each species can be seen as the complex expression of its ecology and evolutionary history, determined by diverse factors operating with different intensities and at different scales (Soberón and Peterson, 2005). Many environmental factors can influence macroalgae but, depending on the spatial and time scales considered, some factors may, more than others, influence the particular distribution of each species. As species differ in their adaptations to particular environments, the outcome of interactions depends on the species identity (Viejo et al., 2008) and, at the end, on its population distribution patterns, either locally or in a broader global geographical scale. In this connection, there are considerable gaps in the understanding about rocky shore communities, including the need of cross country comparisons, the role of key species, the macroalgae vs. filter-feeders interactions, the early life history of the species, the effect of the nearshore upwelling and downwelling water mass or the effect of human impacts (Schiel, 2004).

Undoubtedly, much scientific knowledge has been produced regarding marine macroalgae, including environmental, biogeographical, ecological or ecophysiological issues. Yet complete knowledge of macroalgae distribution patterns (from single species to populations to entire communities) and the prevailing environmental factors driving macroalgae species assemblages to arrange in a particular way on a given shore or in a broader geographical scales, may continue on the marine biologists' agendas as research challenges. Moreover, knowledge of these dynamic patterns grows more uncertain when considering that environmental factors do change, due to complex and interrelated processes that occur both naturally and, not less important, as a result of human activities.

I.3. The concept of reference conditions

Several terms with equivalent meanings have been applied to describe the reference conditions present in a site (e.g., unimpaired, unperturbed, undegraded, unaltered, undisturbed, nearly undisturbed or least affected). The concept of ecological reference conditions has been applied in several contexts, including environmental considerations (from "pristine" to the "best available" state of a water

body, in a historical or a spatial context, etc.) and also including a wide range of economic, social and political considerations (Economou, 2002). Economic and social aspects of marine management may have equal or perhaps even greater weight than the ecological aspects, since we have to protect and maintain the natural ecological characteristics while concurrently delivering the services and benefits required by society (Elliott, 2011).

For water ecosystems such as coastal environments, the ecological reference conditions are associated to a set of biological, physical, chemical and hydrologic quality elements. They should reflect the status of those quality elements under pristine, or not impacted conditions, i.e., conditions to be found in the absence of, or under minimal anthropogenic disturbance. Biological reference conditions (in the sense of the WFD) can be derived from biological communities that inhabit sites without (or with minor) anthropogenic disturbances and should reflect an ecological state that corresponds to very few pressures, i.e., without the effects of major industrialisation, urbanisation or intensification of agriculture and with only very minor modifications of the physical, chemical, hydromorphological and biological characteristics (e.g., WFD CIS, 2009).

This can be interpreted as sites having an absence of pressures or a presence of high ecological quality. However, even large areas such as Europe may not have many pristine places to be used as reference sites. Furthermore, if the human impacts are considered on a global climate change, it can be acknowledged that the pristine marine habitats are currently scarce and may be impossible to achieve (Borja et al., 2013).

Consequently, different approaches can be used in order to define ecological reference conditions. The WFD Common Implementation Strategy (WFD CIS, 2003) suggested an hierarchical criteria for defining reference conditions using various methods in the following order: i) an existing undisturbed (pristine) site or a site with only very minor disturbance; ii) historical data and information; iii) models; and (iv) expert judgment. Also, legislation such as the Marine Strategy Framework Directive (MSFD) is seeking: (v) environmental targets, rather than (undisturbed) reference conditions; this is because it is recognized that humans are part of the marine ecosystem (as users) and, then, their activities can create a certain impact, making it impossible to find pristine areas (Borja et al., 2012).

Important advantages and disadvantages of those methodologies have been recently discussed under the relationships of the DPSIR paradigm (between Drivers-Pressure-State of change-Impact-Responses variables), in assessing the environmental quality status of the marine waters, and having an 'ecosystem approach' or 'holistic approach' methodological point of view (see Borja et al., 2012). Within that framework, each methodological approach (pristine sites, historical data, modelling, best professional judgment, baselines state set at a date in the past or at a current state) aims to relatively different directions (or target goals) for ecological reference and recovery (Fig. I.1).
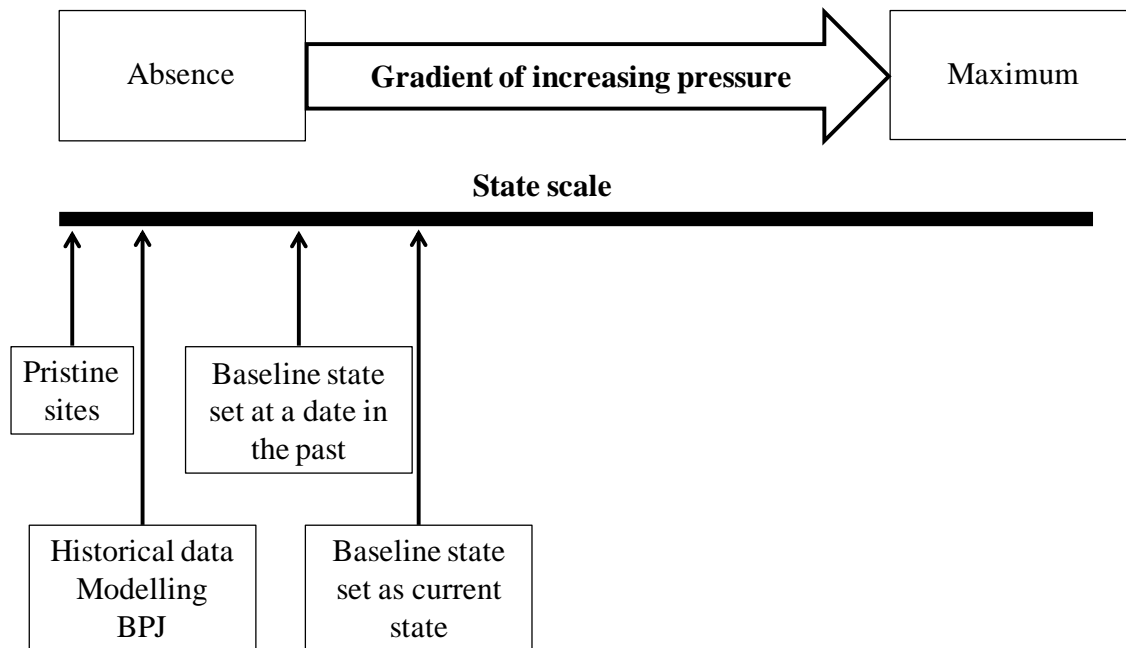
Figure I.1. Environmental status can be regarded as a gradual transition from pristine conditions (high status in the absence of human pressures) to an irrecoverable status (bad status, in a maximum of human pressure). Assessment systems need to set reference conditions (pristine sites, historical data, modelling, best professional judgment—BPJ) or baseline targets (set at a date in the past or as current state) along the pressure (and subsequent state) gradient to assist in status assessments (Adapted from Borja et al., 2012).

Hence, it is difficult to define how far back the baseline has to be set, or how pristine a condition a benchmark should characterize (Hawkins et al., 2010). It depends both on the level of ecological conditions that are aimed by human society and on the availability of historical data and knowledge of the system. In fact, 'pristine state' or 'naturalness' can be best viewed as having dynamic attributes, which may need to be periodically redefined in response to new and better scientific knowledge (van Hoey et al., 2010 and references therein).

Still the need to describe the natural condition of a system — whose features may establish a standard, target goal, or reference condition — becomes as important as science needs controls to compare results (Economou, 2002). Ultimately, to describe a reference condition is to point out directions for ecological restoration and protection (Borja et al., 2012). Reference conditions are just that, a reference, which can be linked to ecological conditions that remain pristine or unchanged, but also as defined environmental goals that can be considered (somehow) reasonable to be achieved.

Regardless of the degree of 'naturalness' or 'pristineness' implied by a specific definition of a benchmark, the accuracy and precision when assessing ecological quality conditions are dependent on the degree to which those benchmarks can be quantified and predicted (Hawkins et al., 2010). Bearing this in mind, several investigation approaches to describe ecological reference conditions regarding the marine macroalgae are briefly presented below.

I.4. Investigation approaches to describe the ecological reference conditions of marine macroalgae

I.4.1. Establishing biotypes ranges for ecological homogeneity: the type-specific reference condition approach

Marine ecosystems are subject to many different changes resulting from both natural processes and human activities (e.g., Aubry and Elliott, 2006). In fact, one of the problems associated with using a reference condition approach is that high natural variability may be present, making it difficult to distinguish between anthropogenic and undisturbed environmental effects on biotic communities. The combination of abiotic and biotic attributes, such as different climates and biotypes, contribute to define different eco-regions, but these eco-regions themselves embrace high biological variability due to environmental heterogeneity or historical/phylogenetic factors (Economou, 2002). For example, the temperature plays an important role on the survival, recruitment, growth and reproduction of macroalgae (Breeman, 1988). This is also reflected by the global geographical distributions of macroalgae species, which are typically delimited by certain seawater isotherms (Lüning et al., 1990). The sea surface temperatures outline boundaries among different bio-geographical regions, preventing the uninterrupted spread of macroalgae species beyond their present distributions to rocky shores all over the world (Eggert, 2012). In this regard, considering for example, the North East Atlantic geographical region by itself, it has heterogeneous coastal waters, with diverse species composition. It includes zones as diverse as Norway, in the North, and the Canary Islands in the South, which makes difficult to establish a common reference condition for the region as a whole (Ramos et al., 2012). Actually, on the Portuguese coastline, several coldwater species (i.e., species abundant in Northern Europe) and several warm-water species (i.e., species that are commonly found in the Mediterranean Sea and in Northwestern Africa) reach their southern or northern distributional limits, respectively (Lima et al., 2007).

To minimize the variability associated with geographical differences, a common approach is to organize the environmental information on a narrower spatial scale. For an effective use of the reference data, the variability within the monitoring network over which the same reference conditions apply must be small enough, in order to enable the effects of anthropogenic activities to dominate (Economou, 2002). Hence, different type-specific reference conditions should be established. The biological reference conditions must summarize a range of possibilities and values over periods of time

and across the geographical extent of each water body type; the reference conditions represent part of nature's continuum and must reflect the natural variability (WFD CIS, 2003).

In fact, in order to predict and better distinguish the anthropogenic effects from the natural environmental effects on biological communities, a more appropriate geographical area or type scale allows to define biological communities with higher homogeneity even though type-specific areas encompass communities presenting high variability features, such as the differences resultant from seasonal species, the effects of phenotypic plasticity, or the intra-specific genetic differences. On the other hand, to describe different reference conditions at higher discrimination levels (small areas like site level) would imply important disadvantages (costs, attainability, data availability, time availability, etc.) (Economou, 2002).

I.4.2. From species richness to grouping species into representative *taxa* listings

It can be agreed that the analysis of species composition and abundance is an unavoidable methodological approach to describe the structure of their community assemblages. In this respect, the WFD outlined the following composition and abundance criteria that should be related to type-specific reference conditions: (1) taxonomic composition corresponds completely to, or almost completely to undisturbed conditions (where all sensitive *taxa* should be present); and (2) there are no detectable changes in macroalgae abundances due to anthropogenic disturbances (WFD CIS, 2003).

Nevertheless, the absence of reference values, or the awareness of the ecological state of a certain system against which comparisons can be made, makes these assessment requirements complicated to address (Borja et al., 2004). The sensitive species are not easy to define and the species composition changes for natural reasons even under undisturbed conditions (Wells et al., 2007). Moreover, to identify all macroalgae to the species level and describe their relative abundance (such as species coverage or biomass) over time and space is a task that involves several problems such as the time consumption involved, the need for taxonomic expertise or the costs of monitoring designs. Representativeness and time consumption are central issues in monitoring designs. Sorting, identifying and quantifying the macroalgae species samples are time and labour demanding and need good taxonomical expertise (Ballesteros et al., 2007) to accurately record their species richness (Wells et al., 2007).

Taxonomical identifications with a species-level resolution are a time-consuming task and therefore, the need to find simpler ways to assess species richness may be inevitable (Wilkinson and Wood, 2003). Macroalgae species richness decreases along increasing disturbance gradients, shifting the composition of their communities (e.g., Díez et al., 2012) while, on the other hand, the numerical macroalgae species richness—not the list of actual species present—remains approximately constant in the absence of disturbance, which provides an excellent rationale for using it as a measure of ecological quality (Wells et al., 2007).

In effect, most of the macroalgae species are easily identifiable in the field with easy-to-acquire expertise and, therefore, allow the monitoring of large areas with relatively little effort (Ballesteros et al., 2007). The use of both non-destructive data collection and easy-to-apply methods has an effective cost-benefit relationship (e.g., Wells et al., 2007; Juanes et al., 2008), being scientifically rigorous and at the same time, allowing the execution of wider monitoring plans (Guinda et al., 2008). An alternative way to record qualitative species data has been achieved by selecting comprehensive and representative species listings that can be more commonly found within certain geographical areas or typologies and simultaneously associated with expected undisturbed conditions (Table I.1) (e.g. Wells et al., 2007; Guinda et al., 2008; Juanes et al., 2008; Gaspar et al., 2012; Bermejo et al., 2012).

When developing a representative and comprehensive species list for a certain area, the species' natural variety must be considered. Also, the list of species must reflect the water conditions over time, and should be sensitive enough to detect those changes in the composition of macroalgae communities. Representativeness can be included by merging the available historical data with the contemporary monitoring data, allowing for the selection of more common *taxa*. Selected *taxa* can be grouped considering taxonomical, morphological or functional similarities (see Section I.4.4 below), while maintaining the natural proportions of the main macroalgae taxonomical groups (Chlorophyta, i.e., green macroalgae, Heterokontophyta-Phaeophyceae, i.e., brown macroalgae and Rhodophyta, i.e., red macroalgae) (see Section I.4.3 below) that are normally associated with a given water body type (Gaspar et al., 2012). Departing from previous specific works done for certain geographical areas, these approaches may define *a priori* which are the main characteristic species of macroalgae that constitute well-defined, conspicuous populations, along with opportunistic species related to anthropogenic disturbances, as well as invasive species (e.g., *Sargassum muticum* in European coastal waters) (Juanes et al., 2008). Furthermore, taking into account the seasonal variability of macroalgae communities, composition data for those selected *taxa* can be studied from undisturbed areas during their local seasonal period of maximum development (spring/summer). Last but not least, during that period of time and at the same undisturbed areas, data concerning macroalgae abundance can be estimated, such as the coverage of characteristic species and the coverage of opportunistic species (Juanes et al., 2008; Gaspar et al., 2012).

Table I.1. Examples of representative species listings established for different geographical areas within European Atlantic coasts, namely for the coasts of Southern England, Republic of Ireland and Wales, Northern Ireland, Scotland and Northern England (Wells et al., 2007), Northern Spain (Cantabrian) (Guinda et al., 2008; Juanes et al., 2008), Northern Portugal (Gaspar et al., 2012), and Southern Spain (Andalusia) (Bermejo et al., 2012). The species' opportunistic character is indicated (Op). Species examples are given for particular species groupings at the table end.

Table I.1.

Southern England, Republic of Ireland & Wales	Northern Ireland	Scotland and Northern England
Chlorophyta	**Chlorophyta**	**Chlorophyta**
Blidingia sp. (Op)	*Blidingia* sp. (Op)	*Blidingia* sp. (Op)
Bryopsis plumosa	*Chaetomorpha linum* (Op)	*Chaetomorpha linum* (Op)
Chaetomorpha linum (Op)	*Chaetomorpha mediterranea* (Op)	*Chaetomorpha melagonium*
Chaetomorpha ligustica (mediterranea) (Op)	*Cladophora albida*	*Cladophora rupestris*
Chaetomorpha melagonium	*Cladophora rupestris*	*Cladophora sericea*
Cladophora rupestris	*Cladophora sericea*	*Ulva* sp (*Enteromorpha* sp.) (Op)
Cladophora sericea	*Enteromorpha* sp. (Op)	*Sykidion moorei*
Enteromorpha sp. (Op)	*Monostroma grevillei*	*Ulva lactuca* (Op)
Ulva lactuca (Op)	*Rhizoclonium tortuosum*	
	Spongomorpha arcta	
	Ulothrix sp.	
	Ulva lactuca (Op)	
Phaeophyceae (Heterokontophyta)	**Phaeophyceae (Heterokontophyta)**	**Phaeophyceae (Heterokontophyta)**
Ascophyllum nodosum	*Alaria esculenta*	*Alaria esculenta*
Chorda filum	*Ascophyllum nodosum*	*Ascophyllum nodosum*
Cladostephus spongious	*Asperococcus fistulosus*	*Asperococcus fistulosus*
Dictyota dichotoma	*Cladostephus spongious*	*Chorda filum*
Ectocarpus sp. (Op)	*Dictyota dichotoma*	*Chordaria flagelliformis*
Elachista fucicola	*Ectocarpus* sp. (Op)	*Cladostephus spongious*
Fucus serratus	*Elachista fucicola*	*Desmarestia aculeata*
Fucus spiralis	*Fucus serratus*	*Dictyosiphon foeniculaceus*
Fucus vesiculosus	*Fucus spiralis*	*Dictyota dichotoma*
Halidrys siliquosa	*Fucus vesiculosus*	*Ectocarpus* sp. (Op)
Himanthalia elongata	*Halidrys siliquosa*	*Elachista fucicola*
Laminaria digitata	*Himanthalia elongata*	*Fucus serratus*
Laminaria hyperborea	*Laminaria digitata*	*Fucus spiralis*
Saccharina latissima (Laminaria saccharina)	*Saccharina latissima (Laminaria saccharina)*	*Fucus vesiculosus*
Leathesia difformis	*Leathesia difformis*	*Halidrys siliquosa*
Pelvetia canaliculata	*Pelvetia canaliculata*	*Himanthalia elongata*
Pilayella littoralis (Op)	*Petalonia fascia*	*Laminaria digitata*
Ralfsia sp.	*Pilayella littoralis* (Op)	*Laminaria hyperborea*
Saccorhiza polyschides	*Ralfsia* sp.	*Saccharina latissima (Laminaria saccharina)*
Scytosiphon lomentaria	*Scytosiphon lomentaria*	*Leathesia difformis*
	Sphacelaria sp.	*Litosiphon laminariae*
	Spongonema tomentosum	*Pelvetia canaliculata*
		Pilayella littoralis (Op)
		Ralfsia sp.
		Scytosiphon lomentaria
		Spongonema tomentosum
Rhodophyta	**Rhodophyta**	**Rhodophyta**
Aglaothamnion/Callithamnion	*Aglaothamnion/Callithamnion*	*Aglaothamnion/Callithamnion*
Ahnfeltia plicata	*Ahnfeltia plicata*	*Ahnfeltia plicata*
Calcareous encrusters	*Audouinella purpurea*	Calcareous encrusters
Catenella caespitosa	*Audouinella* sp.	*Callophyllis laciniata*
Ceramium nodulosum	Calcareous encrusters	*Ceramium nodulosum*
Ceramium shuttleworthianum	*Catenella caespitosa*	*Ceramium shuttleworthianum*
Ceramium sp.	*Ceramium nodulosum*	*Chondrus crispus*
Chondrus crispus	*Ceramium shuttleworthianum*	*Corallina officinalis*
Corallina officinalis	*Chondrus crispus*	*Cryptopleura ramosa*
Cryptopleura ramosa	*Corallina officinalis*	*Cystoclonium purpureum*
Cystoclonium purpureum	*Cryptopleura ramosa*	*Delesseria sanguinea*
Dilsea carnosa	*Cystoclonium purpureum*	*Dilsea carnosa*
Dumontia contorta	*Dilsea carnosa*	*Dumontia contorta*
Erythrotrichia carnea	*Dumontia contorta*	*Erythrotrichia carnea*
Furcellaria lumbricalis	*Furcellaria lumbricalis*	*Furcellaria lumbricalis*
Gastroclonium ovatum	*Gelidium* sp.	*Lomentaria articulata*
Gelidium sp.	*Hildenbrandia rubra*	*Lomentaria clavellosa*
Gracilaria gracilis	*Lomentaria articulata*	*Mastocarpus stellatus*
Halurus equisetifolius	*Mastocarpus stellatus*	*Membranoptera alata*
Halurus flosculosus	*Melobesia membranacea*	*Odonthalia dentata*
Heterosiphonia plumosa	*Membranoptera alata*	*Osmundea hybrida*
Hildenbrandia rubra	*Odonthalia dentata*	*Osmundea pinnatifida*
Hypoglossum hypoglossoides	*Osmundea hybrida*	*Palmaria palmata*
Lomentaria articulata	*Osmundea pinnatifida*	*Phycodrys rubens*
Mastocarpus stellatus	*Palmaria palmata*	*Phyllophora* sp.
Membranoptera alata	*Phyllophora* sp.	*Plocamium cartilagineum*
Nemalion elminthoides	*Plocamium cartilagineum*	*Plumaria plumosa*
Osmundea hybrida	*Plumaria plumosa*	*Polyides rotundus*
Osmundea pinnatifida	*Polysiphonia fucoides*	*Polysiphonia fucoides*
Palmaria palmata	*Polysiphonia lanosa*	*Polysiphonia lanosa*
Phyllophora sp.	*Polysiphonia* sp.	*Polysiphonia* sp.
Plocamium cartilagineum	*Porphyra umbilicalis* (Op)	*Pyropia (Porphyra) leucosticta* (Op)
Plumaria plumosa	*Rhodomela confervoides*	*Porphyra umbilicalis* (Op)
Polyides rotundus	*Rhodothamniella floridula*	*Ptilota gunneri*
Polysiphonia fucoides		*Rhodomela confervoides*
Polysiphonia lanosa		*Rhodothamniella floridula*
Polysiphonia sp.		
Porphyra umbilicalis (Op)		
Rhodomela confervoides		
Rhodothamniella floridula		

Table I.1 (continued)

Northern Spain (Cantabria)
Chlorophyta
Blidingia/Derbesia (Op)
Bryopsis plumosa (Op)
Chaetomorpha spp. (Op)
Cladophora spp. (Op)
Codium adhaerens
Codium tomentosum/ C. fragile
Enteromorpha spp. (now *Ulva* spp.) (Op)
Ulva spp. (Op)

Phaeophyceae (Heterokontophyta)
Bifurcaria bifurcata
Cladostephus spongiosus–verticillatus
Colpomenia spp./*Leathesia* spp.
Cystoseira baccata
Cystoseira tamariscifolia
Dictyota dichotoma
Ectocarpaceae/Sphacelaria spp. (Op)
Fucus spiralis
Fucus vesiculosus
Laminaria spp.
Nemalion elminthoides
Pelvetia canaliculata
Ralfsia verrucosa
Saccorhiza spp.
Sargassum muticum
Scytosiphon spp.
Stypocaulon (Halopteris) scoparia

Rhodophyta
Epiphytic filamentous (a) (Op)
Small folioses (b)
Champiaceae (c)
Calcareous encrusters (d)
Asparagopsis armata
Catenella caespitosa (Op)
Caulacanthus ustulatus
Chondracanthus (Gigartina) acicularis
Chondria coerulescens
Chondrus crispus
Corallina elongata/C. officinalis/Jania spp.
Falkenbergia/Trailliella
Gelidium latifolium
Gelidium pusillum
Gelidium corneum (G. sesquipedale)
Gigartina spp.
Gymnogongrus spp.
Halurus equisetifolius
Hildenbrandia spp.
Lithophyllum byssoides
Mastocarpus stellatus
Osmundea (Laurencia) spp.
Peyssonnelia spp.
Plocamium/Sphaerococcus
Porphyra spp. (Op)
Pterosiphonia complanata

(a) *Ceramium, Pleonosporium, Aglaothamnion, Callithamnion, Antithamnion, Antithamionella, Polysiphonia, Dasya, Pterosiphonia.* (b) *Apoglossum, Hypoglossum, Acrosorium, Nytophyllum, Cryptopleura, Rhodophyllis, Stenogramme, Callophyllis, Kallymenia, Rhodymenia.* (c) *Champia, Lomentaria, Gastroclonium, Chylocladia.* (d) *Lithophyllum, Mesophyllum, Lithothamnion.*

Northern Portugal
Chlorophyta
Bryopsis spp. (Op)
Other Filamentous Chlorophyta (1) (Op)
Cladophora spp. (Op)
Codium spp.
Ulva spp. ('Sheet-type')/*Ulvaria obscura/Prasiola stipitata* (2) (Op)
Ulva spp. ('Tubular-type')/*Blidingia* spp. (3) (Op)

Phaeophyceae (Heterokontophyta)
Bifurcaria bifurcata
Cladostephus spongiosus
Colpomenia spp./*Leathesia marina*
Cystoseira spp.
Desmarestia ligulata
Dictyopteris polypodioides
Dictyota spp.
Filamentous Phaeophyceae (4) (Op)
Fucus spp.
Halopteris filicina/H. scoparia
Himanthalia elongata
Laminaria spp.
Pelvetia canaliculata
Ralfsia verrucosa
Saccorhiza polyschides

Rhodophyta
Acrosorium ciliolatum/Callophyllis laciniata/Cryptopleura ramosa
Ahnfeltia plicata
Ahnfeltiopsis spp./*Gymnogongrus* spp.
Apoglossum ruscifolium/Hypoglossum hypoglossoides
Asparagopsis armata/Falkenbergia rufolanosa
Bornetia spp./*Griffithsia* spp.
Calliblepharis spp.
Catenella caespitosa/Caulacanthus ustulatus
Champiaceae (5)
Chondracanthus acicularis
Chondracanthus teedei
Chondria spp.
Chondrus crispus
Calcareous encrusters (6)
Calcareous erect (7)
Dilsea carnosa/Schizymenia dubyi
Gelidiales (8)
Gigartina pistillata
Gracilaria spp.
Grateloupia filicina
Halurus equisetifolius
Hildenbrandia spp.
Laurencia spp./*Osmundea* spp.
Mastocarpus stellatus/Petrocelis cruenta
Nitophyllum punctatum
Other Filamentous Rhodophyta (9) (Op)
Phyllophora spp./*Rhodymenia pseudopalmata*
Palmaria palmata
Peyssonnelia spp.
Plocamium cartilagineum/Sphaerococcus coronopifolius
Porphyra spp./*Pyropia* spp. (Op)
Pterosiphonia complanata
Scinaia furcellata

(1) *Chaetomorpha, Pseudendoclonium, Rhizoclonium, Ulothricales.* (2) *Ulva* spp. 'Sheet-type' in opposition to (3) 'Tubular-type' in the sense of 'ex-*Enteromorpha* spp.' (4) *Ectocarpales/Sphacelaria* spp. (5) *Champia, Chylocladia, Gastroclonium, Lomentaria.* (6) *Lithophyllum, Melobesia, Mesophyllum, Phymatolithon.* (7) *Amphiroa, Corallina, Jania.* (8) *Gelidium, Pterocladiella.* (9) *Acrochaetium, Aglaothamnion, Antithamnion, Bangia, Boergeseniella, Brongniartella, Colaconema, Callithamnion, Ceramium, Compsothamnion, Dasya, Erythrotrichiaceae, Herposiphonia, Heterosiphonia, Janczewskia, Leptosiphonia, Lophosiphonia, Ophidocladus, Pleonosporium, Plumaria, Polysiphonia, Pterosiphonia* (except *P. complanata*), *Pterothamnion, Ptilothamnion, Rhodothamniella, Streblocladia, Vertebrata.*

Southern Spain (Andalusia)
Chlorophyta
Bryopsis spp. (Op)
Chaetomorpha spp. (Op)
Cladophora spp. (Op)
Codium spp. erect (i)
Codium spp. Encrusting (ii)
Codium bursa
Derbesia spp. (Op)
Flabellia petiolata
Pedobepsia simplex
Enteromorpha spp. (Op)
Ulva spp. (Op)
Valonia utricularis

Phaeophyceae (Heterokontophyta)
Cladostephus spongiosus
Colpomenia sinuosa
Cystoseira compressa
Cystoseira spp.
Cystoseira usneoides
Dictyota dichotoma
Dictyopteris polypodioides
Fucus spiralis
Halopteris spp.
Saccorhiza polyschides
Padina pavonica
Laminaria ochroleuca
Ectocarpus and Sphacelaria (Op)

Rhodophyta
Delesseriaceae (iii)
Asparagopsis armata
Botryocladia botryoides
Caulacanthus ustulatus
Ceramium spp. (Op)
Chondracanthus acicularis
Corallina sp.
Gelidium microdon
Gelidium spinosum
Gelidium corneum
Gelidium pusillum
Gymnogongrus and *Ahnfetiopsis*
Halopithys incurva
Halurus equisetifolius
Hildenbradia rubra
Jania rubens
Laurencia obtusa
Lithophyllum byssoides
Lithophyllum dentatum
Lithophyllum incrustans
Nemalion elminthoides
Lomentaria articulata
Osmundea pinnatifida
Osmundea hybrida
Peyssonnelia spp.
Plocamium cartilagineum
Pterocladiella capillacea
Pterosiphonia complanata
Rhodymenia and *Schottera*
Sphaerococcus coronopifolius

(i) *Codium tomentosum, C. fragile, C. vermilara* and *C. decorticatum.*
(ii) *Codium adhaerens* and *C. effusum.*
(iii) *Acrosorium uncinatum, Cryptopleura ramulosa* or *Haraldiophyllum bonnemaisonnii.*

I.4.3. Green, brown and red species patterns

The use of macroalgae species patterns based on their main taxonomical groups — the Chlorophyta (green macroalgae), the Heterokontophyta – Phaeophyceae (brown macroalgae) or the Rhodophyta (red macroalgae) — can be also an important approach beyond the use of species richness alone when considering the macroalgae composition of a given area.

Green, brown and red macroalgae species have distinct patterns of distribution around the globe. Contrary to most kinds of macroscopic organisms in terrestrial and marine habitats, macroalgae species richness does not always increase towards the tropics. Brown macroalgae species richness increases and green macroalgae species richness decreases towards higher latitudes, while red macroalgae species richness increases from the Arctic to the Tropics and from the Tropics to the Subantarctic (Santelices et al., 2009).

Ratios such as the Feldmann's (1937) R:P ratio (the number of red macroalgae to the number of brown macroalgae), the P:C ratio (the number of brown macroalgae to the number of green macroalgae), the R:C ratio (the number of red macroalgae to the number of green macroalgae) or others possibilities such Cheney's (1977) R+C/P ratio (the number of red and green macroalgae to the number of brown macroalgae) can display changes between geographical areas (e.g., Santelices et al., 2009). These aspects can be very useful, since for sites within a range of a particular area, it is possible that the same ratios might have a narrow range of values over a range of separate shores and so that a departure from that range might indicate adverse influences and therefore, less ecological quality (Wilkinson and Wood, 2003).

In order to fully apply the above ratios, the total species taxonomic identification is needed. Nevertheless, through the use of representative species listings (see previous Section I.4.2) similar range of values for species ratios can be calculated and associated with the particular area to which the species are listed. Not least important, the descriptors 'number' and 'proportion' of red, brown and green macroalgae species (e.g., number of green macroalgae, proportion of green macroalgae, etc.) may also be established individually as local reference values, e.g., data outputs resulting from the use of regional species listings in monitoring undisturbed sites (e.g., Gaspar et al., 2012). The species listings representativeness can be evaluated by comparing the relative proportions of red, brown and green macroalgae species between regional phycological checklists or floras against the *taxa* list selected for the same areas (Fig. I.2).

In northern cold waters, the brown algae are naturally dominant, but at southern temperate waters, the red algae predominate (e.g., Lüning, 1990; Boaventura et al., 2002). Depending on the latitudinal location and on the main macroalgae taxonomical group (red, brown or green species) considered, the use of the descriptor 'number', as the 'number' of red species, may correlate higher with environmental change than the use of the descriptor 'proportion', as the 'proportion' of red species. Considering the green species alone, they may depend less on bio-geographic factors, water quality or intertidal structure than the red and brown species: the proportion of green species decreases in

parallel to the improvement of environmental conditions, where late-successional brown and red species thrive in large number; this can be very useful when results from different areas in the same eco-region are compared (Bermejo et al., 2012).
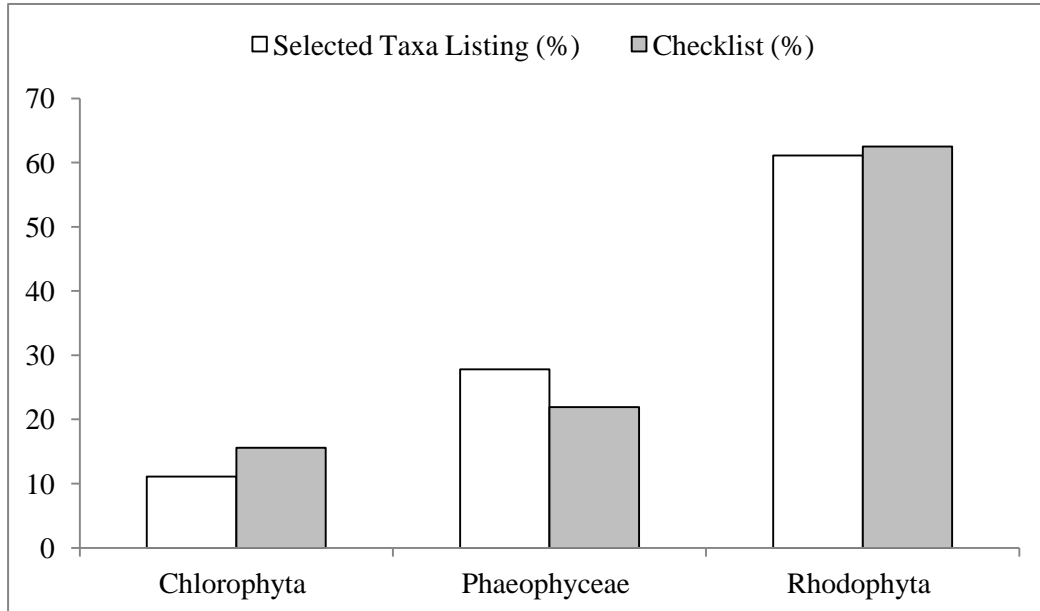

Figure I.2. Comparison among relative proportions (%) of main macroalgal taxonomic groups (Chlorophyta, Phaeophyceae and Rhodophyta) and between the *taxa* list selected for Portuguese Northern Shores (see Table I.1) and the *taxa* recorded by Araújo et al. (2009) regarding the northern Portugal checklist (data from Gaspar et al., 2012).

The number and the proportion of green species are likely to remain constant under stable environmental conditions; although the green species include many opportunistic species, they are also naturally present along the rocky shores. Under good environmental conditions, their presence is not normally dominant, and their biomass can be relatively low. These aspects change when the environmental conditions degrade. The opportunistic green species are able to respond more readily to changes, and dominate in coverage under worst environmental conditions and therefore may ultimately dictate the disappearance of other species (decreasing species richness and increasing the proportion of green species) (Gaspar et al., 2012). The brown species are more likely to remain constant within certain ranges of environmental quality change (Wells et al., 2007). In the northern hemisphere, they show changes with latitude, decreasing from the north to the south in the presence of warmer waters but they decline under deviations from excellent quality standards (Gaspar et al., 2012).

The red species also show changes with latitude, increasing from northern cooler to southern warmer waters and in systems subject to less anthropogenic pressure, where they naturally dominate in numbers over the brown and the green species. Because of this, with increasing disturbances, the number of red species declines, although their proportions may not decline as clearly as in numbers, but ultimately that affects the diversity and the species richness (Gaspar et al., 2012).

I.4.4. The use of biodiversity surrogates and functional traits

The use of biological surrogates as proxies for biodiversity patterns is gaining popularity, namely in marine systems where field surveys can be expensive and species richness high (Mellin et al., 2011). According to Smale (2010), a range of biodiversity (in the sense of species richness or diversity) surrogates have been proposed, which are usually derived by (1) selecting a subset of species (or higher taxon) from the whole assemblage that is thought to represent the richness of the whole assemblage (or another taxon); (2) analysing the entire assemblage to a coarser taxonomic resolution than species, thereby reducing the number of variables and level of expertise required; (3) employing the diversity of morphological or functional groups as proxies for species richness or evolutionary diversity; and (4) using environmental or habitat derived variable(s) to predict biological diversity.

Species identity (in the sense of species-level identifications) can be important for some studies (such as ones concerning biodiversity, conservation or introduced species) and then identification to the species- level will be needed. However, for communitywide impacts, where species identity may not be important (and actually may introduce more variation, making it difficult to find general similarities within regions), higher identification levels may be sufficient to reliably discern the similarities and differences among assemblages. Identification to a higher *taxa* level is cheaper, more efficient, requires less taxonomic expertise, and removes possible errors in identification (Konar and Iken 2009).

In order to adopt biodiversity surrogate measures, it must be assumed that the relationship between the surrogate(s) and the total richness or diversity of the assemblages is consistent in both space and time (Colwell and Coddington, 1994). The use of taxonomical and functional surrogates for macroalgae species-level identifications has been studied (e.g., Goldberg et al., 2006; Konar and Iken, 2009; Smale, 2010; Rubal et al., 2011; Balata et al., 2011; Veiga et al., 2013) with the view to test the reliability of those surrogates in discerning similarities and differences among macroalgae assemblages along different spatial and temporal scales. Within this context, the spatial extent and design of any biodiversity monitoring program should be considered when choosing cost-effective alternatives to species-level data collection as the spatial and temporal scales will influence the efficacy of the biodiversity surrogates (Smale 2010).

In a recent work, Balata et al. (2011) departed from features such as the thallus structure, the growth form, the branching pattern and the taxonomic affinities, to further subdivide the seven traditional

morphological groups proposed by Steneck and Dethier (1994) (Crustose, Articulated calcareous, Leathery, Corticated terete, Corticated foliose and Filamentous macroalgae and also a Microalgae group) in thirty-five newly defined groups (e.g., Siphonus Chlorophyta with thin compact filaments, Filamentous uniseriate Chlorophyta, Kelp-like Phaeophyceae, Prostrate Phaeophyceae not strictly adherent to substrate, Blade-like Rhodophyta with one or few layers of cells, Filamentous uniseriate and pluriseriate Rhodophyta with extensive prostrate filaments, Flattened Rhodophyta with cortication, Filamentous uniseriate and pluriseriate Rhodophyta with erect thallus, Smaller-sized articulated Rhodophyta, etc.). The newly defined groups are expected to show more uniform responses to environmental alterations than the traditional morphological groups, to be easily recognizable, and to represent a compromise between the respective advantages of species and traditional morphological groups (Balata et al., 2011).

Under different levels of environmental disturbance, macroalgae species display characteristic growth patterns, suggesting a link between species morphology and ecological function (e.g., Norton et al., 1982; Steneck and Watling, 1982; Littler and Littler, 1984; Dethier, 1994; Steneck and Dethier, 1994). For example, anthropogenic disturbances all over the world have been linked with the decline of large perennial species such the canopy-forming species of the Genus *Cystoseira*, considered to be sensitive to pollution; the calcareous red algae are considered to be tolerant to pollution; the simple forms such as filamentous and sheet-like algae proliferate in degraded environments (Díez et al., 2012 and references therein). The declining of kelps, fucoids and other complex canopy-forming species affect their role in coastal primary production, nutrient cycling, animal habitat creation or disturbance regulation (e.g., Steneck et al., 2002; Bertocci et al., 2010; Tait and Schiel, 2011; Cheminée et al., 2013). One of the symptoms of the eutrophication in coastal waters and estuaries is the proliferation of fast- growing opportunistic macroalgae, resulting in blooms that change the community structure and function (e.g., Nelson et al., 2008; Teichberg et al., 2010).

If external morphology integrates and reflects the macroalgae function properties (e.g., primary productivity and growth rate, competitive ability, resistance to herbivores, resistance to physical disturbance, tolerance to physiological stress, successional stage, etc.), all of which should be interrelated with each other (Padilla, 1985), then, groups of taxonomically distant species having similar morphological and functional characteristics, may be distinguished. However, the relationship between species and functional diversity remains poorly understood; but to comprehend this relationship is critically important, both for the mechanistic understanding of the community assemblages and for the appropriate expectations and approaches to protect and restore biological communities (Micheli and Halpern, 2005).

An important functional-form model hypothesis became paradigmatic since Littler and Littler (1980) and Littler et al. (1983), having macroalgae species assigned into seven functional-form groups, which respond differently to photosynthesis and productivity (Littler, 1980). Nevertheless, other explanations of the functional-form model in relation to other environmental factors, such as herbivory, succession stages of the community and desiccation stress, do not occur except for some

species-specific interactions, or are explained by other factors regardless of the macroalgae morphology (Santelices et al., 2009 and references therein).

Certainly, grouping *taxa* by a particular function can be very useful and often necessary for many ecosystem-level questions and modeling, and when there are too many species in a system to consider them all individually; however, to make functional group models more useful, groupings should be based on specific functions (e.g., nutrient uptake rates, photosynthesis rates, herbivore resistance, disturbance resistance, etc.) rather than gross morphology (Padilla and Allen, 2000). In fact, two species belonging to exactly the same functional-form group in the sense of Littler et al. (1983) or situated in the same taxonomical group (Genus, Family) can display a completely different response to pollution. For example, *Cystoseira mediterranea* and *Corallina elongata* are perennial species but respond to disturbance in a completely different manner or *C. elongata* and *Jania rubens*, both in the Jointed Calcareous group and members of the Family Corallinaceae, exhibit different patterns of distribution along pollution gradients (Arévalo et al., 2007). As a matter of fact, to take into account species identity (species level identification) might be very important in ecological studies, just like distinguishing among different species of *Cystoseira*, not only because they can respond differently to human impacts, but also because they seem to have different 'engineering' effects on under-storey assemblages (Mangialajo et al., 2008).

Critical aspects to develop predictive measures of functional diversity should include (1) the choice of the functional traits with which organisms are distinguished; (2) how the diversity of that trait information is summarized into a measure of functional diversity; and (3) the measures of functional diversity should be validated through quantitative analyses and experimental tests (Petchey and Gaston, 2006). Last but not least, the level of species diversity necessary for the functional redundancy, i.e., the capacity of one species to functionally compensate for the loss of another (and therefore preventing losses in ecosystem functioning if diversity declines due to disturbance), remains a critical question (Bernhardt and Leslie, 2013).

In a recent approach, Orfanidis et al. (2011) assigned different macroalgae species into five different categories or Ecological Status Groups (ESGs) (Table I.2) by using different traits—morphological (external morphology, internal anatomy, texture), physiological (surface area/volume ratio, photosynthetic/non-photosynthetic ratio, photosynthetic performance, growth, light adaptation) and life history (longevity, succession), all of which are important to nutrient and light responses—that were selected and respond accordingly along distributional data (including from reference sites) and across eutrophication gradients.

1.5. Conclusion

Marine ecosystems are being affected by multiple human impacts, facing ecological degradation

and the concomitant decline of natural or pristine environments. In this connection, the concept of ecological conditions that can be linked to a historic past without significant human pressures—and implied in the idea of a 'natural' or 'pristine' ecosystem—give rise to the idea of ecological reference conditions, against which current degraded ecological conditions can be compared.

Table I.2. Key functional traits used to assign macroalgae into different five Ecological Status Groups (ESGs). Some *taxa* examples are given at the table end (Adapted from Orfanidis et al., 2011).

Functional traits	ESG IA	ESG IB	ESG IC	ESG IIA	ESG IIB
Thallus morphology	thick	thick	calcareous upright and calcareous and non-calcareous crusts	fleshy	filamentous and leaf-like
Growth	slow	slow	slow	fast	fast
Light adaptation	sun-adapted	sun-adapted	shade-adapted	sun-adapted	sun-adapted
Phenotypic plasticity	no	yes	yes	yes	yes
Thallus longevity	perennial	perennial thallus basis or stipe	perennial thallus basis	annual	annual
Succession	late-successional	late-successional	late-successional	opportunistic	opportunistic
Taxa examples	*Chondrus, Cystoseira*	*Aglaozonia, Asperococcus, Cystoseira (C.barbata* and *C.compressa), Culteria, Erythroglossum, Halopitys, Plocamium, Rhodophyllis, Sargassum, Taonia*	*Amphirhoa, Corallina, Dermatolithon, Halimeda, Hydrolithon, Jania, Lithophyllum, Melobesia, Mesophyllum, Peyssonnelia, Ralfsia, Titanoderma*	*Acrosorium, Ahnfeltiopsis, Asparagopsis, Boergeseniella, Caulacanthus, Champia, Chondracanthus, Chondria, Cladostephus, Colpomenia, Dictyopteris, Dictyota, Gastroclonium, Gelidium, Gigartina, Gracilaria, Grateloupia, Halopteris, Hypnea, Hypoglossum, Kallymenia, Laurencia, Lomentaria, Nitophyllum, Osmundea, Phyllophora, Pterocladiella, Schizymenia, Stypocaulon*	*Aglaothamnion, Anotrichium, Antithamnion, Blidingia, Bryopsis, Callithamnion, Ceramium, Chaetomorpha, Cladophora, Codium, Dasya, Derbesia, Ectocarpus, Griffithsia, Halurus, Herposiphonia, Lophosiphonia, Monostroma, Petalonia, Pleonosporium, Polysiphonia, Porphyra, Pterosiphonia, Rhizoclonium, Rhodothamnionella, Scytosiphon, Stylonema, Ulotrix, Ulva, Valonia*

Reference conditions can be linked to ecological conditions that remain pristine or unchanged, but also as defined environmental goals that can be considered (somehow) reasonable to be achieved. It depends both on the level of ecological conditions that are aimed by human society and on the availability of historical data and knowledge of the system. Actually, 'pristine state' or 'natural state' can be best regarded as having dynamic attributes, which may need to be periodically re-defined in response to new and

better scientific knowledge (van Hoey et al., 2010 and references therein).

Regardless of the degree of 'naturalness' or 'pristineness' implied by a specific definition of a benchmark, the accuracy and precision when assessing ecological quality conditions are dependent on the degree to which those benchmarks can be quantified and predicted (Hawkins et al., 2010). Macroalgae communities can reflect the ecological degradation and their study can be useful to quantify the level of integrity of marine ecosystems. Several lines of investigation can be taken into account, and combined in an integrative way, in order to search for marine macroalgae definitions under reference conditions. To search for it can be highly important to indicate directions for the conservation, restoration and management of the marine ecosystems.

Chapter II

Intertidal zonation and latitudinal gradients on macroalgal communities: species, functional groups and thallus morphologies approaches[2]

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[2] Gaspar, R., Pereira, L., Neto, J.M., (submitted to Ecological Indicators). Intertidal zonation and latitudinal gradients on macroalgal communities: species, functional groups and thallus morphologies approaches.

Using macroalgae to assess coastal and estuarine environments

Abstract

Macroalgae are unavoidable biological elements when monitoring and assessing costal environments. Macroalgae assemblages are commonly known to vary across intertidal gradients (vertical variability) in response to small-scale environmental factors and processes. However, macroalgal variability can also be resultant form large-scale processes. On the other hand, the use of biological surrogates for species-level approaches might represent complementary useful measures for monitoring and assessment needs, because surrogates decrease taxonomical expertise needs. Nevertheless, surrogacy must be spatial-explicitly tested, as different environmental factors and processes act at different spatial scales. Under above scopes, this work addresses how the vertical (intertidal zonation) and horizontal (latitudinal gradient) variability of macroalgae assemblages behave across continental Portugal, as well as how some macroalgal biodiversity surrogates (functional groups and thallus morphologies) respond to such large-scale variability. Particularly, it was tested if intertidal zonation patterns is higher than fine-scale horizontal variation, and however, if vertical variation decreases along broad-scale horizontal variation. To do so, coverage per species was taken (using a photographical and GIS methodological approach) from five sites located along the shoreline and along respective upper- mid- and lower-intertidal intertidal zones. The work findings include that both intertidal and latitudinal gradients impose deep structural changes on assemblages. That is, broad-scale process act as strongly as vertical gradients on assemblages patterns. Functional groups and thallus morphologies can complement species-level approaches and can improve ecological synthesis. To generate broader databases on rocky shore assemblages diversity (from species-level to functional groups and thallus morphologies) can be useful for large-scale comparisons and for establishing ecological reference conditions, including for monitoring programs and for environmental impact studies.

Key-words: Marine Macroalgae, Spatial variability, Rocky shores, Community structure, Functional groups, Traits, Intertidal zonation, Latitudinal patterns

II.1. Introduction

Monitoring marine biodiversity of coastal areas is a key activity for conservation and management issues. However, the inherent spatial and temporal complexity and variability of coastal ecosystems will always present problems for meaningful biomonitoring (de Jonge et al., 2006). Difficulties related with the assessment of benthic communities such as marine macroalgae are well known, not only due to their natural variability across a range of spatial and temporal scales, but also due to their high sampling and laboratory processing effort, as well as the insufficient knowledge about the structural and functional characteristics of coastal ecosystems (Puente and Juanes, 2008). Despite that, the successful

management and protection of biodiversity, the assessment of anthropogenic impacts and the restoration of altered ecosystems rely largely on quantifying assemblages variability patterns across a range of relevant scales, and on understanding the processes and factors that structure these assemblages (Underwood et al., 2000; Benedetti-Cecchi, 2001; Díaz-Tapia et al., 2013 and references therein).

Since our understanding of different factors driving ecological patterns and processes is spatial scale-dependent (Wiens, 1989; Levin, 1992; Willig et al., 2003), the analysis of spatial patterns of assemblages contributes to identify the major ecological processes that may determine these patterns. Different ecological processes generate variability at different scales (where some processes can only act at small scales and some only at large scales), and therefore the examination of patterns across a range of spatial scales is a fundamental step before ecological explanations for these patterns can be proposed (Underwood and Chapman 1996; Hewitt et al., 2007; Burrows et al., 2009).

Macroalgae thriving in rocky shores are known to be distributed in a distinct manner, occurring at specific levels along a height axis, from the lower to the upper shore (e.g. Araújo et al., 2005; Martins et al., 2008). These assemblages vertical zonation patterns are particularly visible at the intertidal rocky shore landscapes, and are known to be resultant from the effects of biological factors such as competition and grazing, as well as physical factors such as wave action, aerial exposure, irradiance, temperature ranges and time available for nutrient exchange (Lobban and Harrison, 1994; Choi and Kim, 2004). However, assemblages vertical zonation patterns may vary along the coastline (horizontal variations) due to processes unrelated to vertical gradients (Chapius et al., 2014). In larger geographical scales such as along latitudinal gradients, most species can vary in their ecology in response to large-scale environmental variability (Brown, 1984).

Critically, broad-scale processes may add extra variability to the patchiness commonly observed at fine scales on rocky shores (Martins et al., 2008). Thus, it has been highlighted the need of more knowledge on how vertical variation (intertidal zonation) compares with horizontal variation measured at increasing spatial scales (in terms of sampling interval along the shoreline). Actually, recent studies have been focused on the variability of littoral assemblages at different spatial scales along shores (horizontal variation) taking or not taking into account the intertidal zonation patterns (vertical variation) (e.g. Araújo et al., 2005; Fraschetti et al., 2005; Martins et al., 2008; Burrows et al., 2009; Cruz-Motta et al., 2010; Valdivia et al., 2011; Veiga et al., 2012; Chapius et al., 2014).

On other hand, aiming to find general rules when studying species' distribution (MacArthur, 1972), it must be acknowledge that the conclusions drawn will markedly depend on how communities' biodiversity is measured. Biodiversity measures of marine macroalgae include variables such as structural components (species richness, composition, abundance) as well as other taxa classifications beyond species-level approaches, such as other taxonomic resolutions, morphologies, functional groups, traits (e.g., Steneck and Dethier, 1994; Konar and Iken, 2009; Orfanidis et al., 2011). Particularly, species-level approaches to detect changes are costly because are labor-intensive and high taxonomic expertise is needed. Alternatively, species can be grouped into different functional groups, based on their ecological

and morphological attributes (Litter and Litter ,1980; Steneck and Dethier, 1994; Balata et al., 2011) and act as a surrogate measure for macroalgae (species-level) biodiversity. Biological traits, in the sense of well-defined, measurable properties of organisms, usually measured at the individual level and used comparatively across species (McGill et al., 2006), may be seen as well as complementary surrogate measure of the (species-level measured) biodiversity. However, the use of surrogates' measures implies that the relationship between the assemblage structure considering species and the surrogate is consistent in space (Colwell and Coddington, 1994). These assumptions have, however, rarely been examined explicitly (Smale, 2010; Rubal et al., 2011;Veiga et al., 2012).

Under above scopes, this work hypothesizes that 1) the variability of macroalgal assemblages' structure (species coverage) at vertical gradients (intertidal zonation) is higher than the variability at small-scale horizontal gradients (among transects within each study site); but also, and however, that 2) the assemblages vertical variability will be lower (or will decrease) with increasing broad-scale horizontal gradients (along sites located along different Portuguese latitudes). Furthermore, 3) the same above assumptions will be evaluated when considering macroalgal species assignments into different morphological-based surrogates (morphological functional groups and trait-based thallus morphologies), in order to test their ability to capture the assemblages variability patterns.

II.2. Material and methods

II.2.1. Study area

Five study sites depicting macroalgal assemblages thriving in intertidal rocky shores were selected along the Portuguese continental coastline, distancing apart from each other in about 150 km. According to Bettencourt et al. (2004) Portuguese typologies, Viana do Castelo and Buarcos sites depict wave-exposed conditions (A5 Typology), Ericeira and Queimado sites depict moderately wave-exposed conditions (A6 Typology) and Arrifes site depict wave-sheltered conditions (A7 Typology) (Fig. II.1).

II.2.2. Sampling design and data production

At each study site, the intertidal rocky shore was sampled along three transects perpendicular to the water line, distancing apart from each other in about 50 m. Each defined transect followed a stratified random sampling scheme, where 3 replicates were randomly sampled at each intertidal zone (upper-, mid- and lower-intertidal, i.e., 9 sampling replicates per transect). Replicates were collected as the tide receded, on flattened bedrock (excluding rock pools). Sampling occurred during spring low tides, in 2013

and 2014, and during representative spring/summer months (corresponding to the seasonal period of maximum development of macroalgae at temperate seas).
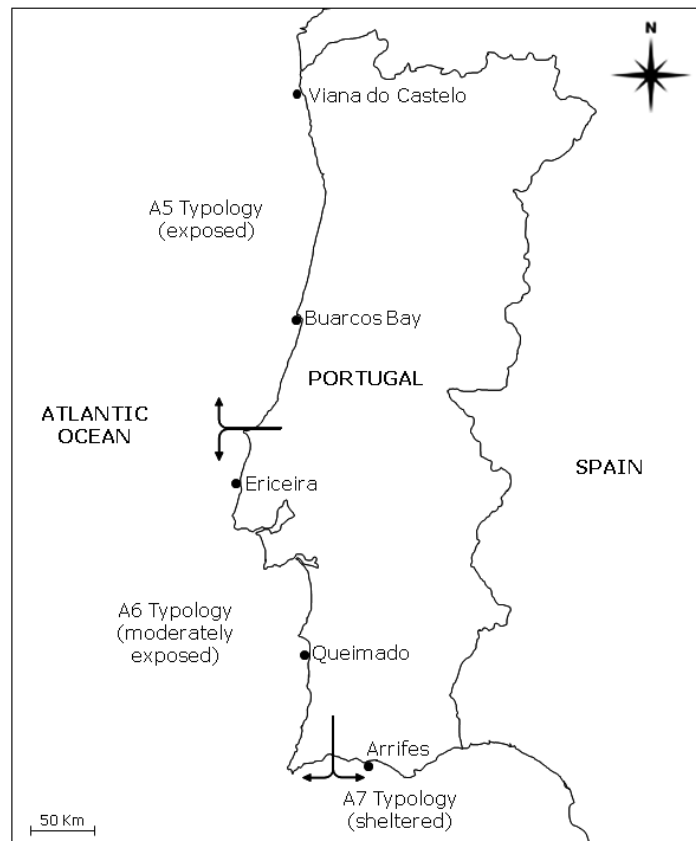
Fig II.1. Study area indicating sampling sites (black dots) located along the Portuguese continental shoreline (Iberian Peninsula, NE Atlantic Ocean). Portuguese typologies (A5, A6 and A7) indicate different wave-exposure conditions.

Each replicate (quadrate, 0.5x0.5 m, i.e., 0.25 m^2), and each of its twenty-five replicate' sub-quadrates (subdivisions of 0.1x0.1 m each) were singly photographed. Whenever necessary, mainly at lower-intertidal habitats, multilayered species were taken into account by setting aside canopy species for secondary photographic records of understory species. Each photo-quadrate replicate (0.5x0.5 m) was geo-referenced using Quantum GIS 1.8.0 Lisboa software. Species coverage areas were calculated by outlining polygons, drawn on each geo-referenced 0.5x0.5 m photo-quadrate. Close-up photographs (for higher image detail) taken from each replicate' sub-quadrates were used aside to allow better accuracy on the polygons' drawing. Sampled taxa were mostly identified to the species level (Fig. II.2).

Fig. II.2. Sampling design of macroalgal assemblages per sampling site: a) three transects taken 50 m apart from each other; each transect = three quadrate replicates (0.5x0.5 m) randomly sampled per intertidal zone (upper-, mid-, and lower-intertidal) = 9 replicates; b) one photograph taken per replicate

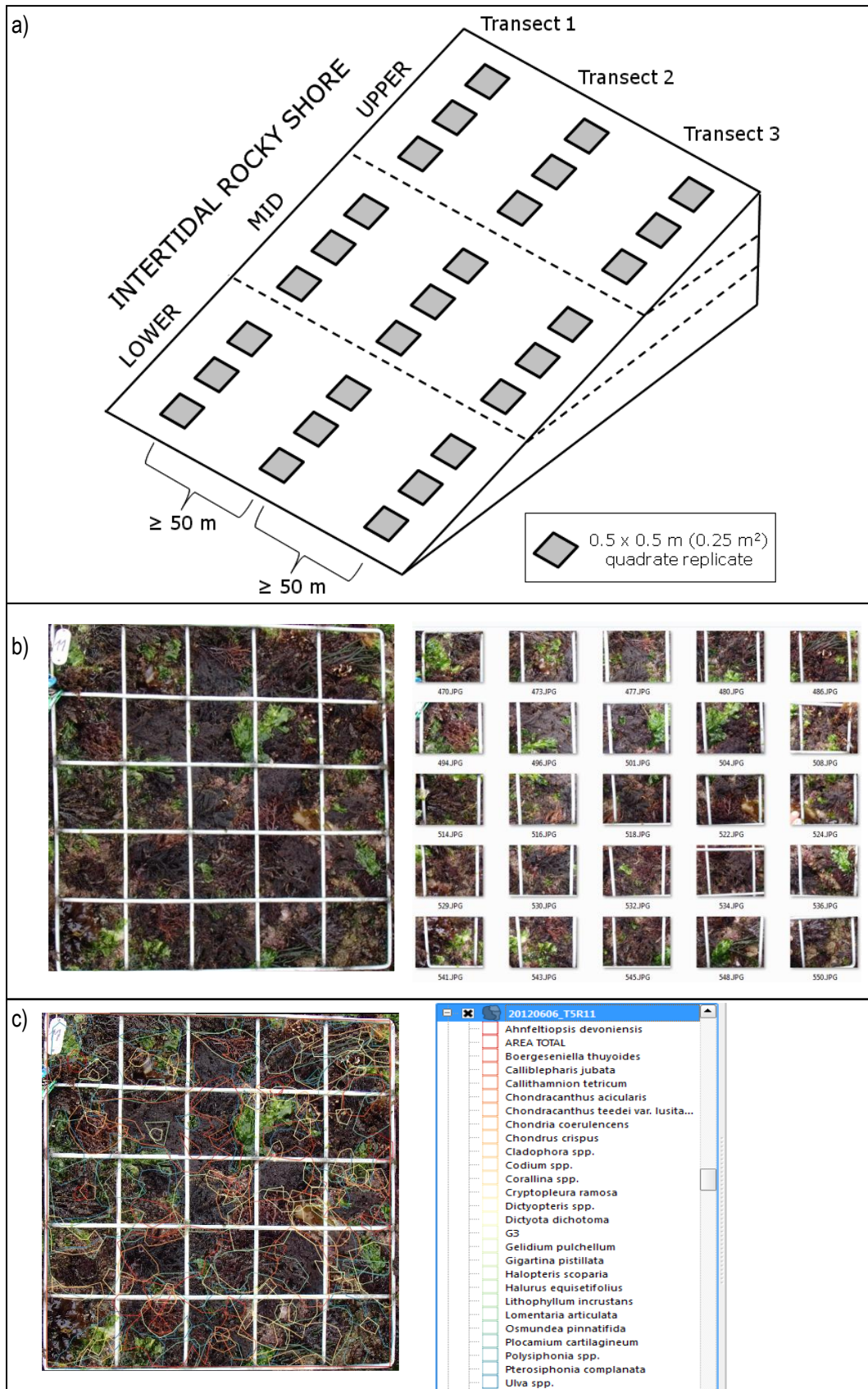(quadrate, 0.5x0.5 m), plus 25close-up photographs (sub-quadrates, 0.1x0.1 m each) taken for higher image detail; c) polygons were drawn (using a GIS software) outlining species coverage areas on the 0.5x0.5 m photo-quadrates (using aside close up sub-quadrate' photographs for better accuracy on the polygons' drawing).

II.*2.3. Taxa assignments*

Firstly, macroalgal taxa (mostly identified at species level) were assigned into different traits (Orfanidis et al., 2011), namely: a) light adaptation (sun-adapted or shade-adapted categories); b) growth (fast- or slow-growth categories), thallus longevity (annuals or perennial categories) and succession (opportunistic or late-successional categories); and c) thallus morphology (TM, in four categories, namely filamentous and leaf-like, fleshy, thick, or calcareous upright and calcareous and non-calcareous crusts, which in turn correspond to different Ecological Status Groups, ESG, Orfanidis et al., 2011). Some assignment exceptions from above literature reference was made, and information from elsewhere was considered (Guinda et al., 2008; MarLIN, 2014; Guiry, 2014) regarding the local/regional perspective on some taxa (e.g., all species form the Order Gigartinales were considered to be late-successional species and as having a thick thallus morphology). Secondly, taxa were assigned according to the different morphological-functional groups (FG), in seven categories, namely, filamentous, foliose, corticated foliose, corticated, leathery, articulated calcareous, and crustose (Steneck and Dethier, 1994). Thirdly, taxonomical Orders were used to subdivide some FG.

II.*2.4. Statistical analysis*

Multivariate analysis of macroalgal assemblages were done using the statistical package PRIMER 6 with the PERMANOVA add-on (Clarke and Gorley, 2006; Anderson et al., 2008). Coverage data based on species and on species assignments into different FG and into different TM were used to construct resemblance matrices with Bray-Curtis as similarity index. The tables allowed to represent the spatial ordination of samples through the nonmetric multidimensional scaling (nMDS) method. Differences in the assemblages along the studied habitats were assessed through using permutational multivariate analysis of variance (PERMANOVA) using 9999 permutations under a reduced model, considering two factors, site (fixed, 5 levels), and intertidal zonation (fixed, 3 levels), and pair-wise tests to analyze the interaction between the factors (i.e., site vs. intertidal zonation); a significance level was accepted at $p < 0.05$.

Similarity percentage analysis (SIMPER routine package) on coverage data without any transformation were conducted, in order to show the species and the species assignments into FG and into TM that mostly contribute to the average similarity within each site and according to each intertidal zone (contribution %; 95% cut-off for lower contributions).

SIMPER analysis (on coverage data without any transformation, using either species, species assignments into FG or into TM matrices) were also conducted in order to compare the assemblages' vertical variability against its horizontal variability at increasing shoreline distances. To do so, a) the average dissimilarity values among zones within each transect and b) the average dissimilarity values between transects were calculated. Then, for pairs of transects located within each site and for pairs of transects located at different sites (located at increasing shore horizontal distances), the following ratio was calculated:

DissRatio (i&ii) = AvDissZones (i&ii) / AvDissTransects (i&ii)

where:
- DissRatio (i & ii) is the dissimilarity ratio between transects i & ii;
- AvDissZones (i & ii) is the mean value obtained from the average dissimilarity values calculated among intertidal zones (of both transects i & ii);
- AvDissTransects (i & ii) is the average dissimilarity value between transects i & ii.

Lastly, in order to assess assemblages variability across space using species, FG or TM, the resultant ratio values (including the ratio values calculated between transects located within the same site) were then plotted against the increasing shoreline distances between sites (using real strait line measures; starting at Viana do Castelo, the northern upper most).

II.3. Results
II.*3.1. Species, functional groups and thallus morphologies assignments*

A total of 91 taxa were found (59 Rhodophyta, 25 Ochrophyta (Phaeophyceae), 6 Cholorophyta and 1 Cyanobacteria), mostly identified to the species level. The macroalgal assemblages' structure (coverage per species data) is given in detail according to its intertidal zonation patterns per site, where each species is classified and scored according to DAFOR (adapted) and Domin-Krajina coverage ranges (Appendix II.1).

Taxa were assigned into different traits and functional groups (FG), highlighting the categories of the trait thallus morphology (TM). Filamentous and leaf-like morphologies and fleshy morphologies are featured by annual, opportunistic and fast-growing species. However, rather than fast-growing fleshy species (encompassing ESG-IIA), most filamentous and leaf-like species (encompassing ESG-IIB) have high reproductive capacity and short life spans and can grow in all environments, often forming blooms in highly degraded environments, e.g. green tides of *Ulva* spp. (Orfanidis et al., 2014). Filamentous and leaf-like species comprise the filamentous group or in the foliose/tubular group. The last group was renamed (after Steneck and Dethier, 1994) to clearly include the tubular-forming species of *Ulva* (such as those

from the former genera *Enteromorpha*). Fleshy species comprise the fleshy corticated foliose group or in the fleshy corticated group. Thick morphologies correspond to slow-growing, late succession and perennial species (encompassing ESG-IA and ESG-IB) although one species in particular, *Sacchoriza polyschides*, is a thick kelp-like, yet an annual, opportunistic and fast growing species (*sensu* ESG-IIA). Actually, *S. polyschides* is not a true Laminariales kelp, being of the Order Tilopteridales; still is a "pseudo-kelp" once can perform a similar ecological role as a dominant canopy former (Smale et al., 2013). Thick species comprise the thick corticated (Gigartinales) group, the thick corticated (other Orthers) group, or the thick leathery group (includes species from Orders such as Fucales, Laminariales, Desmarestiales or Tilopteridales). Calcareous upright and calcareous and non-calcareous crusts morphologies are also featured by slow-growing, late succession and perennial species, although being shade-adapted (encompassing ESG-IC). These species comprise the articulated calcareous group or the crustose group (Table II.1).

II.*3.2. Assemblages patterns*

The structural patterns of macroalgal assemblages (coverage data) differed in terms of species as well as in terms of species assignments into functional groups (FG) or into thallus morphologies (TM), both between sites ($p<0.0001$) and between intertidal zones ($p<0.0001$). Furthermore, differences were also detected for the majority of the interaction of both factors (sites vs. intertidal zones), with only few exceptions ($p>0.05$), namely for FG between the mid-intertidal zones of Ericeira and Arrifes; for TM between upper-intertidal zones of Viana do Castelo and Queimado; for TM between the mid-intertidal zones of Viana do Castelo and Buarcos Bay and of Ericeira and Arrifes; and for TM between lower-intertidal zones of Buarcos Bay and Ericeira and of Ericeira and Arrifes.

The differences detected are shown by the nMDS analysis (Fig. II.3). Particularly, namely when using the species-level approach, the assemblages appear separated in a systematic fashion both in terms of their latitudinal location and their intertidal zonation (Fig. II.3a).

II.*3.4. Intertidal zonation patterns*

The intertidal zonation patterns of macroalgal assemblages are briefly described below according to each site (Table II.2).

Table II.1. Macroalgal taxa assignments. Traits (Orfanidis et al., 2011): a) light adaptation, b) thallus longevity, succession and growth; and c) thallus morphology (corresponding to different Ecological Status Groups = ESG, Orfanidis et al., 2011). And d) functional groups (Steneck and Dethier, 1994). Taxonomical Orders were used to further subdivide the functional groups within thick thallus morphologies.

a) SUN-ADAPTED	
b) ANNUALS AND OPPORTUNISTIC, FAST GROWTH	b) PERENNIALS AND LATE-SUCCESSIONAL, SLOW GROWTH
c) Filamentous and leaf-like (ESG IIB)	c) Thick (ESG IA or ESG IB)
d) Filamentous	d) Thick Corticated (Gigartinales)
Bornetia secundiflora	*Ahnfeltiopsis devoniensis*
Callithamnion tetragonum	*Calliblepharis jubata*
Callithamnion tetricum	*Callophyllis laciniata*
Ceramium spp.	*Caulacanthus ustulatus*
Chaetomorpha spp.	*Chondracanthus acicularis*
Cladophora spp.	*Chondracanthus teedei* *A
Codium adhaerens	*Chondrus crispus*
Codium spp.	*Gigartina pistillata*
Dasya spp.	*Gymnogongrus crenulatus*
Falkenbergia rufolanosa (*Asparagopsis armata*)	*Hypnea musciformis*
Halopteris scoparia/filicina	*Mastocarpus stellatus*
Halurus equisetifolius	d)Thick Corticated (Other Orders)
Ophidocladus simpliciusculus	*Gelidium corneum*
Other Ceramiales *1	*Gelidium pulchellum*
Other Ectocarpales/*Sphacelaria* spp.	*Gelidium pusillum*
Petrospongium berkeleyi	*Halopithys incurva*
Polysiphonia spp. (or other Rhodomelaceae)*2	*Liagora viscida*
Pterosiphonia ardreana/pennata	*Osmundea hybrida*
Rhodothamniella spp./Acrochaetiales	*Osmundea pinnatifida*
Rivularia bullata	*Padina pavonica*
Vertebrata lanosa	*Plocamium cartilagineum*
d) Foliose/Tubular	*Pterocladiella capillacea*
Porphyra spp./ *Pyropia* spp.	*Pterosiphonia complanata*
Ulva clathrata	*Taonia atomaria*
Ulva spp.	d)Thick Leathery (e.g., Fucales, Laminariales, Desmarestiales, Tilopteridales)
c) Fleshy (ESG IIA)	*Ascophyllum nodosum*
d) Fleshy Corticated foliose	*Bifurcaria bifurcata*
Acrosorium ciliolatum	*Cystoseira baccata*
Cryptopleura ramosa	*Cystoseira humilis*
Dictyopteris polypodioides	*Cystoseira tamariscifolia*
Dictyota dichotoma	*Desmarestia ligulata*
Grateloupia turuturu	*Fucus spiralis*
Hypoglossum hypoglossoides (or other Delesseriaceae) *3	*Halidrys siliquosa*
Nitophyllum punctatum	*Laminaria ochroleuca*
d) Fleshy Corticated	*Pelvetia canaliculata*
Asparagopsis armata	*Saccorhiza polyschides* (ANNUAL AND OPPORTUNISTIC, FAST GROWTH) *B
Boergeseniella spp.	*Sargassum flavifolium*
Champia parvula	*Sargassum muticum*
Chondria coerulescens	*Sargassum vulgare*
Chondria dasyphylla/scintillans	a) SHADE-ADAPTED
Cladostephus spongiosus	c) Calcareous upright and calcareous and non-calcareous crusts (ESG IC)
Colpomenia spp./*Leathesia marina*	d) Articulated calcareous
Gastroclonium ovatum	*Amphiroa rigida*
Gracilaria gracilis	*Corallina* spp.
Gracilaria multipartita	*Jania* spp.
Lomentaria articulata	d) Crustose
Scinaia furcellata	*Hildenbrandia* spp.
Scytosiphon spp.	*Lithophyllum byssoides*
	Lithophyllum incrustans
	Mesophyllum lichenoides
	Petrocelis cruenta (*Mastocarpus stellatus*)
	Peyssonnelia spp.
	Ralfsia verrucosa

Taxa groupings can include one or more of the following genera: *1 – *Aglaothamnion*, *Anotrichium*, *Anthithamnion*, other *Callithamnion*, *Compsothamnion*, *Griffithsia*, *Pleonosporium*; *2 – *Aphanocladia*, *Herposiphonia*, *Heterosiphonia*, *Streblocladia*; *3 – *Apoglossum*, *Erythroglossum*. *A – *C. teedeei* founded correspond to *C. teedeei* var. *lusitanicus*, except at the site Ericeira. *B – *S. polyschides* is thick species; yet is an annual, opportunistic and fast-growing species.

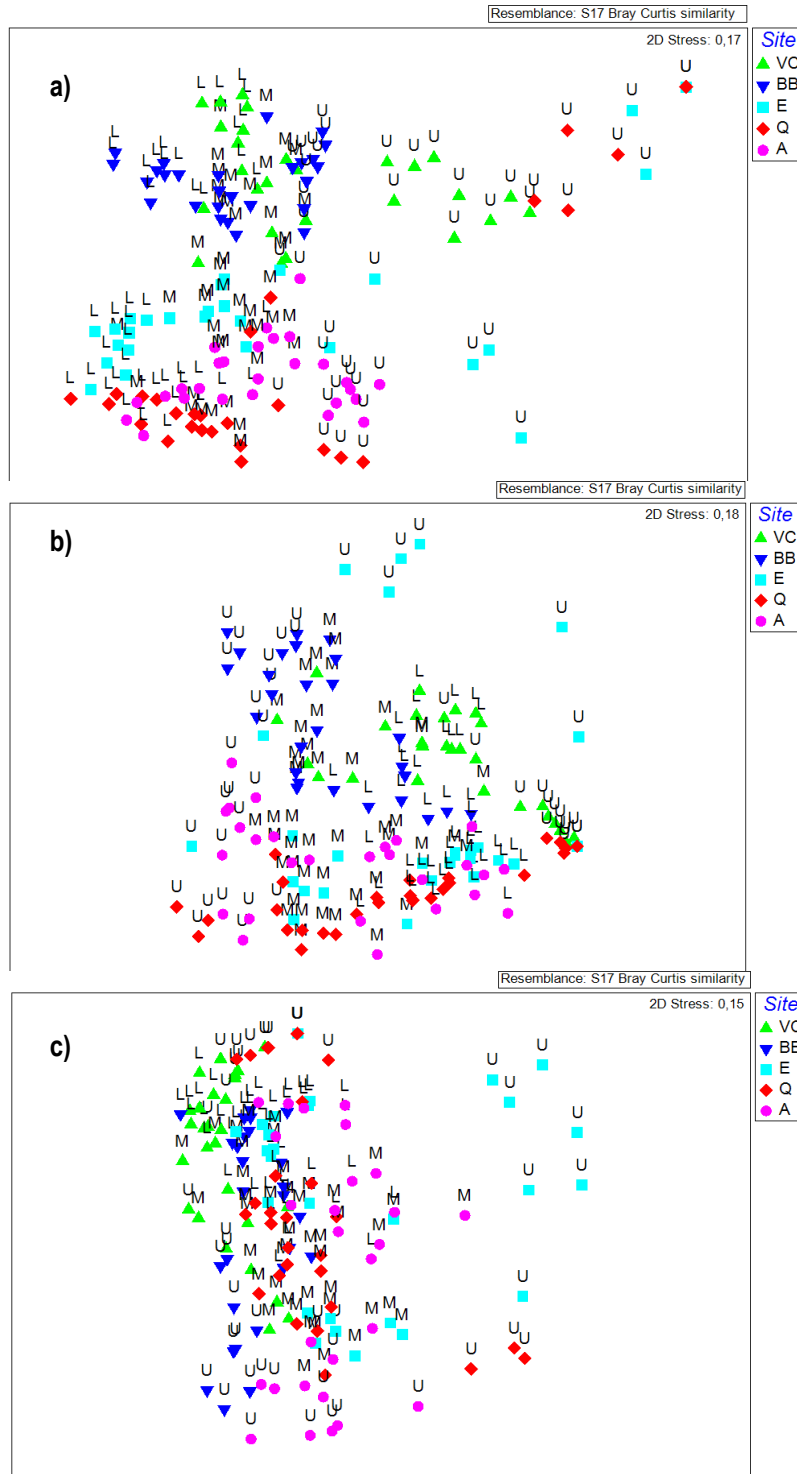**Fig. II.3.** nMDS ordinations of macroalgal assemblages based on Bray-Curtis dissimilarity measures (coverage data) in terms of a) species; b) functional groups; and c) thallus morphologies. Sites (n=27 per site): VC = Viana do Castelo, BB = Buarcos Bay, E = Ericeira, Q = Queimado, A = Arrifes. Intertidal nonation (n=9 per intertidal zone and per site): U = Upper-intertidal, M = Mid-intertidal, L = Lower-intertidal.

Table II.2. Results of SIMPER tests for species, functional groups and thallus morphologies (similarity contribution %, 95% cut-off for lower contributions) within each site (Viana do Castelo, Buarcos Bay, Ericeira, Queimado and Arrifes) and intertidal zonation: (a) upper-intertidal, (b) mid-intertidal, and (c) lower-intertidal.

(a) upper-intertidal

Viana do Castelo	%	Buarcos Bay	%	Ericeira	%	Queimado	%	Arrifes	%
Species		**Species**		**Species**		**Species**		**Species**	
Average similarity: 46.01		Average similarity: 65.11		Average similarity: 16.41		Average similarity: 29.14		Average similarity: 41.50	
Fucus spiralis	52.5	*Ulva* spp.	86.9	*Petrocelis cruenta* (*Mastocarpus stellatus*)	26.5	*Fucus spiralis*	72.6	*Corallina* spp.	26.3
Ascophyllum nodosum	35.4	*Mastocarpus stellatus*	4.1	*Fucus spiralis*	19.8	*Ralfsia verrucosa*	11.1	*Codium adhaerens*	24.5
Ulva spp.	7.4	*Osmundea pinnatifida*	2.7	*Mastocarpus stellatus*	18.2	*Codium adhaerens*	7.5	*Ulva clathrata*	19.2
		Gelidium pulchellum	2.0	*Caulacanthus ustulatus*	12.6	*Corallina* spp.	4.6	*Ceramium* spp.	11.1
				Osmundea hybrida	9.9			Other Ectocarpales/*Sphacelaria* spp.	8.7
				Corallina spp.	6.8			*Dictyota dichotoma*	3.0
				Ulva spp.	4.9			*Gelidium pusillum*	2.7
Functional Groups		**Functional Groups**		**Functional Groups**		**Functional Groups**		**Functional Groups**	
Average similarity: 73.03		Average similarity: 70.91		Average similarity: 23.16		Average similarity: 32.91		Average similarity: 58.96	
Thick Leathery	90.93	Foliose/Tubular	80.15	Thick Corticated (Gigartinales)	43.73	Thick Leathery	69.79	Filamentous	54.34
Foliose/Tubular	8.3	Thick Corticated (Gigartinales)	10.45	Crustose	18.77	Crustose	12.06	Foliose/Tubular	20.42
		Thick Corticated (Other Orders)	5.72	Thick Leathery	14.02	Filamentous	11.51	Articulated calcareous	18.54
				Thick Corticated (Other Orders)	7.47	Articulated calcareous	4.06	Thick Corticated (Other Orders)	2.82
				Filamentous	6.15				
				Articulated calcareous	4.82				
				Foliose/Tubular	3.49				
Thallus Morphologies		**Thallus Morphologies**		**Thallus Morphologies**		**Thallus Morphologies**		**Thallus Morphologies**	
Average similarity:77.15		Average similarity:76.93		Average similarity:42.93		Average similarity:38.94		Average similarity:70.36	
Thick	91.47	Filamentous and leaf-like	80.67	Thick	31.41	Thick	27.97	Filamentous and leaf-like	52.4
Filamentous and leaf-like	8.53	Thick	18.27	Calcareous upright and calcareous and non-calcareous crusts	7.36	Calcareous upright and calcareous and non-calcareous crusts	6.24	Calcareous upright and calcareous and non-calcareous crusts	14.96
				Filamentous and leaf-like	3.79	Filamentous and leaf-like	4.32		

Table II.2. (continued)

(b) mid-intertidal

Viana do Castelo	%	Buarcos Bay	%	Ericeira	%	Queimado	%	Arrifes	%
Species		**Species**		**Species**		**Species**		**Species**	
Average similarity: 40.61		Average similarity: 50.22		Average similarity: 52.52		Average similarity: 52.95		Average similarity: 47.76	
Ulva spp.	58.4	*Ulva* spp.	31.2	*Codium* spp.	22.5	*Falkenbergia rufolanosa* (*Asparagopsis armata*)	38.9	*Dictyota dichotoma*	37.3
Bifurcaria bifurcata	13.6	*Chondrus crispus*	22.6	*Dictyota dichotoma*	20.2	*Osmundea hybrida*	22.7	*Halopteris scoparia/filicina*	16.3
Chondracanthus teedei	5.8	*Osmundea pinnatifida*	10.7	*Asparagopsis armata*	17.4	*Dictyota dichotoma*	12.1	*Corallina* spp.	10.9
Chondracanthus acicularis	3.9	*Corallina* spp.	5.8	*Bifurcaria bifurcata*	10.2	*Corallina* spp.	9.4	*Cystoseira tamariscifolia*	7.1
Halopteris scoparia/filicina	3.3	*Callithamnion tetricum*	4.4	*Ulva* spp.	7.5	*Cystoseira tamariscifolia*	6.0	*Sargassum vulgare*	5.2
Ceramium spp.	2.7	*Boergeseniella* spp.	4.0	*Ceramium* spp.	7.5	*Halopteris scoparia/filicina*	2.8	*Ulva clathrata*	5.0
Corallina spp.	2.0	*Chondracanthus acicularis*	3.9	*Corallina* spp.	6.4	*Codium adhaerens*	1.9	*Ulva* spp.	4.2
Osmundea pinnatifida	1.8	*Codium* spp.	3.4	*Cystoseira tamariscifolia*	2.2	*Asparagopsis armata*	1.7	*Ceramium* spp.	3.6
Ulva clathrata	1.3	*Chondracanthus teedei*	2.7	*Plocamium cartilagineum*	1.1			*Cladostephus spongiosus*	3.0
Dictyota dichotoma	1.2	*Pterosiphonia complanata*	2.4	*Cladophora* spp.	1.1			*Hypnea musciformis*	2.1
Chondria coerulescens	1.2	*Gigartina pistillata*	1.3					*Pterocladiella capillacea*	1.5
		Lomentaria articulata	1.2						
		Gelidium pulchellum	1.1						
		Ahnfeltiopsis devoniensis	0.9						
Functional Groups		**Functional Groups**		**Functional Groups**		**Functional Groups**		**Functional Groups**	
Average similarity: 54.24		Average similarity: 67.35		Average similarity: 62.83		Average similarity: 68.26		Average similarity: 55.76	
Foliose/Tubular	47.14	Thick Corticated (Gigartinales)	38.36	Filamentous	38.2	Filamentous	53.76	Fleshy Corticated foliose	32.84
Thick Leathery	17.47	Foliose/Tubular	23.28	Fleshy Corticated	19	Thick Corticated (Other Orders)	18.8	Filamentous	23.74
Thick Corticated (Gigartinales)	15.11	Thick Corticated (Other Orders)	14.58	Fleshy Corticated foliose	16.89	Fleshy Corticated foliose	9.42	Thick Leathery	12.24
Filamentous	11.07	Filamentous	10.1	Thick Leathery	11.04	Articulated calcareous	7.31	Articulated calcareous	9.37
Fleshy Corticated	2.62	Fleshy Corticated	7.6	Foliose/Tubular	6.3	Thick Leathery	5.22	Foliose/Tubular	9.15
Articulated calcareous	2.48	Articulated calcareous	4.32	Articulated calcareous	5.36	Fleshy Corticated	4.21	Thick Corticated (Other Orders)	6.21
								Fleshy Corticated	3.74
Thallus Morphologies		**Thallus Morphologies**		**Thallus Morphologies**		**Thallus Morphologies**		**Thallus Morphologies**	
Average similarity: 68.93		Average similarity: 76.75		Average similarity: 67.66		Average similarity: 75.26		Average similarity: 64.07	
Thick	48.41	Thick	55.38	Filamentous and leaf-like	43.28	Filamentous and leaf-like	52.71	Fleshy	38.53
Filamentous and leaf-like	43.07	Filamentous and leaf-like	32.03	Fleshy	35.38	Thick	24.69	Filamentous and leaf-like	29.28
Fleshy	5.87	Fleshy	8.60	Thick	14.82	Fleshy	14.45	Thick	21.87
				Calcareous upright and calcareous and non-calcareous crusts	6.53	Calcareous upright and calcareous and non-calcareous crusts	8.15	Calcareous upright and calcareous and non-calcareous crusts	10.32

Table II.2. (continued)

(c) lower-intertidal

Viana do Castelo

Species	%
Average similarity: 58.12	
Laminaria ochroleuca	38.8
Chondrus crispus	19.8
Ulva spp.	16.6
Ahnfeltiopsis devoniensis	9.8
Osmundea pinnatifida	5.4
Gigartina pistillata	3.3
Chondracanthus acicularis	2.6
Functional Groups	
Average similarity: 74.19	
Thick Leathery	39.69
Thick Corticated (Other Orders)	38.84
Thick Corticated (Gigartinales)	14.2
Foliose/Tubular	5.88
Thallus Morphologies	
Average similarity: 88.08	
Thick	86.43
Filamentous and leaf-like	12.72

Buarcos Bay

Species	%
Average similarity: 59.87	
Saccorhiza polyschides	27.4
Pterosiphonia complanata	19.8
Osmundea pinnatifida	11.6
Boergeseniella spp.	8.8
Chondrus crispus	7.3
Callithamnion tetricum	3.5
Ulva spp.	3.4
Gigartina pistillata	2.7
Corallina spp.	2.7
Gymnogongrus crenulatus	2.5
Cryptopleura ramosa	1.8
Halurus equisetifolius	1.4
Other Ectocarpales/Sphacelaria spp.	1.4
Calliblepharis jubata	1.3
Functional Groups	
Average similarity: 71.99	
Thick Corticated (Other Orders)	30.88
Thick Leathery	24.03
Thick Corticated (Gigartinales)	17.61
Filamentous	10.41
Fleshy Corticated	8.94
Foliose/Tubular	2.78
Fleshy Corticated foliose	2.49
Thallus Morphologies	
Average similarity: 82.45	
Thick	74.96
Filamentous and leaf-like	11.99
Fleshy	10.38

Ericeira

Species	%
Average similarity: 72.92	
Cystoseira tamariscifolia	27.4
Asparagopsis armata	19.9
Codium spp.	11.6
Pterosiphonia complanata	8.8
Corallina spp.	7.3
Hypoglossum hypoglossoides (or other Delesseriaceae)	3.5
Functional Groups	
Average similarity: 79.44	
Thick Leathery	65.28
Fleshy Corticated	17.24
Filamentous	7.68
Thick Corticated (Other Orders)	5.39
Thallus Morphologies	
Average similarity: 84.00	
Thick	70.55
Fleshy	18.86
Filamentous and leaf-like	8.47

Queimado

Species	%
Average similarity: 66.10	
Cystoseira tamariscifolia	46.6
Falkenbergia rufolanosa (Asparagopsis armata)	24.9
Asparagopsis armata	5.6
Sargassum flavifolium	1.8
Dictyota dichotoma	1.3
Corallina spp.	1.0
Lithophyllum incrustans	1.6
Codium spp.	0.8
Functional Groups	
Average similarity: 75.06	
Thick Leathery	49.47
Filamentous	27.09
Fleshy Corticated	14.08
Articulated calcareous	3.22
Fleshy Corticated foliose	2.96
Thallus Morphologies	
Average similarity: 78.42	
Thick	50.39
Filamentous and leaf-like	26.10
Fleshy	17.74
Calcareous upright and calcareous and non-calcareous crusts	5.77

Arrifes

Species	%
Average similarity: 54.01	
Dictyota dichotoma	39.0
Cystoseira tamariscifolia	26.0
Sargassum vulgare	20.6
Corallina spp.	5.6
Hypnea musciformis	2.1
Halopteris scoparia/filicina	1.6
Ceramium spp.	1.4
Functional Groups	
Average similarity: 67.15	
Thick Leathery	47.76
Fleshy Corticated foliose	31.58
Filamentous	9.99
Articulated calcareous	4.49
Thick Corticated (Other Orders)	1.96
Thallus Morphologies	
Average similarity: 70.11	
Thick	51.74
Fleshy	32.14
Filamentous and leaf-like	11.24

At Viana do Castelo (VC), the upper-intertidal zone was mostly featured by the thick leathery species (*Fucus spiralis* and *Ascophyllum nodosum*); the mid-intertidal was mostly featured by foliose/tubular species (*Ulva* spp.), thick leathery species (*Bifurcaria bifurcata*), thick corticated (Gigartinales) species (*Chondracanthus teedei*, *Chondracanthus acicularis*) and filamentous species (*Halopteris scoparia/filicina*, *Ceramium* spp.); the lower-intertidal was mostly featured by thick leathery species (*Laminaria ochroleuca*), thick corticated (Gigartinales) species (*Chondrus crispus, Ahnfeltiopsis devoniensis*, *Gigartina pistillata*, *Chondracanthus acicularis*), foliose/tubular species (*Ulva* spp) and thick corticated (Other Orders) species (*Osmundea pinnatifida*).

At Buarcos Bay (BB), the upper-intertidal zone was mostly featured by foliose/tubular species (*Ulva* spp.) and thick corticated (Gigartinales) species (*Mastocarpus stellatus*); the mid-intertidal was mostly featured by thick corticated (Gigartinales) species (*Chondrus crispus*, *Chondracanthus acicularis*, Chondracanthus teedei), foliose/tubular species (*Ulva* spp), thick corticated (other Orders) (*Osmundea pinnatifida*, *Pterosiphonia complanata*) and Filamentous species (*Callithamnion tetricum, Codium* spp.); the lower-intertidal was mostly featured by thick corticated (other Orders) species (*Pterosiphonia complanata, Osmundea pinnatifida*), thick leathery (*Saccorhiza polyschides*) and Thick Corticated (Gigartinales) (*Chondrus crispus*, *Gigartina pistillata, Gymnogongrus crenulatu*s).

At Ericeira (E), the upper-intertidal zone was mostly featured by thick corticated (Gigartinales) (*Mastocarpus stellatus, Caulacanthus ustulatus*), crustose (*Petrocelis cruenta*), thick leathery species (*Fucus spiralis*) and thick corticated (other Orders) (*Osmundea hybrida*); the mid-intertidal was mostly featured by filamentous (*Codium* spp., *Ceramium* spp.), fleshy corticated species (*Asparagopsis armata*), fleshy corticated foliose (*Dictyota dichotoma*) and thick leathery (*Bifurcaria bifurcata*); the lower-intertidal was mostly featured by thick leathery species (*Cystoseira tamariscifolia*), fleshy corticated species (*Asparagopsis armata*), filamentous species (*Codium* spp.).

At Queimado (Q), the upper-intertidal zone was mostly featured by thick leathery species (*Fucus spiralis*), crustose species (*Ralfsia verrucosa)* and filamentous species (*Codium adhaerens*); the mid-intertidal was mostly featured by filamentous species (*Falkenbergia rufolanosa, Halopteris scoparia/filicina*), thick corticated (other Orders) species (*Osmundea hybrida*), fleshy corticated foliose species (*Dictyota dichotoma*), articulated calcareous species (*Corallina* spp.) and thick leathery species (*Cystoseira tamariscifolia*); the lower-intertidal was mostly featured by thick leathery (*Cystoseira tamariscifolia, Sargassum flavifolium*), filamentous species (*Falkenbergia rufolanosa*) and fleshy corticated species *(Asparagopsis armata, Dictyota dichotoma*).

At Arrifes (A), the upper-intertidal zone was mostly featured by filamentous species (*Codium adhaerens*, *Ceramium* spp.), foliose/tubular species (*Ulva clathrata*) and articulated calcareous species (*Corallina* spp.); the mid-intertidal was mostly featured by fleshy corticated foliose species (*Dictyota dichotoma*), filamentous species (*Halopteris scoparia/filicina*), thick leathery species (*Cystoseira tamariscifolia),* articulated calcareous species (*Corallina* spp.) and foliose/tubular species (*Ulva clathrata*, *Ulva* spp.), the lower-intertidal was mostly featured by thick leathery species (*Cystoseira tamariscifolia,*

Sargassum vulgare), fleshy corticated foliose species (*Dictyota dichotoma*) and filamentous species (*Halopteris scoparia/filicina, Ceramium* spp.) (Table II.2).

II.*3.5. Geographical latitudinal patterns*

The geographical patterns in regard to the abundance of FG, TM are briefly described below (Appendix II.2).

Filamentous and leaf-like TM. Filamentous species seem to increase towards southern (upper-intertidal) and mid-to-southern Portuguese latitudes (mid- and lower-intertidal). On the contrary, foliose/tubular species seem to decrease southwards, particularly at mid and also lower-intertidal, whereas at upper-intertidal they were mostly present at BB and A sites.

Fleshy TM. Fleshy corticated foliose species decrease in coverage northwards (particularly at mid intertidal) whereas the remaining fleshy corticated species seemed to increase in coverage towards central Portuguese latitudes (E and Q sites), being mostly present at mid- and lower-intertidal, but also at upper-intertidal.

Calcareous upright and calcareous and non-calcareous crusts TM. Articulated calcareous species seem to decrease in coverage northwards (at each intertidal zone, but particularly at the upper intertidal) and concerning the crustose species, the same seem to be true (although represented by less species coverage).

Thick TM. Thick corticated (Gigartinales) species were mostly present at northern sites (VC and BB), having higher coverage mostly at its lower- and mid-intertidal zones. Thick corticated (other Orders) were mostly present at lower-intertidal, presenting higher coverage values at northern/central Portuguese sites (VC, BB and E), whereas mid-intertidal zones they presented the higher coverage at Q site. Thick leathery species are the most abundant morphologies at site's lower-intertidal, but were also very abundant at upper- and mid-intertidal zones; they were however absent from the upper- intertidal zones of BB and A sites, and also absent from the mid-intertidal of BB site (but see Appendix II.2).

Overall, latitudinal trends seems to indicate that thick TM increase northwards, at the expense of the northwards decrease of both fleshy TM and calcareous upright and calcareous and non-calcareous crusts TM. Particularly, the main FG responsive to above mention latitude trends were the thick corticated (Gigartinales), the fleshy corticated foliose, the articulated calcareous, and the filamentous species. Although filamentous and leaf-like TM do not seem to exhibit a particular trend along latitude as a whole, it should be noted that filamentous species increase towards southern latitudes, while foliose/tubular species seem to predominate in northern sites (VC and BB) (Fig. II.4).
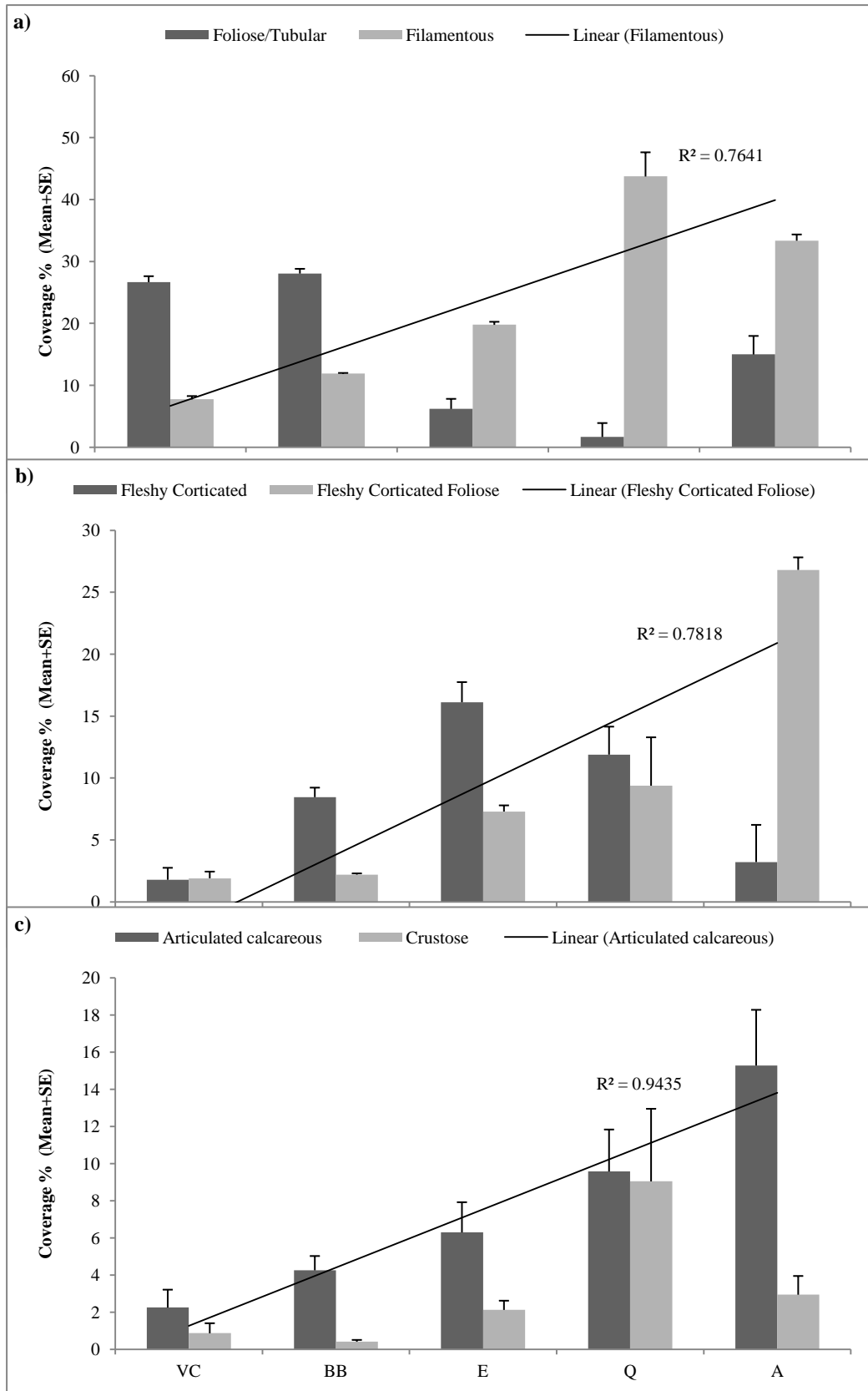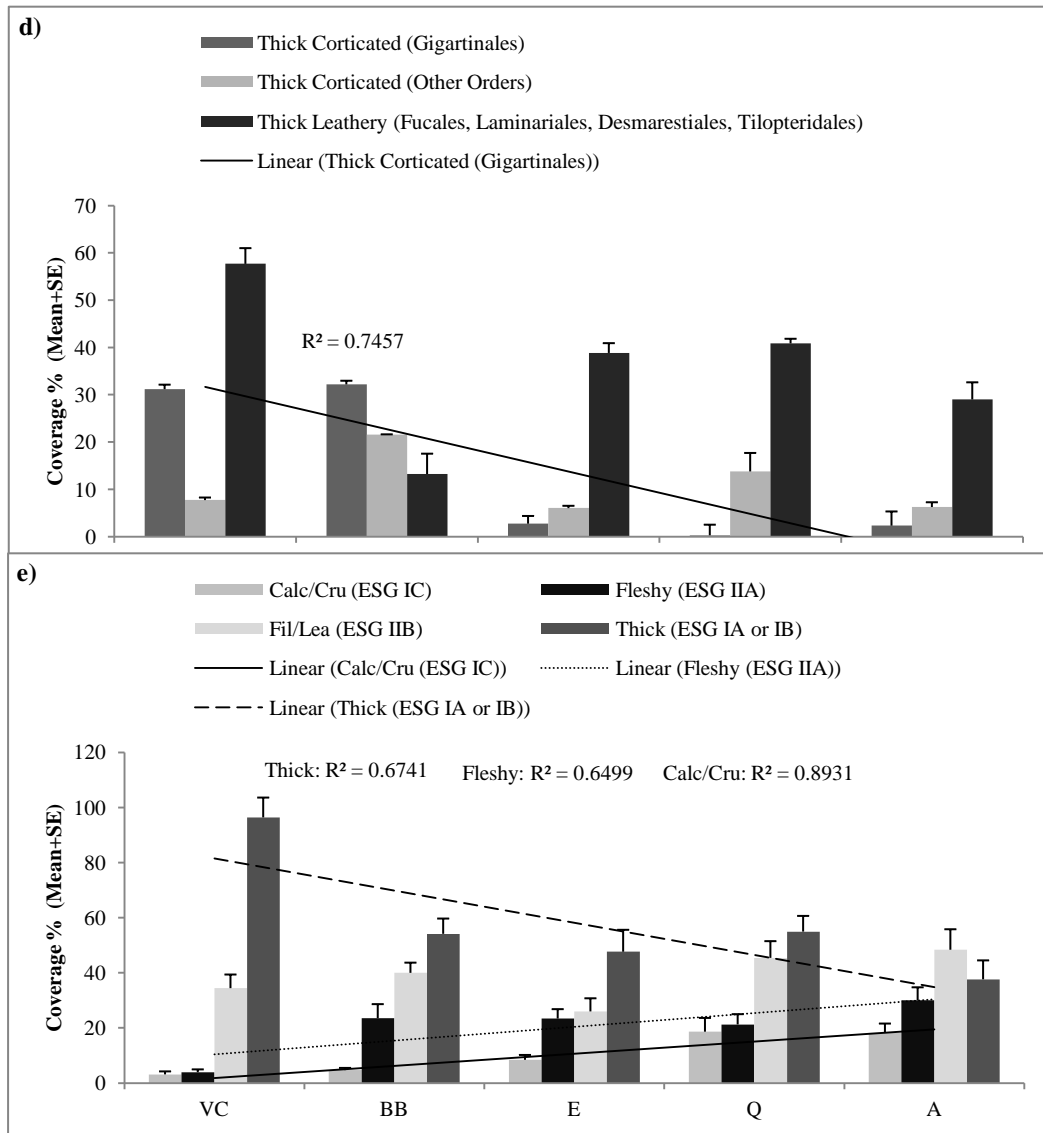
Fig. II.4. (continued)

Fig. II.4. Latitudinal trends of macroalgal assemblages (coverage %, Mean +SE, n=27 per site) Functional groups per thallus morphology: a) filamentous and leaf-like; b) fleshy; c) calcareous upright and calcareous and non-calcareous crusts; d) thick morphologies. And e) thallus morphologies. Regression lines (linear trends) are shown if $R^2 > 0.5$. VC = Viana do Castelo, BB = Buarcos Bay, E = Ericeira, Q = Queimado, A = Arrifes. Note different scales on the graphics.

II.3.6. Vertical versus horizontal variability patterns

The assemblages' vertical variability (intertidal zonation) is compared against its horizontal variability (increasing shoreline distances) according to the dissimilarity ratio in Fig. II.5.
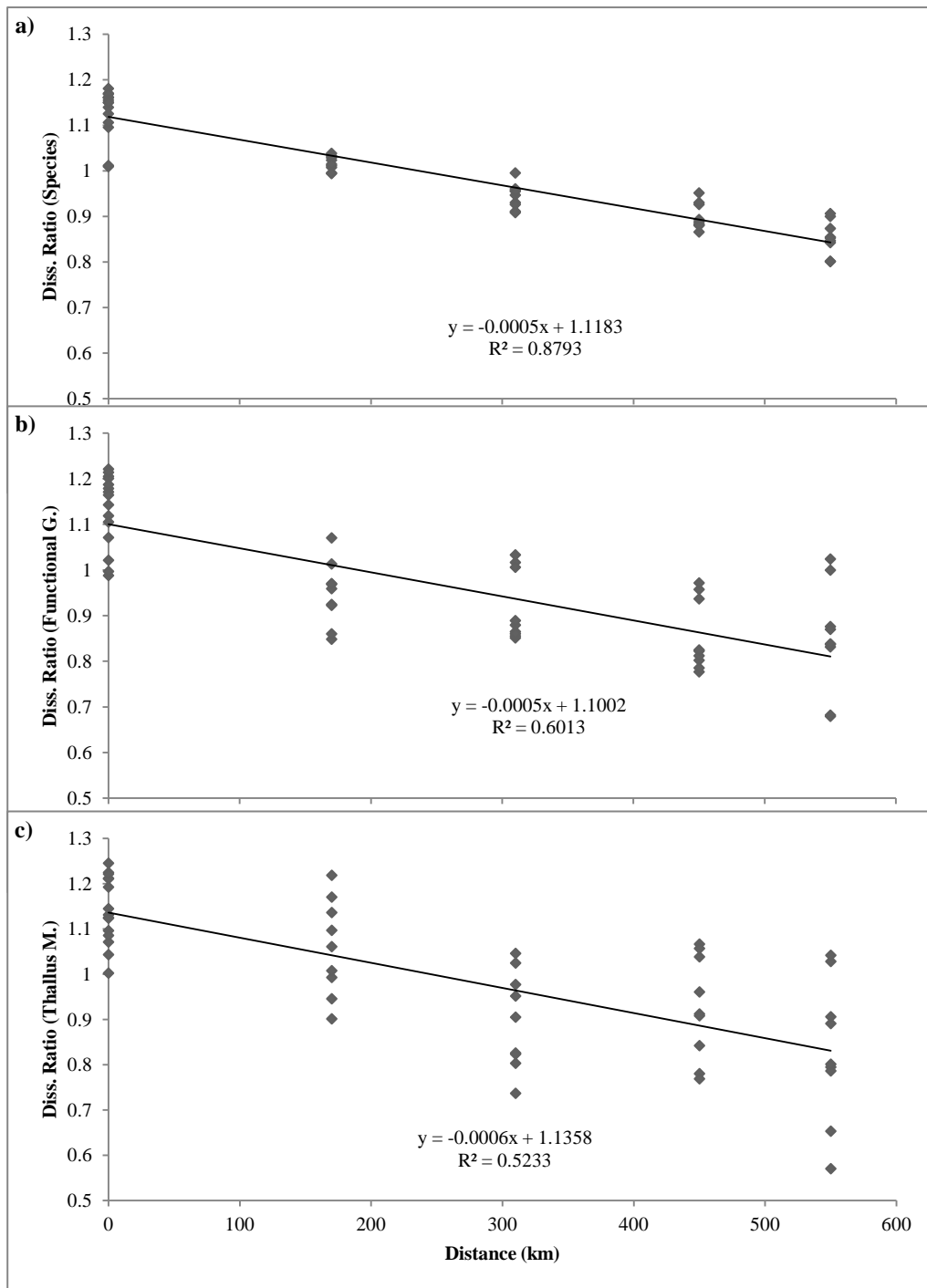
Fig. II.5. Dissimilarity ratio at increasing shoreline distances, considering a) species, b) functional groups and c) thallus morphologies. 0 km = all transects within each site, including Viana do Castelo; 170 km = Buarcos Bay; 310Km = Ericeira; 450 km = Queimado; 550 km = Arrifes.

Higher ratio values indicate that the assemblages vertical variability is higher/stronger than the horizontal variability, i.e. higher dissimilarity values among intertidal zones against lower dissimilarity values between nearby transects located within the same site. However, at increasing shoreline distances,

ratio values decrease as assemblages' horizontal variability increases (i.e. increasing distance between transects of different sites), where the dissimilarity values among intertidal zones get less stronger against increasing dissimilarity values between transects located at increasing shoreline intervals. This was mostly true when considering a species level approach to describe changes in the assemblages structure (R^2 = 0.8793). Nevertheless the FG approach (R^2 = 0.6013) as well as, although in less extent, the TM approach (R^2 = 0.5233), where also able to detect the influence of increasing latitudinal distances on the assemblages structure (Fig. II.5).

II.4. Discussion

The role of biotic interactions and physical factors in structuring intertidal assemblages patterns along its vertical gradient of stress has long been studied by many authors (see Araujo et al., 2005 and references therein; see Díaz-Tapia et al., 2013 and references therein). In rocky intertidal habitats, the pronounced increase in environmental stress from low to high elevations greatly affects the macroalgal community structure, that is, the combined measure of species identity (composition) and their relative abundance (Valdivia et al, 2011; Scrosati et al., 2011). Nevertheless, environmental factors variability influence assemblages' heterogeneity at different scales, ranging from local patchiness to variation along biogeographic gradients (e.g. Levin, 1992; Fraschetti et al., 2005; Tuya and Haroun, 2006).

As our results showed, vertical variation in community structure across intertidal zones is very strong. In fact, it is higher than the variability at fine-scale horizontal gradients (variability among transects within the same site). Even though, fine-scale horizontal variation in species assemblages is a common attribute of marine benthic habitats. Overall, in areas harboring the same basic biota, the highest variation in assemblages structure often occurs at fine resolutions, i.e. small- (10s to 100s of centimetres) to middle-scale (10s to 100s of meters) variation (Valdivia et al, 2011; Fraschetti et al., 2005). Potential processes responsible for variability at these spatial scales include differences in the substrate slope and pre-emption, recruitment, grazing, competition, wave action, aerial exposure, irradiance, temperature ranges or time available for nutrient exchange (e.g. Benedetti-Cecchi et al. 1999, 2001; Coleman, 2003; Lobban and Harrison, 1994; Choi and Kim, 2004). Nevertheless, broad-scale processes can generate geographic patterns in community structure. In this regard, our results also showed that the assemblages intertidal vertical variability decreases with increasing broad-scale horizontal gradients along Portuguese latitudes. That is, the assemblages' structural changes along sites is very pronounced. From northern to southern sites, the studied assemblages were progressively different from each other, where some macroalgae species decline in coverage or disappear, while and others emerge and gain relative importance or dominate.

At broader resolutions (differences among sites, each one commonly meant to be between 100s and 1000s of m in width), variation in community structure has been related to broad-scale changes in environmental variables such as wave-exposure and ice-scour, and recruitment (Valdivia et al, 2011 and references therein). From northern to southern Portugal, the broad latitudinal gradient can be linked with the decreasing wave-exposure, as well as with changes in ocean climate, with increasing sea surface temperature and photosynthetically active radiation and decreasing nutrients, and this drives changes in the distribution of macroalgal assemblages (Tuya et al., 2012; Ramos et al., 2012).

In fact, the overlapping distributions of macroalgal species of both boreal and Lusitanian origins have been documented (Lima et al., 2007), where a large number of cold- and warm-water species have their southern or northern distributional range edges (Ardré, 1971), and while other species show latitudinal clines in abundance (Boaventura et al., 2002; Lima et al., 2007; Pereira et al., 2006). In this context, to evaluate intertidal macroalgae species distribution along latitudinal gradients, such as along continental Portuguese coast, may be useful to document global warming scenarios, as some cold-water species can retreat polewards against some warm-water species that can start colonize new areas (Tuya et al., 2012 and references therein). The concomitant decrease in nutrient concentrations might also affect negatively the performance of some macroalgae across Portuguese latitudes, such as Laminariales or other kelps (thick thallus morphology, thick leathery functional group, e.g. *Ascophyllum nodosum, Laminaria ochroleuca, Saccorhiza polyschides*) which may lose their performance under reduced nutrient availability and increasing temperature southwards (Tuya et al., 2012 and references therein). On the other hand, other adapted thick leathery species (such as *Cystoseira tamariscifolia, Sargassum flavifolium*, *S. vulgare*) may emerge southwards in order to compensate such important niche gaps.

Hence, broad-scale processes arise as being important (or more) in determining community structure along the studied latitudinal gradient; as important as the abiotic stresses that act so pervasively on marine rocky shores across the vertical elevation gradient (Valdivia et al., 2011). As both vertical and horizontal axes gradients impose important changes in determining the species composition and abundance, both gradients should be considered in studies of spatial patterns of distribution of organisms within this area.

Critically, such variable community patterns may raise somehow problems for monitoring programs and environmental impact studies purposes, namely at the regional, country level scale. As in the Portuguese realms, the need to establish different type-specific reference conditions has been previously recognized (e.g. Borja et al., 2012; Gaspar et al., 2012). In this context, ecological reference conditions must summarize a range of possibilities and values over periods of time and across the geographical extent of each water body type; it should represent part of nature's continuum and must reflect the natural variability (WFD CIS, 2003). To minimize the variability associated with geographical differences, a common approach is to organize the environmental information on a narrower spatial scale. In order to predict and better distinguish the anthropogenic effects from the natural environmental effects on biological communities, a more appropriate geographical area or type scale allows to define

biological communities with higher homogeneity. Even though, type-specific areas encompass communities presenting high variability features, such as the differences resultant from seasonal species, the effects of phenotypic plasticity, or the intra-specific genetic differences. On the other hand, to describe different reference conditions at higher discrimination levels (small areas like site level) can imply important disadvantages (costs, attainability, data availability, time availability, etc.) (Economou, 2002).

Thus, the better the control or starting point descriptions to compare results, the better our ability to detect impact changes, that is, the departure from assemblages natural variability ranges that might indicate ecological degradation. In order to recognize chance, it should be highlighted the importance of generating biodiversity broader databases of rocky intertidal assemblages to be used in large-scale comparisons. The value and interpretation of other datasets are frequently predicated on the existence of such baseline datasets. From the ability to recognize change, to establish cost-effective biomonitoring at a range of scales, or to use biodiversity surrogates, all require that there is baseline knowledge of the biota (Cruz-Motta et al., 2010; Neill and Nelson, 2016).

Still, representativeness and time consumption are central issues in monitoring designs (Ballesteros et al., 2007). Species-level approaches to detecting change are costly because are labor-intensive and need high taxonomic expertise. Actually, species-level changes may have little overriding effect on whole assemblages (Littler and Littler, 1980; Steneck and Dethier, 1994). On the other hand, although morphological/functional group hypothesis may provide broad insight into community structure (Roberts and Connell, 2008), such approaches may be pointed out to lose sensitivity in detecting change along environmental gradients, compared to species-level approaches (Phillips et al., 1997; Padilla and Allen, 2000).

We believe however that, depending on the aim, morphological/functional groups may be useful as complementary biodiversity measures rather than actually act as a surrogate measures of (species-level) biodiversity. After all, in order to use such surrogates, taxonomic expertise is needed as well. Ultimately, to quantify the patterns of variation under different biodiversity attributes and measures may provide complementary information, which might be crucial to understand the effects of both natural processes and anthropogenic impacts on natural assemblages (Bevilacqua et al., 2012).

As our results show, both functional groups and trait-based thallus morphologies approaches were useful to generalize latitudinal patterns in the assemblages, where some groups emerge at the expense of others. Both approaches had the ability to be sensitive to the spatial variability of macroalgal assemblages and, as complementary measures, may be helpful when studying macroalgal assemblages. Actually, each macroalgal species change in its worldwide distribution, but each one can be easily assign into such reduced grouping categories, and this may allow comparisons among regions having different sets of species, which in turn may improve ecological synthesis.

Particularly, trait-based approaches (such as considering different thallus morphologies) may improve biodiversity understanding (Ackerly and Cornwell, 2007; Vandewalle et al., 2010) because traits

may reflect species ecological roles, namely how species interact or are adapted to their physical and chemical environment and with other species, and species roles in maintaining and regulating ecosystem processes (Díaz and Cabido, 2001). While the loss of species can alter entire systems, the detection of change through the use of broader taxonomic classifications such as morphological groups hypothesis can be informative or potentially more predictive because of the fundamental nature of change brought by human-induced disturbance (Roberts and Conell, 2008 and references therein).

II.5. Conclusions

Ecological systems are heterogeneous and structured by complex and dynamic processes that operate at a range of spatial and temporal scales. Understanding such complexity is essential when wanting to identify the impact of anthropogenic activities, which can impose changes in community structure and functioning (Martins et al., 2016 and references therein). To understand and quantify the magnitude of the natural variability of assemblages is thus imperative for implementing suitable monitoring programs and environmental impact studies (Veiga et al., 2012). This study indicates that broad-scale processes are very important to determine the community structure along the studied latitudinal gradient; as important (or more) as the abiotic stresses that act so strongly on the vertical axis of shores (Valdivia et al, 2011). Both vertical and horizontal axes gradients impose deep changes in determining the species composition and abundance, and thus both gradients should be considered in studies of spatial patterns of distribution of organisms within this area. From northern to southern Portuguese shores, the studied assemblages were progressively different from each other, where some macroalgae species decline in coverage or disappear, while others emerge and gain relative importance or dominate. Beyond species-level approach descriptions, both functional groups and thallus morphologies approaches were useful to generalize latitudinal pattern changes in the assemblages, where some groups emerge at the expense of others. Depending on the aim, functional groups and trait-based approaches may be useful as complementary biodiversity measures rather than actually act as surrogate measures, and may improve biodiversity understanding and ecological synthesis. Ultimately, to quantify the patterns of variation under different biodiversity measures may provide complementary information, which is crucial to understand the effects of both natural processes and anthropogenic impacts on natural assemblages (Bevilacqua et al., 2012). To generate broader databases on rocky shore assemblages diversity (from species-level to functional groups and thallus morphologies approaches) can be useful for large-scale comparisons and for establishing ecological reference conditions, including for monitoring programs and environmental impact studies.

Chapter III

The trait-based approach "thallus morphology" as a surrogate measure of macroalgal diversity[3]

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[3] Gaspar, R., Pereira, L., Neto, J.M., (submitted to Ecological Indicators). The trait-based approach "thallus morphology" as a surrogate measure of macroalgal diversity.

Using macroalgae to assess coastal and estuarine environments

Abstract

Trait-focused and environmental-pattern approaches may improve biodiversity understanding and ecological synthesis, and biological surrogates may improve the cost-effectiveness of biodiversity monitoring. The ability of the trait-based thallus morphology to be a complementary surrogate measure of macroalgal diversity was studied. The approach categorizes species morphologies as filamentous/leafy, fleshy, thick or calcareous/crusts, differing themselves within other traits: the first two are fast-growing, annual, opportunistic species (most filamentous/leafy are bloom-forming species); the last two are slow-growing, perennial, late-successional species (calcareous/crusts are shade-adapted species). Trait patterns were studied between intertidal rocky shores of wave-exposed with sand-free habitats and of wave-sheltered with sand-covered habitats, and along respective upper-, mid-, and lower-intertidal zones. Assemblages' composition and abundance (coverage per specie) were previously measured using a photographic sampling and GIS methodology. Species-level diversity increased from upper- to mid- to lower-intertidal, against an inverse relationship of decreasing species dominance; diversity was however similar between sites of different wave-exposure/sand-deposition. Nevertheless, this study suggests a relationship between different patterns of species having different thallus morphologies with different wave-exposure/sand-deposition disturbance regimes and with intertidal zonation stress gradients. Filamentous/leafy and also fleshy species (early dominants) dominate at wave-sheltered with sand-covered habitats (sand disturbed), exhibiting higher diversity and higher average abundance. Particularly, filamentous/leafy species dominate towards upper areas of the intertidal (increasing stress gradients). In opposition, thick and also calcareous/crusts species (late-successional dominants) dominate at wave-exposed with sand-free habitats. Particularly, thick species dominate towards lower areas of the intertidal (decreasing stress gradients). The trait-based approach thallus morphology may be a complementary tool for monitoring programs and environmental impact studies involving macroalgal assemblages. Under naturally varying environmental conditions, studies may allow to establish certain distribution pattern ranges of different species morphologies, so that the departure from those ranges may be useful to distinguish sites affected by human impacts – which likely shift towards increasing dominance of small-term competitors, such as opportunistic bloom-forming species and other filamentous/leafy species, at the expense of decreasing dominance of long-term competitors, such as late-successional canopy-forming and other thick species.

Key-words: biodiversity; intertidal zonation; rocky shores; macroalgae; sedimentation; wave-exposure

III.1. Introduction

A major goal of communities' ecology is to explain why species change in a systematic fashion across space (McGill et al., 2006). Yet to evaluate biodiversity patterns is a contemporary challenge for ecology, especially in marine environments (Anderson, 2011; Bevilacqua et al., 2012; Villéger and Brosse, 2012). Marine species are high in number and vary at different spatial (and temporal) scales, being subject to many complex and interrelated factors (including species interactions, dispersal, physical disturbance, and environmental stress), complicating the efforts to understand the basis of patterns genesis, maintenance and diversity (Menge et al., 2005 and references therein). To assess all aspects of biodiversity in an ecosystem can represent an impossible task and thus, different methods and measurements can be considered for a relevant, still relative, evaluation of the biological diversity. Any indicator of biodiversity can be used, in the sense of any measurable variable that can help to estimate or monitor a particular component of the biodiversity, such as information on genes, abundances of species, or phylogenetic or functional relationships among species (Vandewalle et al., 2010 and references therein, Anderson, 2011 and references therein).

The biodiversity (in the sense of species-level distribution patterns) is commonly expressed by measures of species richness and composition and of species relative abundances (Purvis and Hector, 2000). However, instead of species to be measured, other biological surrogate or complementary measures of biodiversity can be considered, such as other taxonomic resolutions, morphologies, functional groups or traits (e.g., Steneck and Dethier, 1994; Konar and Iken 2009; Orfanidis et al., 2011). Under that perspective, biological traits, in the sense of well-defined, measurable properties of organisms, usually measured at the individual-level and used comparatively across species (McGill et al., 2006), may be seen as well as complementary surrogate measures of the (species-level measured) biodiversity.

The presence, abundance and diversity of a given set of functional traits (e.g., morphological, ecophysiological and life history characteristics) can be used for estimating particular components of biodiversity, in the sense of "functional indicators of biodiversity". The use of biodiversity indicators based on the functional traits of species and communities, have as yet been rarely considered in current common monitoring schemes (Vandewalle et al., 2010 and references therein).

Under these realms, the use of biological surrogates as proxies for biodiversity patterns is gaining popularity, namely in marine systems where field surveys can be expensive and species richness is high (Mellin et al., 2011). Concomitantly, community ecologists are increasingly considering that trait-based approaches may improve biodiversity understanding (Ackerly and Cornwell, 2007; Vandewalle et al., 2010), particularly because traits may reflect species ecological roles, namely how species interact or are adapted to their physical and chemical environment and with other species, and species roles in maintaining and regulating ecosystem processes (Díaz and Cabido, 2001).

Under a functional diversity perspective, ecosystem properties depend greatly on biodiversity in terms of the functional characteristics (traits) of organisms present in the ecosystem, as on the distribution and abundance of those organisms over space and time (Hooper et al., 2005). However, before

attempting to draw any ecological (or functional) explanations, it must be stressed that the assemblages' spatial patterns should be explicitly described (Menge et al., 2005), hopefully in a manner that can answer what major processes determine the assemblages' patterns (Hewitt et al., 2007; Burrows et al., 2009). Small-scale variability is an intrinsic property of marine benthic assemblages; if that patchiness variation goes undetected, differences due to human impacts may be confused with differences due to natural spatial variability (Coleman, 2002; Fraschetti et al., 2005).

Critically unavoidable, traits should be studied in their patterns along environmental gradients (McGill et al., 2006). It means that in order to adopt surrogate measures of diversity it must be assumed that the surrogate is strongly correlated (not just significantly) with the 'actual' (species-level) patterns of richness or diversity, and where that correlation is consistent in both space and time. Regardless the measures to be used, the spatial and temporal scales considered to be measured are critically determinant, as will influence the efficacy of any particular surrogate measure (Smale, 2010).

Since certain patterns of macroalgal growth forms have been linked to certain levels of environmental disturbance, a link between morphological habit and ecological role or function has been suggested (e.g. Littler and Littler, 1980; Littler and Littler, 1984). Macroalgae' functional-form groups (Steneck and Dethier, 1994) have been widely used, categorizing species differing in morphological features (e.g., filamentous, corticated, leathery among seven others), and where these form-based features are supposedly linked to different ecological functions. Recently, Orfanidis et al. (2011) assigned macroalgal species into five ecological status groups (ESG) categories, based on different traits (morphological, physiological and life history), which responded along eutrophication gradients. In this context, we hypothesize that different macroalgal trait-based thallus morphologies (which encompasses different ESG and categorizes species as filamentous and leaf-like, fleshy, calcareous upright and calcareous and non-calcareous crusts or thick; Orfanidis et al., 2011) may respond also to natural varying small-scale gradients, and therefore it should be explicitly tested. In fact, different thallus morphologies seem to respond along large-scale latitudinal process and gradients and, as a complementary information for species-level approaches, may be useful to improve biodiversity understanding and ecological synthesis (Gaspar et al., in prep.).

Under above scopes, it is here hypothesized that macroalgal trait-based thallus morphology approach will be a useful as a complementary information, able to broaden the ecological perception of macroalgal community patterns under natural varying small-scale gradients.

Particularly, this work aims to test the ability of different categories encompassing different thallus morphologies to detect differences in the macroalgae assemblages patterns, namely between intertidal rocky shores of wave-exposed with sand-free habitats and of wave-sheltered with sand-covered habitats (this study particular environmental disturbance gradient), and among its upper-, mid- and lower-intertidal zones (a commonly studied coastal environmental stress gradient). On this matter, the assemblages patterns regarding other macroalgal traits (thallus longevity, succession and growth, light adaptation;

Orfanidis et al., 2011), ecological status groups (ESG, Orfanidis et al., 2011) and functional-form groups (FG, Steneck and Dethier, 1994) will be considered herein.

III:2. Material and methods

III.2.1. Study area

This study was conducted at the intertidal rocky shores of Buarcos Bay (40°10'2.84"N, 8°53'16.96"W; NW Portuguese coast, Iberian Peninsula, NE Atlantic region). Its rocky platforms emerge during low tides as being relatively long and narrow, arranged parallel to each other, and perpendicularly to the NW-SE coast line, creating an intercalation of exposed habitats (facing the prevailing NW oceanic swell at Portuguese western coast, Boaventura et al., 2002), with sheltered habitats (facing SE). Sand is commonly deposited in the supratidal areas and in the subtidal areas between rocky platforms. Notably, platforms' exposed habitats are typically sand-free, in opposition to platforms' sheltered habitats, which commonly present a thin deposition layer of fine sand (about 0.01 m high) that is usually trapped within macroalgal species.

III.2.2. Sampling design and data production

Three rocky platforms distancing at least 50 m apart were previously selected. Each platform exhibited wave-exposed with sand-free habitats (side of the platforms facing the NW), and wave-sheltered with sand-covered habitats (other side of the platforms facing the SE). Each side of each platform featuring above mention habitats was sampled along a single transect perpendicular to the waterline, following a stratified random sampling scheme: 3 replicates per each intertidal zone (upper-, mid- and lower-intertidal), i.e., 9 replicates per transect. Replicates were collected as the tide receded, on flattened bedrock (excluding rock pools). Sampling occurred on spring low tides, in 2012, and during the spring/summer season, corresponding to the seasonal period of maximum development of macroalgae at temperate seas. Each replicate (quadrate, 0.5x0.5 m, i.e., 0.25 m^2), and each of its twenty-five replicate' sub-quadrates (subdivisions of 0.1x0.1 m each) were singly photographed. Whenever necessary, mainly at lower-intertidal habitats, multilayered species were taken into account by setting aside canopy species for secondary photographic records of understory species. Each photo-quadrate replicate (0.5x0.5 m) was geo-referenced using Quantum GIS 1.8.0 Lisboa software. Species coverage areas were calculated by outlining polygons, drawn on each geo-referenced 0.5x0.5 m photo-quadrate. Close-up photographs (for higher image detail) taken from each replicate' sub-quadrates were used aside to allow better accuracy on the polygons' drawing. Sampled taxa were mostly identified to the species level (Fig. III.2).
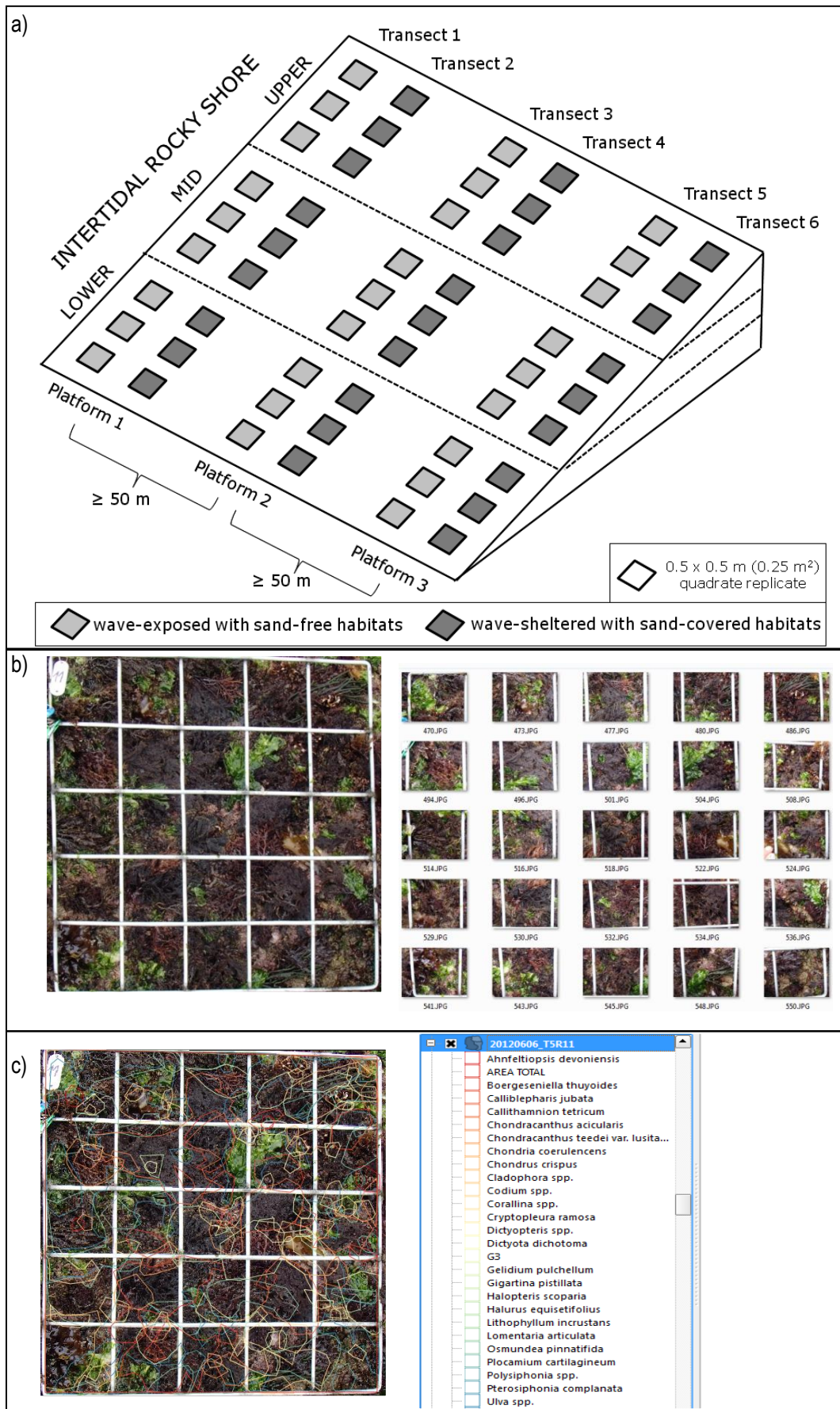
Fig. III.2. Sampling design of macroalgal assemblages (a); photographed sampling replicate (quadrate,

0.5x0.5 m) and 25close-up photographs (sub-quadrates, 0.1x0.1 m each) (b); polygons drawn using a GIS software, outlining species coverage areas on the 0.5x0.5 m photo-quadrates (c).

III.2.4. Species traits, functional groups and ecological status groups

Sampled species were assigned into different traits, namely: a) thallus morphology (Orfanidis et al., 2011), in four categories, the filamentous/leafy (including filamentous and leaf-like), fleshy, thick, or the calcareous/crusts (including calcareous upright and calcareous and non-calcareous crusts; b) life history, comprising thallus longevity (annuals or perennial categories) and succession (opportunistic or late-successional categories) (Orfanidis et al., 2011); c) physiology, comprising growth (fast- or slow-growth categories) and light adaptation (sun-adapted or shade-adapted categories (Orfanidis et al., 2011); as well as into different d) ecological status groups (ESG) (Orfanidis et al., 2011); and e) functional groups (FG) (Steneck and Dethier, 1994).

Some assignment exceptions from above literature references were made, and information from elsewhere was considered (Guinda et al., 2008, MarLIN, 2014, Guiry, 2014) regarding the local/ regional perspective on some species (e.g., all species from the Order Gigartinales were considered to be late-successional species and as having a thick thallus morphology; some taxonomical Orders were used to subdivide some FG).

III.2.5. Statistical analysis

Coverage data and presence/absence data (both based on species and on species assignments into different thallus morphologies) were used to construct resemblance matrices with Bray-Curtis as similarity index. The tables based on species allowed to represent the spatial ordination of samples through the nonmetric multidimensional scaling (nMDS) method. Differences in the assemblages along the studied habitats were assessed using PRIMER V. 6. PERMANOVA software package (Clarke and Gorley, 2006; Anderson et al., 2008) through:

a) permutational multivariate analysis of variance (PERMANOVA) using 9999 permutations under a reduced model, considering two factors, wave-exposure/sand-deposition (fixed, 2 levels), and zonation (fixed, 3 levels), and pair-wise tests to analyze the interaction between the factors, i.e., wave-exposure/sand-deposition vs. zonation; a significance level was accepted at $p < 0.05$;

b) univariate analysis using the DIVERSE routine package, namely the Shannon-Wiener (H', $\log_e$) and Simpson (λ'=SUM (N_i*(N_i-1)/(N*(N-1)) diversity indices;

c) multivariate analysis of dissimilarities between sites, using similarity percentage analysis (SIMPER routine package) on coverage data without any transformation.

III.3. Results

III.3.1. Species traits, functional groups and ecological status groups

A total of 53 taxa were found, mostly identified to the species level. Sampled taxa were assigned into different traits and classification method groups, highlighting the categories of the trait thallus morphology (adapted from Orfanidis et al., 2011) (Table III.1).

Taxa were assigned into different traits and functional groups (FG), highlighting the categories of the trait thallus morphology (TM). Filamentous and leaf-like morphologies and fleshy morphologies are featured by annual, opportunistic and fast-growing species. However, rather than fast-growing fleshy species (encompassing ESG-IIA), most filamentous and leaf-like species (encompassing ESG-IIB) have high reproductive capacity and short life spans and can grow in all environments, often forming blooms in highly degraded environments, e.g. green tides of *Ulva* spp. (Orfanidis et al., 2014). Filamentous and leaf-like species comprise the filamentous group or in the foliose/tubular group. The last group was renamed (after Steneck and Dethier, 1994) to clearly include the tubular-forming species of *Ulva* (such as those from the former genera *Enteromorpha*). Fleshy species comprise the fleshy corticated foliose group or in the fleshy corticated group. Thick morphologies correspond to slow-growing, late succession and perennial species (encompassing ESG-IA and ESG-IB) although one species in particular, *Sacchoriza polyschides*, is a thick kelp-like, yet an annual, opportunistic and fast growing species (*sensu* ESG-IIA). Actually, *S. polyschides* is not a true Laminariales kelp, being of the Order Tilopteridales; still is a "pseudo-kelp" once can perform a similar ecological role as a dominant canopy former (Smale et al., 2013). Thick species comprise the thick corticated (Gigartinales) group, the thick corticated (other Orthers) group, or the thick leathery group (includes species from Orders such as Fucales, Laminariales, Desmarestiales or Tilopteridales). Calcareous upright and calcareous and non-calcareous crusts morphologies are also featured by slow-growing, late succession and perennial species, although being shade-adapted (encompassing ESG-IC). These species comprise the articulated calcareous group or the crustose group (Table 1).

Table III.1. Macroalgal taxa assignments according to different traits (Orfanidis et al., 2011): a) light adaptation; b) thallus longevity, succession and growth; and c) thallus morphology (corresponding to different Ecological Status Groups = ESG, Orfanidis et al., 2011); and d) functional groups (Steneck and Dethier, 1994). Taxonomical Orders were used to further subdivide the functional groups within thick thallus morphologies.

Table III.1 (continued)

a) SUN-ADAPTED	
b) ANNUALS AND OPPORTUNISTIC, FAST GROWTH	b) PERENNIALS AND LATE-SUCCESSIONAL, SLOW GROWTH
c) Filamentous and leaf-like (ESG IIB)	c) Thick (ESG IA or ESG IB)
d) Filamentous	d) Thick Corticated (Gigartinales)
Bornetia secundiflora	*Ahnfeltiopsis devoniensis*
Callithamnion tetricum	*Calliblepharis jubata*
Ceramium spp.	*Callophyllis laciniata*
Cladophora spp.	*Caulacanthus ustulatus*
Codium spp.	*Chondracanthus acicularis*
Dasya spp.	*Chondracanthus teedei* var. *lusitanicus*
Halopteris scoparia	*Chondrus crispus*
Halurus equisetifolius	*Gigartina pistillata*
Ophidocladus simpliciusculus	*Gymnogongrus crenulatus*
Other Ceramiales *1	*Mastocarpus stellatus*
Other Ectocarpales/*Sphacelaria* spp.	d)Thick Corticated (Other Orders)
Simple Filamentous Chlorophyta*2	*Gelidium pulchellum*
Polysiphonia spp. (or other Rhodomelaceae)*3	*Osmundea pinnatifida*
Pterosiphonia ardreana/pennata	*Plocamium cartilagineum*
d) Foliose/Tubular	*Pterosiphonia complanata*
Porphyra spp./*Pyropia* spp.	d)Thick Leathery (e.g., Fucales, Laminariales, Desmarestiales, Tilopteridales)
Ulva spp.	*Cystoseira baccata*
c) Fleshy (ESG IIA)	*Saccorhiza polyschides* (ANNUAL AND OPPORTUNISTIC, FAST GROWTH) *A
d) Fleshy Corticated foliose	a) SHADE-ADAPTED
Acrosorium ciliolatum	c) Calcareous upright and calcareous and non-calcareous crusts (ESG IC)
Cryptopleura ramosa	d) Articulated calcareous
Dictyopteris polypodioides	*Corallina* spp.
Dictyota dichotoma	d) Crustose
Grateloupia turuturu	*Hildenbrandia rubra*
Hypoglossum hypoglossoides (or other Delesseriaceae) *4	*Lithophyllum incrustans*
Nitophyllum punctatum	*Peyssonnelia* spp.
d) Fleshy Corticated	
Boergeseniella spp.	
Chondria coerulescens	
Chondria dasyphylla/scintillans	
Cladostephus spongiosus	
Gastroclonium ovatum	
Gastroclonium reflexum	
Gracilaria gracilis	
Gracilaria multipartita	
Lomentaria articulata	
Scinaia furcellata	

Taxa groupings can include one or more of the following genera: *1 – *Aglaothamnion*, *Anotrichium*, *Anthithamnion*, other *Callithamnion*, *Compsothamnion*, *Griffithsia*, *Pleonosporium*; *2 – *Pseudendoclonium*, *Rhizoclonium*, *Ulothricales* *3 – *Aphanocladia*, *Herposiphonia*, *Heterosiphonia*, *Streblocladia*; *4 – *Apoglossum*, *Erythroglossum*. *A – *S. polyschides* is thick species; yet is an annual, opportunistic and fast-growing species.

III.3.2. Composition and abundance patterns

Out of the 53 sampled taxa, 49 and 48 taxa where respectively present at wave-exposed with sand-free (hereafter E.) habitats, and at wave-sheltered with sand-covered (hereafter S.) habitats. Forty-four (44) taxa were simultaneously present at E. and S. habitats; 5 and 4 taxa were only found at E. and at S. habitats, respectively. However, the assemblages patterns differed (p<0.001, both in terms of composition and abundance) between sites with different wave-exposure/sand-deposition conditions, namely between mid- and lower-intertidal zones, and also in terms of composition at the upper-intertidal zones (Fig. III.2, Appendix III.3).

Similarities between E. and S. habitats and along respective intertidal zonation patterns were found in terms of a) the total number of taxa (in the sense of species richness), where upper-intertidal areas from both E. and S. habitats presented relatively lower species richness than mid- and lower-intertidal areas (Fig. III.3a); and b) in terms of the total species coverage, which increased towards the lower-intertidal due to multilayered species (Fig. III.3b).

Furthermore, similarities were also found in terms of species richness having different thallus morphologies between E. and S. habitats according to each intertidal zone, where all morphologies decreased in richness at the upper-intertidal, and where only calcareous/crusts species presented lower richness values than the remaining species morphologies (Fig. III.3a).

However, in terms of abundance, filamentous/leafy species dominated at S. habitats, decreasing in abundance from upper- to mid- to lower-intertidal, being significantly more abundant at the mid- and lower-intertidal zones of S. habitats. In opposition, thick species increased in abundance from upper- to mid- to lower-intertidal, being significantly more abundant at the mid- and lower-intertidal of E. habitats. Fleshy species were significantly more abundant at the mid- and lower-intertidal zones of S. habitats. Calcareous/crusts species were significantly more abundant at the lower-intertidal zone of E. habitats, yet being relatively less abundant than remaining species morphologies (Fig. III.3b).
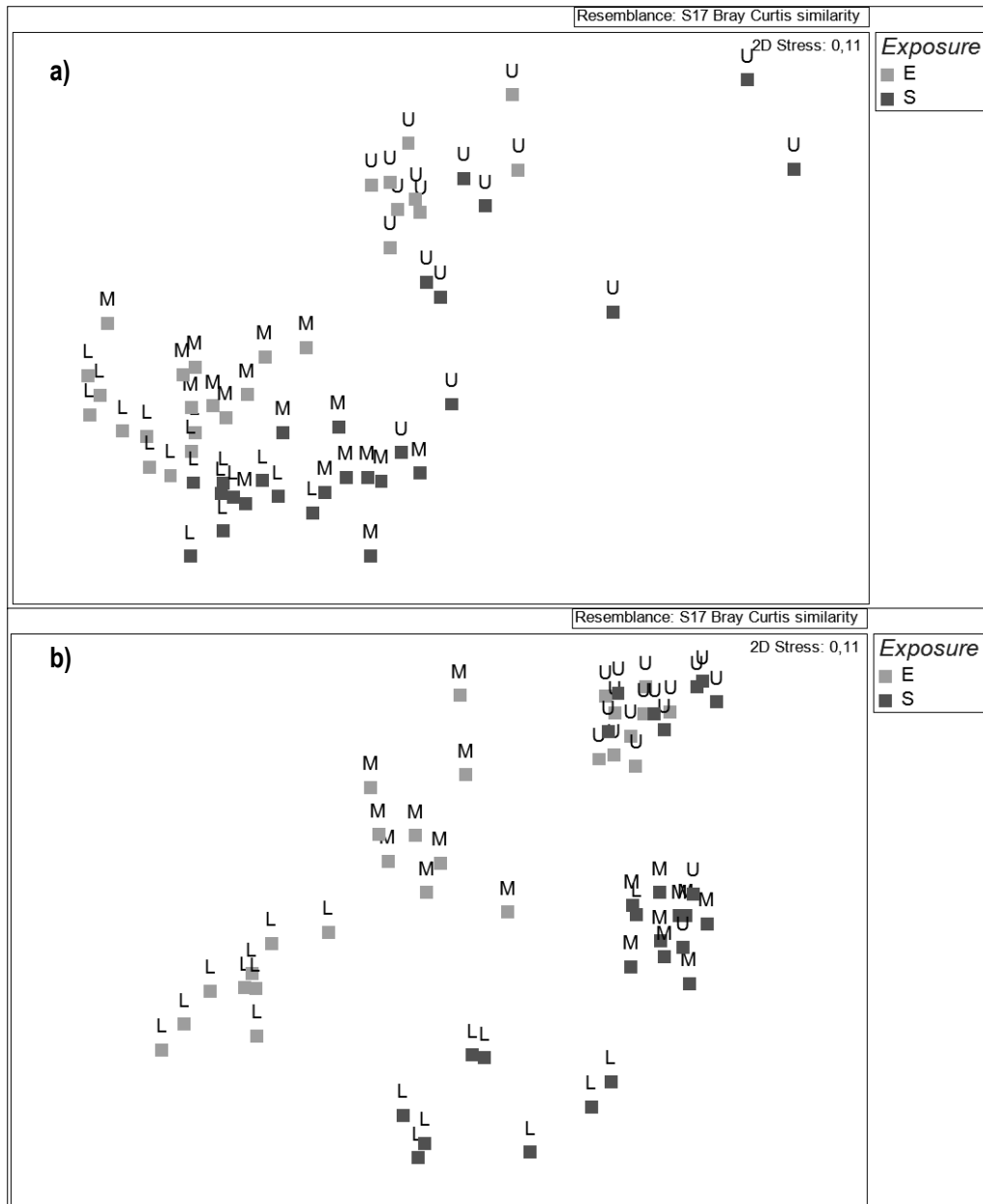
Fig. III.2. nMDS ordinations of macroalgal assemblages based on Bray-Curtis dissimilarity measures of (a) composition (species richness) and of (b) abundance (species coverage) data between wave-exposed with sand-free habitats (n=27; E) and wave-sheltered with sand-covered habitats (n=27, S): U = upper-intertidal zone (n=9); M = mid-intertidal zone (n=9) and L = lower-intertidal zone (n=9).
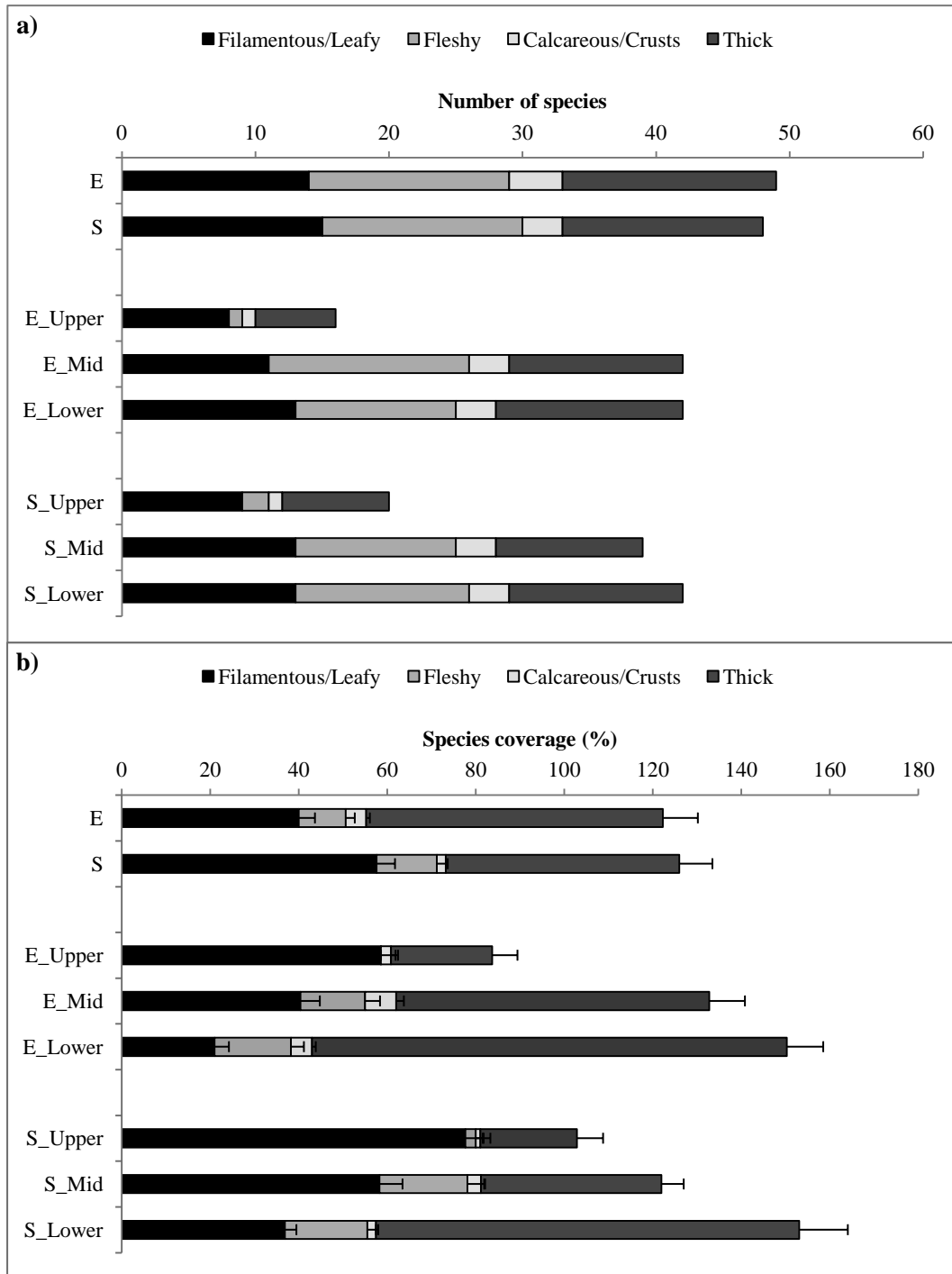
Fig. III.3. Distribution patterns of different thallus morphologies between wave-exposed with sand-free habitats (E, n=27) and wave-sheltered with sand-covered habitats (S, n=27), and along their upper-, mid- and lower-intertidal zones (n=9 per intertidal zone), based on a) species richness and b) species coverage (%).

III.3.3. Diversity indices

The species diversity (Shannon-Wiener index) increased from upper- to mid- to lower-intertidal (Fig. III.4a), against an inverse relationship of decreasing species dominance (Simpson index) (Fig. III.4b);

species diversity was however similar between habitats of different wave-exposure/sand-deposition conditions (Fig.III.4a and b); only mid-intertidal zones presented significantly higher diversity at E. habitats (Fig. III.4a).
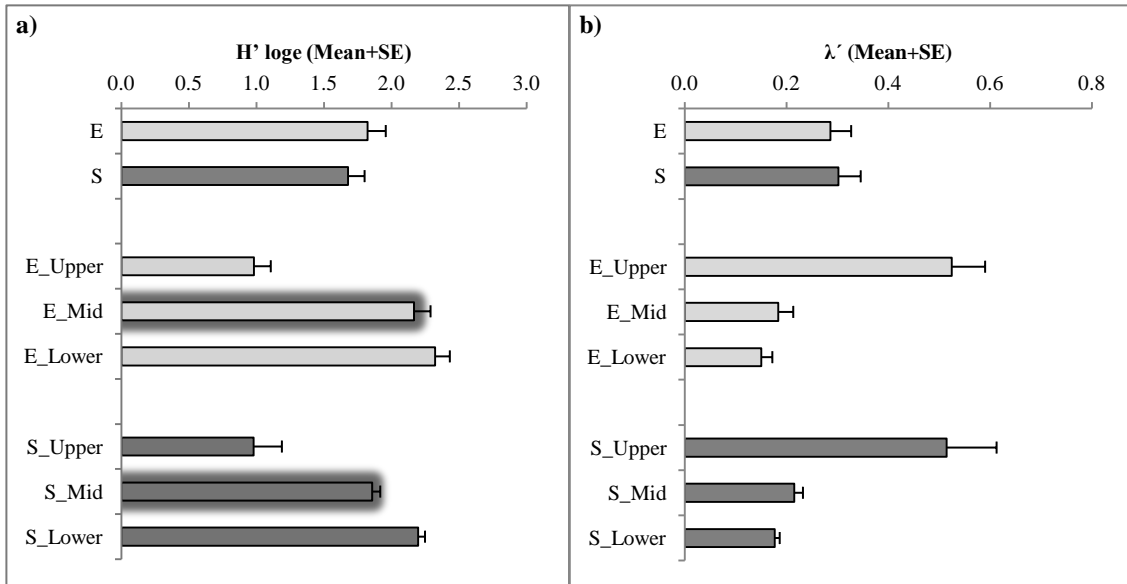

Fig. III.4. Shannon-Wiener (H' log$_e$, mean +SE) (a) and Simpson (λ', mean +SE) (b)diversity indices for wave-exposed (sand-free habitats, E, n=27) and wave-sheltered (sand-covered habitats, S, n=27), along upper-, mid- and lower-intertidal zones (n=9 per intertidal zone). Glow effect = significant difference (p<0.05).

However, in terms of thallus morphologies, filamentous/leafy species presented higher diversity at S. habitats. In opposition, thick species presented higher diversity at E. habitats. Furthermore, diversity of thick species was significantly higher at upper- mid- and lower-intertidal zones of the E. habitats. Diversity of fleshy species was significantly higher at the mid-intertidal of the E. habitats and at the lower-intertidal of the S. habitats (Fig. III.5).
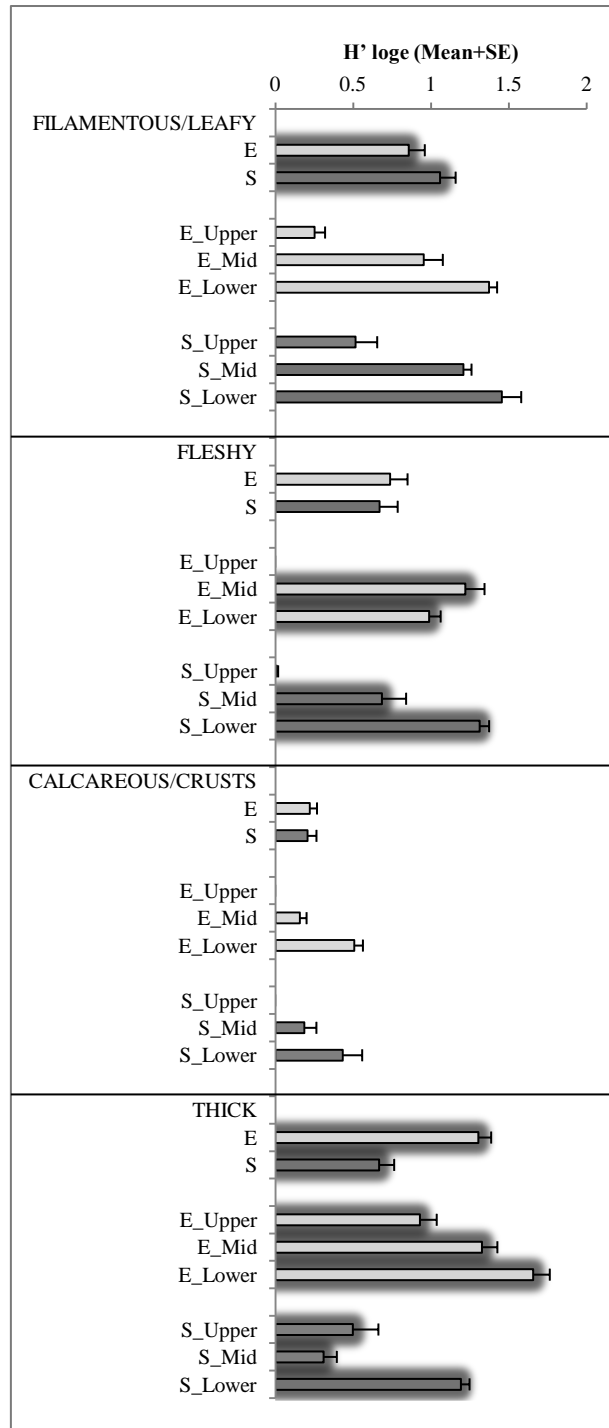
Fig. III.5. Shannon-Wiener diversity index (H' $\log_e$, mean +SE) based on thallus morphologies, for wave-exposed (sand-free habitats, E., n=27) and wave-sheltered (sand-covered habitats, S., n=27) sites, along upper-, mid- and lower-intertidal zones (n=9 per intertidal zone). Glow effect = significant difference (p<0.05).

III.3.4. Multivariate analysis

The species that contributed mostly to the overall dissimilarity patterns between sites showing different wave-exposure/sand-deposition and according to each intertidal zone is given by Appendix III.4.

Thick species contributed mostly to the overall dissimilarity patterns between sites with different wave-exposure/sand-deposition, followed by filamentous/leafy, then by fleshy, and lastly by calcareous/crusts species. E. habitats exhibited relatively higher average abundances of thick species, and also of calcareous/crusts species, in opposition to S. habitats, which exhibited relatively higher average abundances of filamentous/leafy species, and also of fleshy species. Filamentous/leafy species, and also fleshy species, presented always higher average abundances at all intertidal zones of S. habitats. In opposition, thick species, and also calcareous/crusts species, presented always higher average abundances at all intertidal zones of E. habitats (Appendix III:5; Fig. III.6).
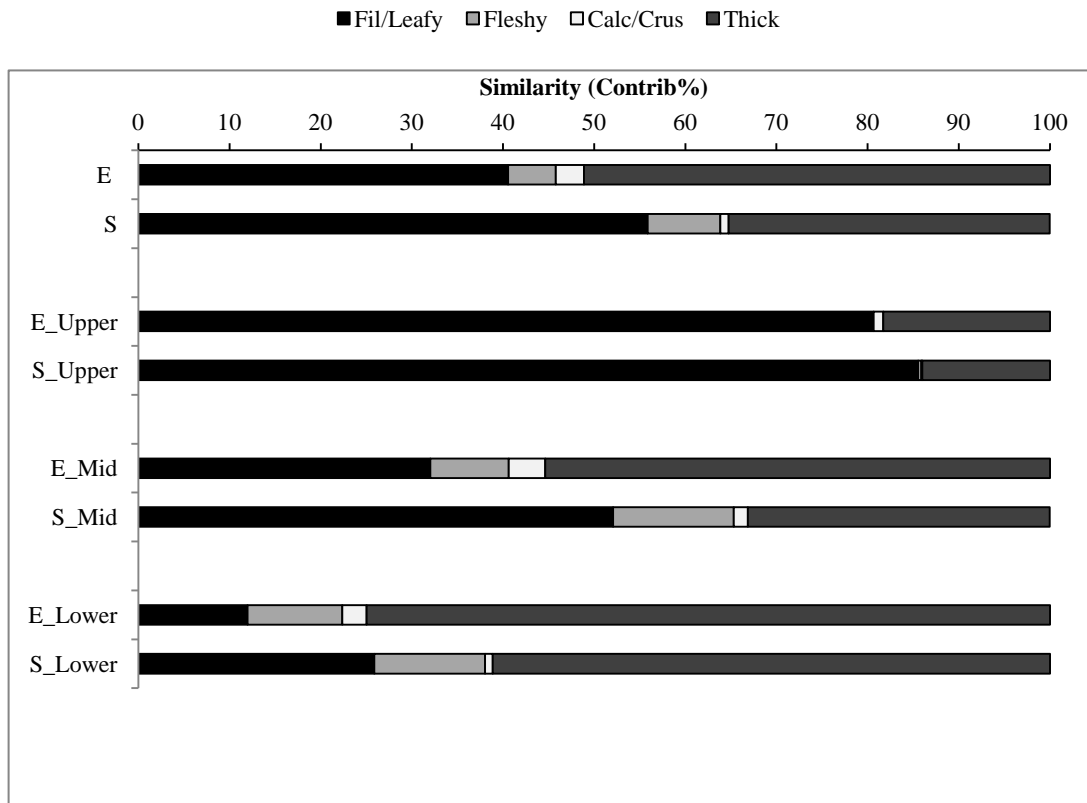

Fig. III.6. Graphical representation of SIMPER results showing the contribution of thallus morphologies for general similarity (%; without cut off for low contributions) between wave-exposed (sand-free habitats, E., n=27) and wave-sheltered (sand-covered habitats, S., n=27), and for intertidal zones (n=9 per intertidal zone).

III.4. Discussion

This work aimed to study the ability of the trait thallus morphology to be a surrogate complementary measure of macroalgal diversity patterns. In order to study the thallus morphologies' distribution patterns, two different intertidal rocky shore habitats were selected featuring different wave-exposures associated with or without sand-deposition influences.

The effects of wave action on intertidal organisms can include damage, detachment and displacement; which indirectly can involve interactions with sediments and adult populations (Taylor and Schiel, 2003). Sites with different levels of wave-exposure can differ in their sediment sizes and effects: sheltered areas are subject to more settlement and accretion of fine sediments, whereas exposed areas are subject to more resuspension and abrasion by coarse sediments (Airoldi, 2003). Sediment deposition alone is considered to be one major source of physical perturbation in marine habitats (e.g. burial, abrasion, stress, disturbance), whose effects on species and assemblages can be a major source of spatial (and temporal) heterogeneity for rocky coastal organisms (as it can interfere with settlement, recruitment, and growth processes, and also indirectly by unbalancing biological interactions). When sediments are abnormally added to rocky shores due to human actions, this ultimately may drive shifts in the composition, structure, dynamics and diversity of rocky shore assemblages (Littler et al., 1983b; Airoldi, 2003, Irving and Connell 2002).

It should be noted though that some studies support the negative effect of sedimentation on assemblages spatial heterogeneity, although they have been focused on the impact of human related sediments on subtidal assemblages (Díaz-Tapia et al., 2013 and references therein). In fact, even the most tolerant hard-bottom organisms may eventually suffer inhibition and mortality above certain degrees of sedimentation; furthermore, there is evidence that "excessive" sediment loads can be a threat to diversity and functioning of coastal rocky assemblages, being a prime driver of shifts between alternate states in the species distribution (Airoldi, 2003).

Nevertheless, sedimentation effects on species diversity may still need to be clarified. On one hand, high sediment loads related to anthropogenic activities have been associated with detrimental of the overall diversity of rocky coast organisms. On the other hand, the effects of the natural presence of sediments support the hypothesis that it promotes species diversity. However, the effect of natural sedimentation in the structure of benthic assemblages has received little attention. (Díaz-Tapia et al 2013). Sedimentation effects on species and assemblages may vary at different spatial and temporal scales, depending on the characteristics of the depositional environment, life histories of species and the stage of development of individuals and assemblages, and in relation to a panoply of variable physical factors (Airoldi, 2003). Nevertheless, studies on the effects of sedimentation on macroalgal diversity in combination with physical factors, such as wave-action and bathymetric level, seem to be lacking (Díaz-Tapia et al., 2013).

In this work, the inverse relationship between the degree of wave-exposure and the amount of sediments was found, as well as its linkage to differences found in the dominant macroalgal assemblages (McQuaid and Dower, 1990; Menge et al., 2005; Schiel et al., 2006. Furthermore, the macroalgal assemblages also exhibited intertidal zonation patterns, whose differences can be commonly associated to other environmental stress gradients such as temperature, desiccation, and solar irradiation (e.g. Menge and Olson, 1990) (Appendix III.3 and III.4).

The photographic and GIS method used allowed a spatial-explicit, low-cost, non-destructive, fast and reliable approach for fieldwork sampling of the intertidal macroalgae assemblages (Fig. III.1). The method was able (to a very small area precision level) to detect differences in the assemblages patterns along the studied habitats, both in terms of composition and abundance measures (Fig. III.2). In terms of total values of species richness and of species coverage, the assemblages patterns observed look outwardly similar (Fig. III.3), i.e., the available intertidal space of both sites is occupied by similar species numbers and species abundances. However, under composition and abundance measures, species assemblages clearly varied between sites of different wave-exposure/sand-deposition, namely at mid- and lower-intertidal zones; only upper-intertidal assemblages seem to be similar between sites in terms of abundance (Fig. III.2). On one hand, species diversity measures increased from upper- to mid- to lower-intertidal, against an inverse relationship of decreasing species dominance. On the other hand, assemblages diversity measures resulted somehow similar between the two sites of different wave-exposure/sand-deposition regimes (Fig. III.4). Only mid-intertidal zones presented significantly higher diversity at wave-exposed with sand-free habitats (Fig. III.4a). Moreover, no relevant pattern differences between sites were found in terms of species richness within different thallus morphologies, except that calcareous/crusts species presented lower species richness values that the others, and that all morphologies decreased in species richness at the upper-intertidal (Fig. III.3a).

Nevertheless, the overall data analysis (Figs. III.3, III.5 and III.6, Appendix III.5) suggests a relationship between different patterns of species encompassing different thallus morphologies with different wave-exposure and sand disturbance conditions and along its respective intertidal zonation stress gradients. The studied habitats can be seen as differing in both wave-exposition and sand-deposition regimes, and both habitats simultaneously accommodate perennial, slow-growing, late-successional dominants (encompassing thick and calcareous/crusts species) and fast-growing, annuals, opportunistic early dominants (encompassing filamentous/leafy and fleshy species). However, wave-sheltered with sand-covered habitats may serve as a refuge for those early dominants ("fugitive species", Dayton, 1975) such as fast-growing, annual and blooming opportunistic species like *Ulva* spp.. Nevertheless "fugitive species" (and other small-term competitors with higher dispersal capabilities) can coexist along with long-term competitors (slow-growing, perennial and late-succession dominants such as thick and calcareous/crusts species) at both wave-exposed with sand-free habitats and wave-sheltered with sand-covered habitats (Littler et al., 1983b, Menge et al., 2005). Therefore, filamentous/leafy and also fleshy species tend to thrive and dominate at wave-sheltered with sand-covered habitats, by exhibiting

higher diversity and higher average abundance. Particularly, bloom-forming species of filamentous/leafy species dominated towards upper areas of the intertidal, where environmental conditions tend to be more "harsh" (increasing stress). In opposition, long-term competitors tend to dominate at wave-exposed with sand-free habitats, outcompeting the early dominants, namely those "fugitive species". Particularly, thick species dominated towards lower areas of the intertidal (decreasing stress).

Ultimately, sedimentation effects alone strongly influence the assemblage composition, involving effects that are species-specific and morphology-dependent (Díaz-Tapia et al., 2013 and references therein). Higher sedimentation rates may promote tolerant species, with morphological and physiological adaptations to external constraints that limit productivity, as well as opportunistic species with life-history adaptations to disturbances (Eriksson and Johansson, 2005). For example, under those realms, filamentous algal turfs have been documented as the dominant type of assemblages in habitats influenced by sediments, or calcareous Corallinaceae have been reported to dominate at wave-exposed shores (Díaz-Tapia et al., 2013 and references therein).

Depending on the point of view, our data may suggest two contrasting views. At shore level, comprising different transects of different wave-exposure and sedimentation regimes, the habitat heterogeneity is increased and hence, the structural heterogeneity of macroalgal assemblages is increased along space (McQuaid and Dower, 1990). In other words, the natural intercalation of different habitats presented by the rocky platforms' arrangement (i.e. with and without sand deposition resultant from the platform' sides being subjected to lower or higher wave-exposure) at Buarcos Bay, enhances macroalgae diversity. Nevertheless, at habitat level, the overall diversity was somewhat higher at wave-exposed with sand-free habitats, rather than at wave-sheltered with sand-covered habitats (Fig. III.4). In fact, rather than associated with shores covered by sand, the general marine macroalgal species are typically attached to hard, *sensu* sand-free, rocky bottoms. Yet at sheltered habitats with a certain natural, not excessive, amount of sand deposition, wave-sheltered with sand-covered adapted species might also thrive. Thus, at wave-sheltered with sand-covered habitats, wave-exposed with sand-free adapted species may tend to be excluded whereas the wave-sheltered with sand-covered adapted species may tend to thrive (and vice-versa for wave-exposed with sand-free habitats, where its adapted species may tend to outcompete the wave-sheltered with sand-covered adapted species).

It should be further noted that both positive and negative effects of sediments are possible depending on levels of intensity and temporal variance of disturbance (Vaselli et al., 2008). Although disturbance rates may be similar across wave-exposures, where most loss results from wave action at wave-exposed areas, and from sediment burial and substratum failure at wave-protected areas, there are higher rates of change in the assemblages elements at wave-exposed than at wave-sheltered areas. Furthermore, the processes underlying assemblages' structure at wave-exposed areas include competition for space, and variable algal life histories, such as canopy (thick species) competition and synergistic effects among macroalgae (Melville and Connell, 2001; Menge et al., 2005). This may explain the higher diversity detected at the mid-intertidal of wave-exposed with sand-free habitats (Fig. III.4a),

where inclusive fleshy species were able to reach higher diversity (Fig. III.5). In contrast, at wave-sheltered areas, the persistence and standoff competition and differential colonization among species likely occurs (Menge et al., 2005), where opportunistic species dominate (such as filamentous/leafy species). Particularly in this study, sand is deposited at supratidal areas, probably having a strong influence to the upper-intertidal assemblages than one due to wave-exposure conditions. This may be the cause of filamentous/leafy species dominance at those upper-intertidal areas (Fig III.6).

If species dominance shifts under changing environmental conditions, this shifts should affect functional roles as well (Walker, 1999). For example, canopy-forming macroalgae are linked to key ecological roles, once providing food, habitat, protection, structural complexity, and enhancing biodiversity and productivity in coastal ecosystems (Tait and Schiel, 2011). It is found however that impacts such as sediment loads, supposedly anthropogenic, are shifting macroalgal communities from canopy-forming (late-successional thick species) to turf-forming, filamentous, leaf-like or other ephemeral species (early dominants with lower structural complexity such as filamentous/leafy and probably fleshy species). Actually, these canopy-to-turf shifts seem to be a common pattern of biodiversity replacement caused by negative synergistic anthropogenic impacts on these assemblages, such as sediment deposition loads or nutrient pollution effects (Benedetti-Cecchi et al., 2001; Airoldi et al., 2008; Perkol-Finkel and Airoldi, 2010; Strain et al., 2014).

The relationship between diversity and ecosystem function can be supported either by the traits diversity, through the categorization of species (or of species attributes) that can be related to function (Walker, 1999) or by the functional groups diversity (Petchey and Gaston, 2006). Yet it must be stressed that the relationship between species and its functional attributes remains poorly understood, although to comprehend these relationships is critically important, both for the mechanistic understanding of the community assemblages and for the appropriate expectations and approaches to protect and restore biological communities (Micheli and Halpern, 2005). Different functional groups within the generalized and indistinct category of "turf-forming species" (Irving and Connell, 2002; Gorgula and Connell, 2004) can show different responses to stress (Balata et al., 2011); or describing macroalgal assemblages with functional groups can produce results that, although interesting, can raise doubts (Benedetti-Cecchi et al., 2001; Piazzi et al., 2004). Critically, the need to improve functional groupings on specific functions (e.g. nutrient uptake rates, photosynthesis rates, disturbance resistance) has been underlined (Padilla and Allen, 2000).

Thereby, it must be acknowledged that the importance of species identity to ecosystem processes (Bruno et al., 2005) as well as the identity of functional groups, which can be more important than the functional richness itself when studying macroalgal assemblages (Arenas et al., 2006). For example, thick canopy-forming *Cystoseira* species seem to have an important role in maintaining the structure of macroalgal assemblages, but different *Cystoseira* species have different 'engineering' effects on understory assemblages (Benedetti-Cecchi and Cinelli, 1992; Mangialajo et al., 2008). Particularly, *Cystoseira* species (thick morphology, thick leathery functional group, Table III.1) have been linked to

wave-exposed habitats (e.g. Nishihara and Terada, 2010). However, in this work, similarly to others (Bárbara et al., 1995) the species *Cystoseira baccata* in particular appear typically at the lower-intertidal of wave-sheltered with sand-covered habitats, rather than at wave-exposed with sand-free habitats (Appendix III.4). In the same way, different morphological functional groups within the generalized and indistinct category of "turf-forming species" (e.g. Irving and Connell 2002, Gorgula and Connell, 2004) can show different responses to stress (Balata et al, 2011).

Critically, the need to better understand of how ecological functions are linked to the diversity and identities of species, i.e., the relationships between species and functional diversity, and between functional diversity and ecosystem functioning, seems unavoidable for the management and conservation of marine ecosystems (Micheli and Halpern, 2005). After all, the different expression of species attributes under different regimes of disturbance (such as wave-exposure and sand-deposition) and intertidal zonation stress gradients produces much of the spatial and temporal heterogeneity one observes in natural assemblages (Sousa, 1984). As species differ in their adaptations to particular environments, the outcome of interactions depends on the species identity (Viejo et al., 2008), which, in the end, produce their realized niches or patterns (Hutchinson, 1957). Undoubtedly, there is a long term need to link physiology to population dynamics and communities (Lawton, 1991). Yet until then, approaches that are focused on a few species at a time or ignore the environment cannot be able to predict the effects of a changing environment on the biosphere (McGill et al., 2006).

In conclusion, if assemblages' diversity patterns are studied using species-level diversity measures alone, the species adaptive attributes (traits) to the environment are overlooked. Yet, the patterns analysis of those different adaptive attributes (traits distribution and abundance) may bring an important key to both use previous works and explain and predict species diversity patterns, as those adaptive attributes intrinsically tend to vary along different environmental conditions and gradients. The importance of how organisms are classified to be studied along their given patterns has been highlighted, which can be critical to understand the ecological consequences of human impacts affecting species patterns (Roberts and Connell, 2008). Although the macroalgal trait thallus morphology has categorized species in a rather simple manner (only four different categories), each category is linked to other species adaptive attributes or traits (life history, succession and physiology) (Orfanidis et al., 2011). Thus, under the umbrella of the trait thallus morphology, a rough but plausible link between different species having different traits was made with the different studied small-scale environmental gradients. Particularly, within each thallus morphology category, studies may allow to establish certain natural ranges of distribution patterns of species, so that the departure from those ranges may be useful to distinguish those sites affected by human impacts. For example, synergistic human related environmental changes on macroalgae diversity (e.g., high sedimentation loads, nutrient pollution) may characteristically tend to be felt towards increasing dominance of small-term competitors, such as opportunistic bloom-forming species and other filamentous/leafy species, concomitantly with the decreasing dominance of long-term competitors, such as late-successional canopy-forming and other thick species. Hence, the trait-based

approach thallus morphology may be a complementary tool for monitoring programs and environmental impact studies involving macroalgal assemblages, where to detect early stages of environmental changes is of utmost importance. Ultimately, the understanding of community ecology is crucial to deal with many key environmental issues (Simberloff, 2004). If species patterns are a consequence of their geographical range, stochastic events, biotic interactions and stress gradients, their responses may be confounded and thus, to identify generalized patterns may be difficult (Balata et al., 2011). The linkage between ecological functions and the diversity and identities of species remains poorly understood (Micheli and Halpern, 2005), but a trait-focused and environmental-pattern approach may enhance biodiversity understanding and ecological synthesis, being a subject to be explored (McGill et al., 2006).

Chapter IV

Origin here, impact there – The need of integrated management for river basins and coastal areas[4]

Using macroalgae to assess coastal and estuarine environments

Abstract

This study highlights the effect that estuarine polluted waters may have on adjacent coastal waters and the need of an integrated management of the coastal area. Pollution of land-to-sea water plumes varies spatially and temporally, being difficult, costly and time consuming to determine. However, the reduction in water quality of both estuarine and coastal environments and the consequent degradation of its biological communities is at issue. Chlorophyll-a analysis from water and stable nitrogen isotopic analysis (δ^{15}N) from opportunistic macroalgae *Ulva* species were respectively used as proxies to detect phytoplankton proliferation and nitrogen related nutrient fluxes in the water. These analytical techniques were combined with the use of three-dimensional hydrodynamic models, and revealed to constitute reliable early warning instruments, able to identify coastal areas at risk, and supporting an integrated management of coastal and river basin areas. The approach detected synchronized δ^{15}N signal variations along time between estuarine sites (Mondego estuary, Portugal) and nearby adjacent coastal shore sites (NE Atlantic coast). The higher values recorded by macroalgal tissues' δ^{15}N signals, which occurred simultaneously to higher chlorophyll-*a* values, were linked to the anthropogenic contamination of the water, probably related with the Mondego valley land use patterns throughout the year (reflecting the opening of sluices that drain agriculture fields). Modeling scenarios point to a Mondego's influence that is able to reach its adjacent coastal shores in about 7 km from its river mouth. The methodology used here is replicable elsewhere and allowed to track nutrients from the source, inside the estuary, until the final area of impact, where primary producers may use those for growth, and to define vulnerable areas on adjacent coastal zones.

Keywords

Nutrient pollution; Nitrogen isotopes; Estuarine plumes; Biomonitoring; *Ulva*; Coastal waters

IV.1. Introduction

Detecting the origin, fate and distribution of anthropogenic discharges in the sea is of critical importance for management of coastal zones (Orlandi et al., 2014). River basins, although comparatively smaller than open sea, have significant influence on coastal zones, and management of these two areas should not be considered separately (Gowing et al., 2006). In fact, ecosystems do not have rigid boundaries, on the contrary, ecosystems blend into each other and their components can overlap or interact at different scales. Consequently, integrated ecosystem assessments must identify a spatial scale in the context of the issues and problems under consideration (Levin et al., 2009).

Notably, increasing human-derived pollution has been compromising the water quality of *both* coastal and estuarine environments. The increasing nutrient pollution inputs from rivers to the coastal zone (Barile, 2004, Boyer and Howarth, 2008), such as agricultural and untreated sewage outlets, tend to increase the rate of primary production in both estuarine and coastal waters. There is an excessive

proliferation of phytoplankton and/or of fast-growing macroalgae, (e.g., opportunistic macroalgae blooms), which ultimately may degrade seagrass meadows, macroalgae and other benthic communities, altering nitrogen cycling and decreasing water quality (Orlandi et al., 2014; Teichberg et al., 2010). One problem, when trying to identify the spatial footprint of land-derived nutrient plumes at the adjacent coastal area, is linked to monitoring difficulties because of their inherent high-frequency of temporal and spatial variability (Fernandes et al., 2012). To overcome this issue, rather than the occasional measurements made directly in the water, one solution may be the use the of the ratio of stable nitrogen isotopes ($\delta^{15}N$) technique in benthic sessile species such as macroalgae, as they integrate the spatial and temporal variability of the dissolved nitrogen in the water (e.g. Costanzo et al., 2001, 2005; Gartner et al., 2002; Savage and Elmgren, 2004), and allow to identify the source of such elements in the system. The technique is able to detect nitrogen assimilated by macroalgae occurred back in time, from times scales of months (Savage and Elmgren, 2004; Deutsch and Voss, 2006; Thornber et al., 2008; Viana and Bode, 2013). Depending on the objective of the study, different species may be more appropriate than others. Particularly for opportunistic macroalgae, the genera *Ulva* (Chlorophyta) may represent the most advantageous option. *Ulva* species constitute a useful biological model to make geographical comparisons, because even represented by a relatively few taxa, these are broadly widespread in both coastal and estuarine systems. Furthermore, these species have proven to be useful as a proxy for locating anthropogenic sources of nitrogen in disturbed coastal and transitional waters (e.g., Cohen and Fong, 2006; Teichberg et al., 2010; Barr et al., 2013; Orlandi et al., 2014).

On the other hand, when the ecological and the analytical processes to quantify them are known, management can gain from the implementation of simulation tools (e.g., MOHID hydrodynamic model). Ecological models have the ability to merge a lot of information and scientific knowledge, and also the potential to produce hypothetical scenarios without needing to wait for its real occurrence. Thus models can provide relevant information of the system that cannot be achieved by other ways and that information can be used in management tools to support decision-makers.

Located at the Portuguese western coast (NE Atlantic Ocean, Iberian Peninsula), the Mondego estuary has been profusely studied under many aspects, and a considerable number of studies have reported its eutrophication problems and its consequent blooms of *Ulva* species within estuarine boundaries (Martins et al., 1999, 2001; Neto et al, 2008; Baeta et al., 2009; Marques et al., 2003, 2013). However, studies on the influence of the Mondego estuarine discharges on the quality of adjacent coastal waters are almost absent (see Martins et al., 2007).

Therefore, this work aims to contribute for the development of a more efficient management system of the estuary (Mondego) and its adjacent coastal waters. Specifically, this study should provide answers to questions like: i) it is the estuarine upstream water pollution and its seasonal dynamics an important source of the nutrients assimilated by macroalgae inside the estuary and at adjacent coastal areas?; ii) it is possible to relate the dynamics of nutrients discharged from upstream areas in the estuary (e.g., agriculture fields) with the nutrient's cellular content on macroalgae?; and also iii) how adequate is

the use the modeling tools to support stakeholders and policy makers on their decisions and mitigation measures?

IV.2. Material and methods

IV.2.1. Study area

The Mondego River drains a 6670 km^2 basin and supports a population of about 885 thousand inhabitants (2006 data; Pinto et al., 2010). Its estuary, located at the NE Atlantic coast of Portugal (40º08'N, 8º50'W) (Fig. IV.1), is a polyhaline intertidal system with around 1600 ha and 21 km long, influenced by a warm temperate climate. The estuary's terminal part is divided in two arms, north and south, separated by a small alluvial plain (Morraceira Island) of about 6 km long. The north arm is deeper (8–12 m at high tide) and receives most of the freshwater discharged from the river. The south arm is shallower (2–4 m at high tide) and receives the freshwater from a small tributary of the Mondego, the Pranto river. The tidal range varies inside the estuary between 0.35 and 3.3 m, while water residence time goes from one day in winter to five days in summer, at north arm, and three days in winter to nine days in summer, at south arm. The estuary receives agricultural runoff from upstream 15,000 ha of cultivated land (mainly rice fields), it supports a substantial population, industrial activities, saltpans, and aquaculture farms, and is also the place of the commercial and fishing harbors of Figueira da Foz city (Martins et al., 1999, 2001; Neto et al, 2008; Baeta et al., 2009; Kenov et al., 2012; Marques et al., 2013).

Rice fields left resting during winter are drained, plowed and then refilled with water and revolved in mid-Spring (from early April), in order to receive the rice plantation in late April/May. In mid-June fertilizers are spread across the fields. In September, fields are drained to harvest rice, and the nutrient enriched water discharged into the estuary reaches the adjacent coastal areas (http://bordadocampo.com/arroz/cultivo-arroz/; visited in 2015-04-09).
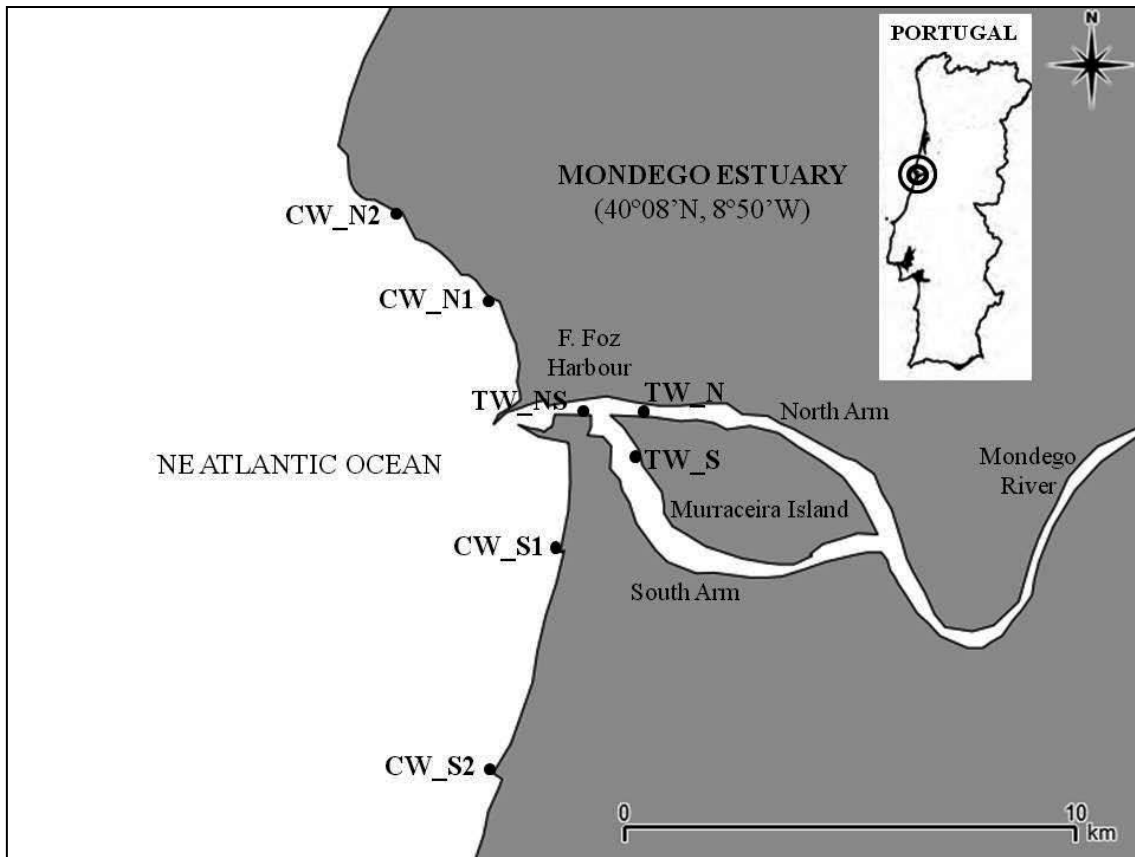
Fig. IV.1. Study area. TW = Transitional waters; CW = Coastal waters; N = Northern sites; S = Southern sites; NS = downstream site, after reconnection of north and south estuarine arms.

At coastal area, sandy beaches and marine soft bottoms constitute mostly the surrounding habitats. Southward, the nearest rocky shores distance at 26 km (Pedrógão) and 43 km (São Pedro de Moel) from the Mondego Estuary entrance and, apart from these natural substrata, other hard structures are constituted by few breakwaters and jetties created to sustain coastal erosion. Facing north through the Figueira da Foz sandy beach and the Buarcos Bay rocky shore, there are several tiers of intertidal rocky platforms, as distant as 2 km, which constitute a natural rocky substratum for macroalgae to attach on. The tidal regime is semidiurnal, with the coastal largest tidal range occurring during spring tides of 3.5–4 m. The coast is exposed to the prevailing northwest (NW) oceanic swell, which can reach values over 5 m in the winter, when most frequent storms occur from WNW (Boaventura et al., 2002).

IV.2.2 Macroalgae and water sampling

Sampling sites were selected to collect green macroalgae growing inside the estuary (transitional waters, TW) and at adjacent coastal waters (CW), where the signal of inland nutrients' (quantified in

tissues) could be different. Seven sites were selected in total: a) at the TW (3), one north arm' site (hereafter TW_N), one south arm' site (hereafter TW_S) and one downstream site located after merging north and south arms (hereafter TW_NS); b) at the CW (4), two sites northwards (CW_N_1 and CW_N_2, respectively at about 2.5 km and 6 km away from river mouth) and two sites southwards (CW_S_1 and CW_S_2, respectively at about 2.5 km and 6 km away from river mouth) (Fig. IV.1). Both CW northern sites comprise Buarcos Bay' rocky shores. Both CW southern sites comprise artificial structures made of a concrete blocks and natural rock mixture, breakwaters built in the sandy shore perpendicularly to coast line.

Along 1-year period, each site was sampled twice a season during spring low tides (total of eight sampling occasions), in November and December 2013 for the autumn (Aut_1 and Aut_2), in February and March 2014 for winter (Win_1 and Win_2), in May and June 2014 for spring (Spr_1 and Spr_2), and in August and September 2014 for summer (Sum_1 and Sum_2). Two different specimens of the genera *Ulva* spp. were collected on the upper-intertidal zone at each sampling site and moment and labelled separately for analysis (i.e., n = 2) of stable nitrogen isotopic ($\delta^{15}N$) in the tissues (as a probe or proxy to detect the nutrient fluxes in the system).

Simultaneously, during the same sampling occasions, sub-surface water samples were collected at each sampling site for Chlorophyll-a analysis (as a proxy to detect phytoplankton proliferation). Biological and water samples were transported to the laboratory in thermal boxes under dark and low temperature conditions.

IV.2.3 Laboratory procedures

IV.2.3.1 Analysis of water samples

Water samples were filtered using a low-pressure vacuum pump and a Whatman GF/C glass–fiber filter. Chlorophyll-a concentration was determined from pigments retained on the filter, after acetone (90%) extraction, and measured in a spectrophotometer (Parsons et al., 1985). To estimate the amount of N discharged from the estuary, water samples collected from the most downstream estuarine site (i.e., TW_NS, Fig. IV.1) were further analyzed following Greenberg et al. (1992) procedures for N-compounds.

IV.2.3.2 Analysis of ratio of stable nitrogen isotopes ($\delta^{15}N$) in macroalgal tissue

Biological samples (specimens of the genera *Ulva,* Chlorophyta) were immediately rinsed with Milli-Q water to remove potential detritus (e.g., sediments, other organisms, salts), being simultaneously identified as blade-forms (e.g., *U. rigida* or *U. lactuca*) or as tubular-forms (e.g., *U. intestinalis* or *U. compressa*, corresponding to the previously genera *Enteromorpha*, Hayden et al., 2003). Species level identification was avoided because of the complicated taxonomy of these genera, in which polymorphism (and misidentification) is common (Malta et al., 1999). Samples were then oven dried (60 ºC, minimum of 48 h) and, once dry, were ground into a homogenous fine powder (using mortar and pestle), weighed and

loaded into tin capsules. The nitrogen isotopic composition of the samples was then determined using a Flash EA 1112 Series elemental analyser coupled on line via Finningan conflo II interface to a Thermo delta V S mass spectrometer. The ratios of stable nitrogen isotopes ($\delta^{15}N$) are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (atmospheric nitrogen); $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where $R = {}^{15}N/{}^{14}N$. Precision in the overall preparation and analysis was 0.2‰.

IV.2.4. Data analysis

The chlorophyll-a data (56 samples) were used as a proxy of primary production of phytoplankton to study the spatial and temporal patterns associated to nutrients variability in the water. Similarly, the spatial and temporal patterns of *Ulva* isotopic signals (112 samples) were analyzed to detect nitrogen nutrient fluxes.

Data of macroalgae isotopic signals were used to construct resemblance matrices with Euclidian distance as similarity index. Differences in the isotopic values were assessed using PRIMER-6 + PERMANOVA software package (Clarke and Gorley, 2006; Anderson et al., 2008) through the permutational multivariate analysis of variance (PERMANOVA) using 9999 permutations under a reduced model; factors considered were: forms of *Ulva* species (fixed, 2 levels, blade- versus tubular-forms); waterbody (fixed, 2 levels, coastal waters versus transitional waters); and season (fixed, 8 levels: two monthly sampling occasions per season, namely Autumn, Winter, Spring and Summer); a significance level was accepted at $p<0.05$. Preliminary analyses were conducted aiming to compare pairs of isotopic signals 1) between estuarine transitional waters (TW) and coastal waters (CW) (regardless sampling time and forms of *Ulva* species) (factor: waterbody); 2) between blade- and tubular-forming *Ulva* species (regardless sampling time and location) (factor: forms of *Ulva* species); 3) between TW and CW within each individual form of *Ulva* (blade and tubular) (regardless time of sampling) (pair-wise tests conducted between factors waterbody and forms of *Ulva* species). Secondly, 4) in order to detect the seasonal dynamics of isotopic signals, as the ultimate influence of estuarine waters in coastal areas (regardless forms of *Ulva* species), those were analyzed between waterbody along seasons (pair-wise tests conducted between factors waterbody and season).

The variation of the nutrient, sourced inside the estuary, assimilated by macroalgae at TW and CW or by different forms of *Ulva* along the year was evidenced by the isotopic content of macroalgal tissues collected on each sampling site. Depending on the aim, the average ratio of isotopic signal was calculated for each water body to estimate the dilution from inside to out side the estuary, or for different *Ulva* forms from each site to compare the nutrient assimilated by each of them.

IV.2.5. Modelling

In order to identify the coastal area influenced by the estuary, the MOHID modeling software was used. It constitutes a modular 3D water modelling system (Miranda, et al. 2000), able to simulate the dynamics of water masses inside (TW) and outside the estuary (CW), at the coastal area. A nested configuration was used to downscale the boundary conditions from the large scale to local scale, i.e. to simulate hydrodynamics on a local scale, based on information provided by large-scale models (Yu et al., 1998; Kenov et al., 2012). The Mondego hydrodynamic model is linked to a large-scale model, the Portuguese Coastal Operational Model (PCOMS; http://forecast.maretec.org/) (Mateus et al., 2012), which provides the boundary conditions (velocity field, temperature, salinity, and water levels) for Mondego application and allows the coastal conditions to have influence on the conditions inside the estuary. The spatial resolution of the Mondego estuary model is variable, between 50 - 400 m, in order to represent in more detail the areas with stronger geomorphologic gradients. Freshwater inflow data used in the present study have been retrieved from the SNIRH database (http://snirh.pt/) for the station called Açude-Ponte Coimbra, located next to the diversion dam Açude-Ponte de Coimbra, with geographic coordinates 40°12' N and 8°26' W (CGS-WGS84). In this study, the Mondego Estuary was represented with a 2-D configuration, justified by the shallowness of the estuary, with an average depth of around 2 m.

The model simulated the dilution of freshwater and the salinity results were used to infer about the influence of nutrients transported out of the estuary into the coastal area. North and south directions were analyzed and the dilution value of 80-85% of freshwater (< 28-30 salinity) were used to determine the extension of adjacent coastal zone potentially influenced by estuarine waters.

During the study period and coinciding with sluices' manipulation time, two low tide situations were selected, April the 5th (1 am) and the 17th (11 am), respectively to illustrate the influence of the estuary water on the north and south adjacent coastal zones. Furthermore, the amount of water discharged during each ebbing tide was simulated (using MOHID) and used to calculate the export of DIN. For that, the water discharged during those ebbing occasions was multiplied by DIN concentration measurements (average value from samples collected in March and May).

IV.3. Results
IV.3.1. Chlorophyll-a

Chlorophyll-a values were always higher at TW sites than at the CW sites, and two higher values can be highlighted at TW, namely those registered in May (Spr_1) and in September (Sum_2) (Fig. IV.2).

Those peaks were coincident in time with the operation of the sluices placed upstream, opened to allow plantation (in April-May) and crop of rice (in September) at the agriculture fields.
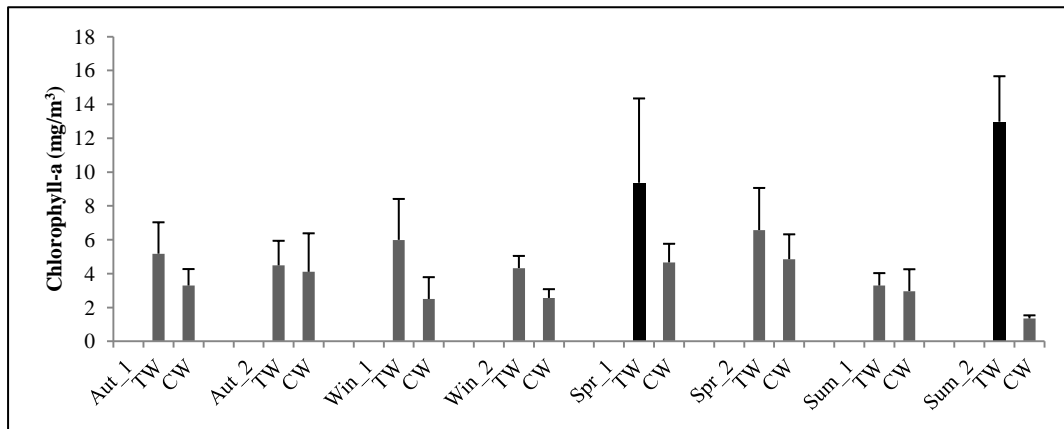

Fig. IV.2. Chlorophyll-a (mg/m^3, mean+SD). Transition waters = TW (n=3); Coastal waters = CW (n=4); Aut = autumn; Win = winter; Spr = Spring; Sum = Summer; 1 = first seasonal sampling event; 2 = second seasonal sampling event.

IV.3.2. Stable isotope analysis

Overall, significant differences were detected when comparing the $\delta^{15}N$ values from *Ulva* species a) between transitional waters (TW) and coastal waters (CW) (i.e. regardless time of sampling), b) between its blade- and tubular-forming *Ulva* species (i.e. regardless time and location of sampling), and c) between TW and CW within each form (blade and tubular) of *Ulva* species (i.e. regardless time of sampling) (Fig. IV.3).

However, no significant differences were detected in the $\delta^{15}N$ signals between blade- and tubular-forming *Ulva* species that were sampled simultaneously at the same time (monthly sampling) and location (site). Still the isotopic signals of blade- and tubular-forms seemed to exhibit a different pattern at the same sampling areas and seasonal sampling occasions, with tubular-forms presenting always higher $\delta^{15}N$ values than those concerning blade-forms (Fig. IV.4).

Furthermore, a spatial and temporal pattern can be identified between months sampled sequentially, where $\delta^{15}N$ mean values increased or decreased from the previous month simultaneously at TW and CW. For each sampled occasion, TW $\delta^{15}N$ mean values were always higher than those recorded at CW and, from month to month, as the TW $\delta^{15}N$ values increase or decrease, the CW $\delta^{15}N$ values seemed to follow the same tendency. This was more evident when $\delta^{15}N$ signals of blade-and tubular-forming *Ulva* species were pooled and analyzed together (Fig. IV.5).
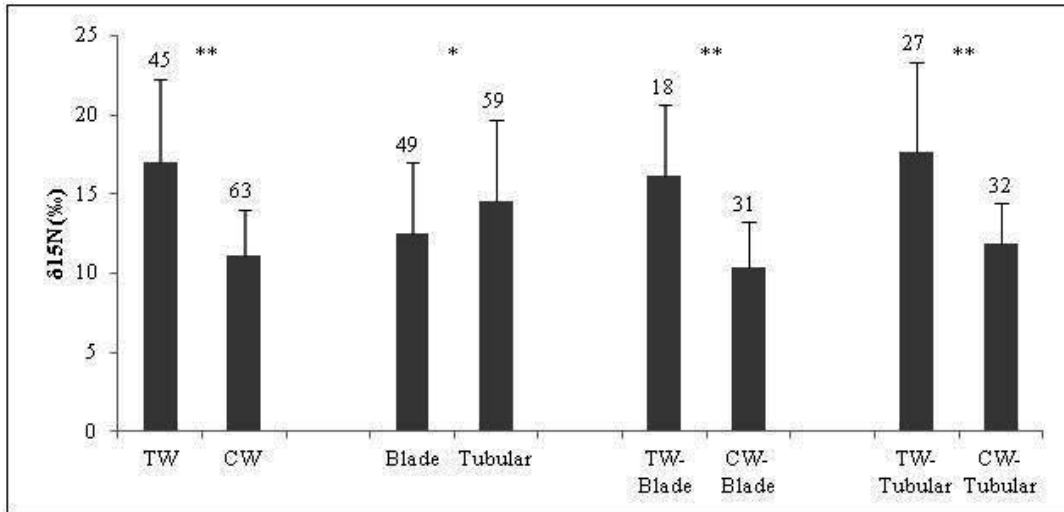
Fig. IV.3. Ratio of stable nitrogen isotopes ($\delta^{15}N$, mean+ SD ‰). TW = transitional waters; CW = coastal waters; Blade = blade-forming *Ulva*; Tubular = tubular-forming *Ulva*. Numbers above bars = number of samples (n); PERMANOVA, * p = 0.0336, ** p = 0.0001.
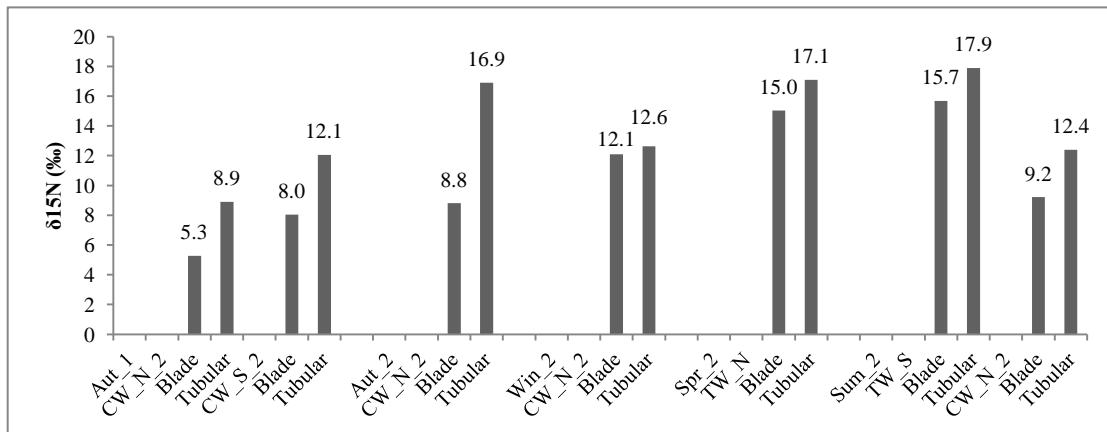

Fig. IV.4. Ratio of stable nitrogen isotopes ($\delta^{15}N$ ‰) between blade- and tubular-forming *Ulva* species sampled simultaneously at the same time (monthly) and location (site) (p> 0.05). Aut = autumn; Win = winter; Spr = spring; Sum = summer; 1 = first seasonal sampling event; 2 = second seasonal sampling event; CW = Coastal Water; TW = Transitional Water; N = North; S = South.
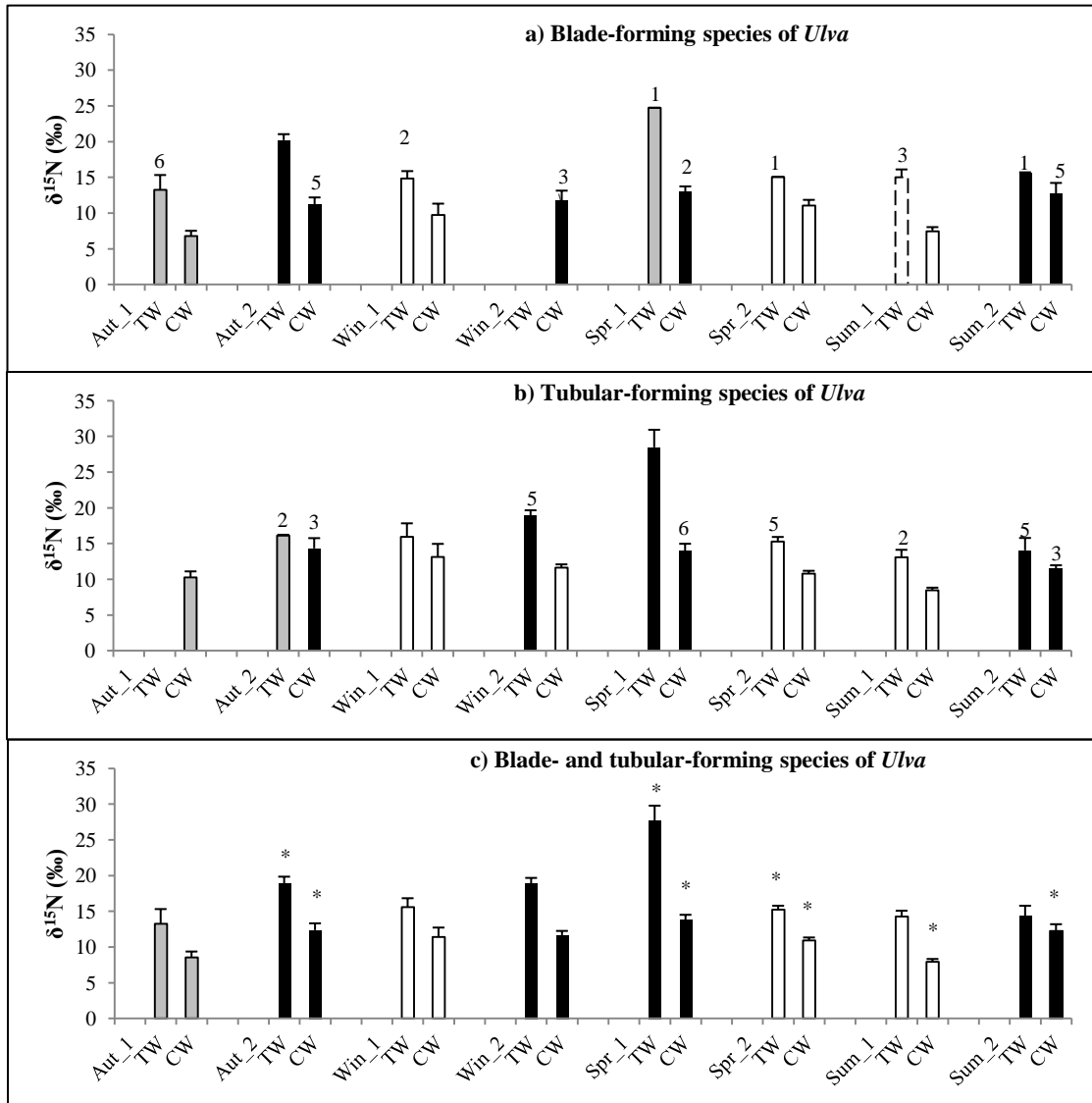
Fig. IV.5. Ratio of stable nitrogen isotopes ($\delta^{15}N$, mean+SE ‰) from blade- (a), tubular- (b) and both blade and tubular-forms of *Ulva* species (c). TW = transitional waters; CW = Coastal Waters; Aut = autumn; Win = winter; Spr = spring; Sum = summer; 1 = first seasonal sampling event; 2 = second seasonal sampling event. a) and b): number above bars = number of samples (n) when different from n=4. c): * = significant differences from the previous month (p<0.05). Black columns = mean values increased from the previous month; White columns = mean values decreased from the previous month, Dashed columns = mean value is equal to the previous month; Grey columns = lack of data for comparisons.

For CW and TW (including sites and using both blade and tubular-forms), the mean values of $\delta^{15}N$ signals calculated for each sampling event allowed estimating a high correlation (r > 0.7) between nutrient contents presented by macroalgae at the estuary (TW) and on its adjacent areas (CW). Overall,

δ15N signals form macroalgae collected at CW were always lower (about 0.66 ± 0.11) than the δ15N signals measured from macroalgae collected at TW.

IV.3.3. Mondego estuary model

The influence of the Mondego estuary on the adjacent coastal areas can be reached through the analyses of the salinity maps obtained by the simulations for April 2014 of the Mondego estuary model, which occurred previously to the time when the highest values of δ15N were recorded (in Spr_1, i.e. May 2014) (Fig IV.6).
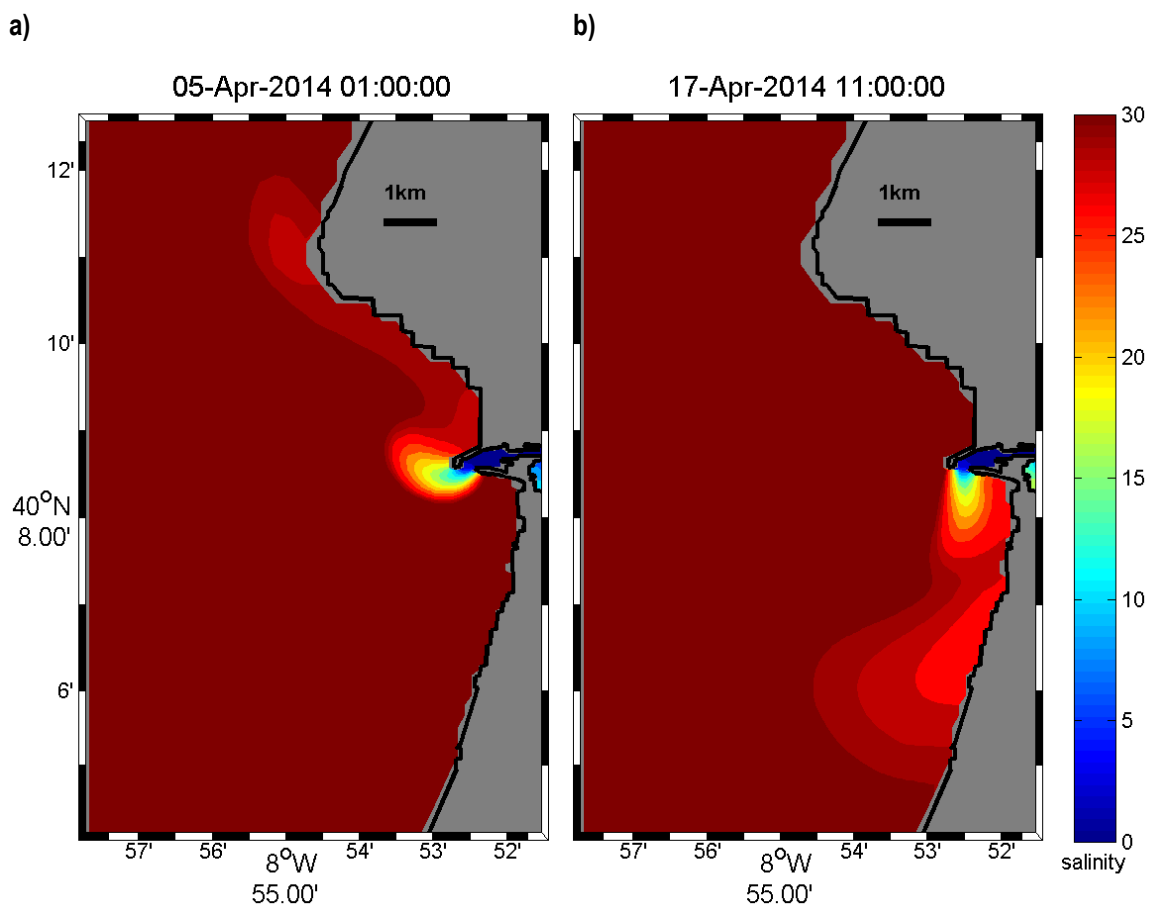
a)   b)

Fig. IV.6. Mondego estuary model (MOHID model) maps of different salinity ranges illustrating Mondego estuary plumes (from April) reaching (a) northern and (b) southern adjacent coastal areas.

The Mondego estuary modeling scenarios show that the Mondego's influence may be significant at least at about 7 km north and south from the river mouth, which was responsible for discharging around

2×10^7 m^3 of water (2.1×10^7 m^3 in 4-5 April and 1.8×10^7 m^3 in 17 April) during the identified ebbing events. During these ebbing tides, around 7388 kg of N were discharged from the estuary into the adjacent coastal areas.

IV.4. Discussion

Water pollution is variable in space and time, but is now widely accepted that pollution (e.g., nutrient loading) flowing from rivers or from urban areas near the margins into estuaries ends almost invariably in coastal zones (Fernandes et al., 2012). Mostly as pulses, these estuarine nutrient outflows were identified as important elements in the ecological balance of coastal areas. Although pollution issues have in the late decades retained scientists' attention, its quantification is still difficult, costly and time consuming (Orlandi et al., 2014). Emerging from the implementation of the Water Framework Directive (WFD, 2000/60/EC) in Europe, the influence of CW and TW on each other earned importance, and managing nutrients (or resources and services) on those systems may reveal incomparably difficult without an Ecosystem Based Approach (Leslie and McLeod, 2007). Combining the information provided from an integrated management of coastal areas is thus of utmost importance on the elaboration of comprehensive river basin management plans, where mitigation measures may really have positive feedback on coastal ecosystems recovery.

For the Mondego Estuary, is known that nutrients affecting the water quality inside the system are mostly originated on the Lower Mondego agriculture fields, located upstream the estuary (Martins et al., 2001; Marques et al.2003, Neto et al., 2008). But its catchment area (6670 km^2), featured by highly diverse characteristics in terms of hydrology, topography and land use (Pinto et al., 2010), is also responsible for other significant pressures affecting the system (e.g., water abstraction and damming, sediment loads, channelization, reduction of riparian corridors, invasive species, etc.) (ARH, 2012; Teixeira et al., 2014).

In this study, it was possible not only to trace down nutrient's path, from upstream agriculture pulses source until its assimilation by estuarine and adjacent coastal primary producers, but also to determine the influence of the estuary on the adjacent coastal area. Environmental parameters, such as Chl *a*, were measured on water samples and isotopic ratios were quantified on macroalgae tissues. The influence on the coastal area was inferred by using model simulation (MOHID model).

The sluices management, specifically the opening occurred in April and September to drain the water from agriculture fields and to allow rice seedling and crop, consequently released nutrient enriched waters into the estuary. Synchronously, high chlorophyll-a values were quantified in water samples collected in the estuary (Fig. IV.2), especially during spring (Spr_1; Fig. IV.2), when primary producers were able to use more efficiently the available dissolved nutrient for biomass production. On the other hand, to show the linkage between river basins and adjacent coastal waters, where nutrients released

inside the estuary may have effects on organisms located outside the estuary, green macroalgae were used as sentinels for registering such possibility. Stable isotopic ratios were analyzed on *Ulva* spp. tissues sampled inside the estuary and at adjacent coastal rocky areas. The use of this technique enabled the quantification, across time and space, of nitrogen related nutrients (dissolved inorganic nitrogen, DIN) that flow from estuarine to coastal waters, rather than typical measurements done directly in the water (Costanzo et al., 2001, 2005). The approach has proven to be advantageous when applied in species of *Ulva* as a proxy or probe organisms because: 1) these benthic sessile species are able to integrate the DIN fluxes in a highly responsive way, and 2) they are commonly widespread in marine and estuarine ecosystems (Cohen and Fong, 2006; Teichberg et al., 2010; Gartner et al., 2002; Dailer et al., 2010, 2012; Barr et al., 2013, Orlandi et al., 2014).

The opportunistic fast-growing *Ulva* species have high N demand and uptake rates, allowing a fast metabolic response to environmental changes, and thus their $\delta^{15}N$ signal changes may occur in a fast and pronounced way (Raimonet et al., 2013). Nevertheless, the interpretation of δ15N values requires a good knowledge of local and regional factors affecting the isotopic signatures (Viana and Bode, 2013). Critically, the knowledge of the regional δ15N reference values for macroalgae may help to provide a more objective interpretation of the results obtained in local studies. Because of the large natural variation in δ15N values of macroalgae, it appears that the reference range must be established for each species in a particular region (Carballeira et al., 2013). In this sense, the present results showed that different species of the same genera *Ulva* tend to exhibit somehow a different 'integrative' pattern in terms δ15N signals. For simplicity, here we have considered blade-forming (*U. rigida* or *U. lactuca*) and tubular-forming (*U. intestinalis* or *U. compressa*). Although tubular-forming species showed higher δ15N values than the blade-forming species (Figs. IV.3 and IV.4), and significant differences were overall detected between the two forms (regardless time and location of sampling), no significant differences in δ15N values were detected between the tubular-forms and blade-forming *Ulva* species sampled at the same time and location (Fig. IV:4). In an opposite manner Deutsch and Voss (2006) recorded, at the same sampling sites, large differences in the δ15N values between those two forms of *Ulva* species, i.e., higher $\delta^{15}N$ signals in blade-forming than in tubular-forming species. Thus, further attention should exist in future monitoring schemes, but it is here highlighted that the simultaneous use of both blade- and tubular-forming *Ulva* species can be helpful, reducing the risk of having one or the other form temporarily absent from the monitoring site.

Different approaches in the analysis of the $\delta^{15}N$ signals of the *Ulva* species, either separately by its tubular-forms or its blade-forms, or by combining its isotopic signals values from both forms, followed apparently the same synchronized pattern along time. First of all, it should be mentioned that macroalgae tissues' δ15N signals, similarly to chlorophyll *a* results, recorded high values in May and September (Spr_1 and Sum_2; Fig. IV.5), at the same time when the sluices were opened. Secondly, the effect of estuarine plume and its nitrogen pulse was captured at adjacent coastal areas, with significant differences in δ15N values detected in general between TW and CW within each *Ulva* form (i.e. regardless time of sampling)

(Fig. IV.3). TW $\delta^{15}N$ mean values were always higher than those recorded at the CW and, from month to month, as the TW $\delta^{15}N$ values increased or decreased, the CW $\delta^{15}N$ values seem to follow the same trend in a systematic manner. In the absence of reference values, $\delta^{15}N$ values alone may not provide unequivocal evidence about the amount of anthropogenic nitrogen affecting the coastal zone (Lamb et al., 2012), but this was particularly evident when pooling the $\delta^{15}N$ signals of both blade-and tubular-forms of *Ulva* species (Fig. IV.5), where around 60% of the signal identified on tissues from macroalgae collected in TW could still be captured on those collected at the CW, adjacent to the estuary. It was evident that on a month timescale, the macroalgae $\delta^{15}N$ signals reflected the nitrogen available at TW and CW and assimilated by the exposed organisms. Nutrients flowing through the estuary to coastal areas were tracked and evidenced their presence on local food webs (assimilation by primary producers). The part of assimilated nutrients with origin inside the estuary decreased in concentration proportionally to the distance to source.

The next point was to estimate the extension of estuarine influence over the coastal area. For that work an existing modeling software (MOHID Mondego estuary model) was used and updated to give desirable detail for the area. The model simulated the dilution of freshwater into marine water, and the coastal area influenced by estuarine nutrients was defined as the area on the graph where salinity dropped down to 28. The modeling scenarios showed that the Mondego's influence may be significant at least at about 7 km north and south from the river mouth (Fig. IV.6). The simulations showed an average discharge of around 2×10^7 m^3 of water during the identified ebbing tide events, which corresponds to a discharge of 7.4 tones of DIN (in average) from the estuary into the adjacent coastal areas. Pereira and Mesquita (2004) have already identified the possible influence of estuarine nutrients on specific macroalgae growing on the Buarcos Bay, where the variability of its colloid contents were related with the variability of nutrients concentration on water discharged from the Mondego estuary. In fact, the estuary may discharge annually something like 8.5×10^9 m^3 of water, 120 tones of nitrogen and 15 tones of phosphorus into the adjacent area (Flindt et al., 1997). Neto et al. (2008) also simulated the nutrients mass balance along the year for the south arm of the estuary. There, an export of 7.9 tones was estimated for entire April, a similar value of the calculated for a single ebbing tide in the present study for the entire estuary. Even knowing that part of the water discharged during the ebb tide returns into the estuary in the following flood, these figures clearly show the importance of the north arm on estuarine nutrients mass balance. River flow varies along the year, as well as nutrients export of the estuary. If the concentration of dissolved nutrient is high in the water discharged during the growing season (March to September), opportunistic species present at the adjacent coastal area may benefit from the enrichment, being then essential to have lower concentration of nutrients in discharged water.

Bad practices inside the river basin may drive to serious degradation of coastal rocky shore communities, difficult to recover and representing, in some situations, an interruption on the coastal continuum. From its location, Buarcos Bay may constitute an important spot ensuring the coastal connectivity. Actually, along the coastline, similar areas of natural rocky shore can only be found at about

100 km northwards (Aguda rocky shore site), and at 26 km (Pedrógão), 43 km (São Pedro de Moel) and 70 km southwards (São Martinho do Porto), dominated in between by sandy beaches. Buarcos Bay, with a record of 177 different species (MACOI website; Pereira et al., 2014), constitutes also an important area on the preservation of macroalgal biodiversity. On a biogeographic context, Buarcos Bay may represent an important role for the biological connectivity and ecological resilience (e.g. Bernhardt and Leslie, 2013), not only for macroalgae but also for the associated communities of these seashore organisms and its populations along the Iberian Peninsula.

More than the local importance of excessive nutrients exported from the estuary on the water quality from adjacent coast, this study highlights the importance of considering the effect on adjacent coastal areas and on the need of having an integrated management for TW and CW. These areas are not independent from each other and CW may present degradation of biological communities originated on anthropogenic pollution released through the estuaries. In a long run, problem analysis may depend upon recognizing and evaluating interactions (and trade-offs) among agriculture, aquaculture and fisheries in these environments, aiming a more sustainable use of resources and where the footprint of anthropogenic inland activities may not have such negative impacts over coastal environments. While negative impacts of change arise, sustainable development goals need to identify possibilities for co-existence and win-win scenarios for future resources use (Gowing et al., 2006).

The methodology used here is replicable elsewhere and aimed to provide a contribution for the integrated management of estuarine and coastal areas. It allowed to track nutrients from the source, inside the estuary, until the final area of impact, where primary producers are able to experience enhanced growth. Defining vulnerable areas on adjacent coastal zones, where the proliferation of opportunistic organisms (e.g., macroalgae) may have negative impact on the ecology and on other services provided by coastal systems, constitutes a valuable support for local and regional administrations.

Concluding remarks

Coastal and estuarine ecosystems are some of the most heavily used and threatened natural systems worldwide. Anthropogenic pressures on such systems include global warming, erosion, sea level rise, habitat change, invasive species, eutrophication, chemical pollution and overexploitation (Halpern et al., 2008). Facing such awareness justifies a scientific quest: the development of ecological indicators to assess ecosystems health (see Rombouts et al., 20013 for a review).

Yet, all environments are heterogeneous systems, a fact that complicate the efforts to understand the genesis, maintenance and diversity patterns of ecological systems. Particularly, the spatial structure of organisms living in coastal and estuarine environments can vary as a function of a panoply of factors, including species interactions, dispersal, physical disturbance, and environmental stress (Menge et.al., 2005 and references therein). As far as macroalgae is a concern, much scientific knowledge has been produced, including environmental, biogeographical, ecological or ecophysiological issues. Still the complete knowledge of macroalgae distribution patterns (from single species to populations to entire communities) and the prevailing environmental factors driving macroalgae species assemblages to arrange in a particular way on a given shore or in a broader geographical scale, may continue on the biologists' agendas as research challenges. Moreover, knowledge of these dynamic patterns grows more uncertain when considering that environmental factors do change not only due to complex and interrelated processes that occur naturally, but also duo to anthropogenic impact changes (Gaspar et al., 2015).

To understand coastal and estuarine ecosystems complexity is therefore essential when wanting to identify the impact of anthropogenic activities, which can impose changes in community structure and functioning (Martins et al., 2016 and references therein). The successful management and protection of biodiversity, the assessment of anthropogenic impacts and the restoration of altered ecosystems rely largely on quantifying assemblages variability patterns across a range of relevant scales, and on understanding the processes and factors that structure these assemblages (Underwood et al., 2000; Benedetti-Cecchi, L., 2001, Díaz-Tapia, et al 2013 and references therein). Thus, to understand and quantify the magnitude of the natural variability of assemblages is thus imperative for implementing suitable monitoring programs and environmental impact studies (Veiga et al., 2012).

The better the control, benchmark, ecological reference or starting point descriptions to compare results, the better our ability to detect impact changes, that is, the departure from assemblages natural variability ranges that might indicate ecological degradation due to human impacts. Macroalgae communities can reflect the ecological degradation and their study have been useful to quantify the level of integrity of coastal and estuarine ecosystems. Several lines of investigation can be taken into account, and combined in an integrative way, in order to search for such marine macroalgae definitions (Chapter I).

Regardless of the degree of 'naturalness' or 'pristineness' implied by a specific definition of a benchmark, the accuracy and precision when assessing ecological quality conditions are dependent on the degree to which those benchmarks can be quantified and predicted (Hawkins et al. 2010). In order to recognize change, it should be thus highlighted the importance of generating biodiversity broader databases of rocky intertidal assemblages to be used in large-scale comparisons. The value and interpretation of other datasets are frequently predicated on the existence of such baseline datasets. From the ability to recognize change, to establish cost-effective biomonitoring at a range of scales, or to use biodiversity surrogates, all require that there is baseline knowledge of the biota (Cruz-Motta et al., 2010; Neill and Nelson, 2016).

Representativeness and time consumption are central issues in monitoring designs (Ballesteros et al., 2007). Species-level approaches to detect change are costly because are labour-intensive and need high taxonomic expertise. Actually, species-level changes may have little overriding effect on whole assemblages (Littler & Littler 1980, Steneck & Dethier 1994). On the other hand, although morphological/functional group hypothesis may provide broad insight into community structure (Roberts and Connell, 2008), such approaches may be pointed out to lose sensitivity in detecting change along environmental gradients, compared to species-level approaches (Phillips et al. 1997, Padilla and Allen 2000). However, it may be recognised that, depending on the aim, morphological/functional groups may be useful as complementary biodiversity measures, rather than actually act as a surrogate measures of (species-level) biodiversity. After all, in order to use such surrogates, taxonomic expertise is needed as well. Still, as each macroalgal species change in its worldwide distribution, each one can be easily assign into such reduced grouping categories, and this may allow comparisons among regions having different sets of species, which in turn may improve ecological synthesis. Grouping species such as by a particular function can be very useful and often necessary for many ecosystem-level questions and modeling, especially when there are too many species in a system to consider them all individually (Padilla and Allen 2000).

To quantify the patterns of variation under different biodiversity attributes and measures may provide complementary information, which might be crucial to understand the effects of both natural processes and anthropogenic impacts on natural assemblages (Bevilacqua et al., 2012). As some results of this work showed (Chapters 2 and 3), both morphological-functional groups and trait-based "thallus morphology" approaches were useful to generalize vertical (intertidal zonation) and horizontal (latitudinal) patterns of macroalgal assemblages, where some groups emerge at the expense of others, in a simultaneous response to both large- and small-scale factors and processes. At both large- and small-scale environmental variability, both surrogate measures (functional groups and thallus morphologies) had the ability to be sensitive to the assemblages (species-level) spatial variability considered and, as complementary measures, may be helpful when studying the large group of species named macroalgae.

Particularly, trait-based approaches (such as considering different thallus morphologies) may improve biodiversity understanding (Ackerly and Cornwell, 2007; Vandewalle et al., 2010) because traits

may reflect species ecological roles, namely how species interact or are adapted to their physical and chemical environment and with other species, and species roles in maintaining and regulating ecosystem processes (Díaz and Cabido, 2001). For example, synergistic human related environmental changes on macroalgae diversity (e.g., high sedimentation loads, nutrient pollution) may characteristically tend to be felt towards increasing dominance of small-term competitors, such as opportunistic filamentous/leafy (bloom-forming) species and fleshy species, concomitantly with the decreasing dominance of long-term competitors, such as late-successional thick (canopy-forming) species and calcareous/crusts species. Hence, the trait-based approach thallus morphology may be a complementary tool for monitoring programs and environmental impact studies involving macroalgal assemblages, where to detect early stages of environmental changes is of utmost importance.

Ultimately, the understanding of community ecology is crucial to deal with many key environmental issues (Simberloff, 2004). If species patterns are a consequence of their geographical range, stochastic events, biotic interactions and stress gradients, their responses may be confounded and thus, to identify generalized patterns may be difficult (Balata et al., 2011). The linkage between ecological functions and the diversity and identities of species remains poorly understood (Micheli and Halpern, 2005), but a trait-focused and environmental-pattern approach may enhance biodiversity understanding and ecological synthesis, being a subject to be explored (McGill et al., 2006). While the loss of species can alter entire systems, the detection of change through the use of broader taxonomic classifications such as morphological-functional groups hypothesis can be informative or potentially more predictive because of the fundamental nature of change brought by human-induced disturbance (Roberts and Conell, 2008 and references therein). For example, focusing on functional rather than species-level diversity, the loss of a whole functional group may seem unlikely due to impacts such as invasive species, where one or a few members of that group may disappear (Arenas et al., 2006). Nevertheless, impacts such as reductions in water quality are causing the widespread loss of canopy-forming (late-sucessional) species, while turf-forming (early dominants) species expand in cover (Benedetti-Cecchi et al., 2001; Gorgula and Connell 2004).

Critically, the need to better understand of how ecological functions are linked to the diversity and identities of species, i.e., the relationships between species and functional diversity, and between functional diversity and ecosystem functioning, seems unavoidable for the management and conservation of marine ecosystems (Micheli and Halpern, 2005). After all, the different expression of species attributes under different regimes of disturbance (such as wave-exposure and sand-deposition) and intertidal zonation stress gradients produces much of the spatial and temporal heterogeneity one observes in natural assemblages (Sousa, 1984). As species differ in their adaptations to particular environments, the outcome of interactions depends on the species identity (Viejo et al., 2008), which, in the end, produce their realized niches or patterns (Hutchinson, 1957). Undoubtedly, there is a long term need to link physiology to population dynamics and communities (Lawton, 1991). Yet, until then, approaches that are

focused on a few species at a time or ignore the environment cannot be able to predict the effects of a changing environment on the biosphere (McGill et al., 2006).

In order to understand biotic patterns and their changes in nature there is an obvious need for high-quality seamless measurements of such patterns. The vast majority of studies have been conducted on limited spatial scales even though the studies covered larger areas than the grain size i.e. the size of sampling units still remained small and vast areas between grains are left unstudied. However, due to a wide range of spatial and complexity scales, the grain size profoundly affects how we see the world around us. Hence flagging this issue as one of the critical problems in ecology (Kotta et al., 2013 and references therein). Nevertheless, contemporary tools such as geographical information systems (GIS, e.g. Ramos et al., 2015), and remote sensing (e.g. Kotta et al., 2013) may enhance the efficacy in fulfilling such vast areas between grains that are still left to be studied. Furthermore, the ability to describe sampling units can be also improved using contemporary tools coupled with high resolution photography. For example, some of this study methodological approaches (Chapter II and III) took advantage from coupling replicates photography with GIS tools (Quantum GIS), where assemblages structural patterns were able to be studied at a very fine area scale level, allowing a spatial-explicit, low-cost, non-destructive, fast and reliable fieldwork sampling approach.

Moreover, contemporary analytical techniques such as stable isotopic analysis on macroalgae may be particularly useful as well, allowing to track impacts from the source, such as nutrients pollution from inland estuarine areas, until the final area of impact, where macroalgae, as primary producers, may use those for growth, and thus, such approaches can be helpful to define vulnerable areas on the adjacent coastal zones (Chapter IV). These analytical techniques, combined with the use of three-dimensional hydrodynamic models, may constitute reliable early warning instruments, able to identify coastal areas at risk, and supporting an integrated management of coastal and river basin areas. Defining vulnerable areas on adjacent coastal zones, where the proliferation of opportunistic bloom-forming macroalgal species may have negative impact on the ecology and on other services provided by coastal systems, constitutes a valuable support for local and regional administrations.

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Appendix

Appendix II.1. Macroalgal assemblages patterns along North to South Portuguese latitudinal gradient (study sites: Viana do Castelo, Buarcos Bay, Ericeira, Queimado and Arrifes) and respective intertidal zones: (a) Upper-intertidal; (b) Mid-intertidal; (c) Lower-intertidal. Each taxa average coverage (%) is scored according to DAFOR (adapted) and Domin-Krajina coverage ranges (n=9 per intertidal zone and per site).

a) Upper-intertidal

DAFOR (Adapted)	Domin-Krajina Coverage ranges	Domin-Krajina score	Viana do Castelo	Buarcos Bay	Ericeira	Queimado	Arrifes
(Absolute Dominant)	100%	10					
Dominant	>75%	9					
Abundant	>50%	8					
(Highly Frequent)	>33%	7					
Frequent	>25%	6	*Fucus spiralis, Ascophyllum nodosum*	*Ulva* spp.		*Fucus spiralis*	*Corallina* spp.
Occasional	>10%	5	*Ulva* spp.	*Mastocarpus stellatus*		*Ralfsia verrucosa*	*Codium adhaerens, Ulva clathrata, Ceramium* spp., Other Ectocarpales/*Sphacelaria* spp.
Rare	>5%	4	*Pelvetia canaliculata*		*Fucus spiralis, Corallina* spp., *Ulva* spp.	*Codium adhaerens, Corallina* spp.	*Gelidium pusillum, Ralfsia verrucosa, Ulva* spp.
(Rare scattered)	>1%	3	*Ulva clathrata, Vertebrata lanosa, Mastocarpus stellatus*	*Osmundea pinnatifida, Gelidium pulchellum, Ceramium* spp., *Corallina* spp., *Chondracanthus acicularis, Chondrus crispus*	*Petrocelis cruenta* (*Mastocarpus stellatus*), *Caulacanthus ustulatus, Osmundea hybrida, Asparagopsis armata*	*Lithophyllum byssoides, Osmundea hybrida, Falkenbergia rufolanosa* (*Asparagopsis armata*), *Cladostephus spongiosus, Cystoseira humilis, Halopteris scoparia/filicina*	*Dictyota dichotoma, Padina pavonica, Polysiphonia* spp. (or other Rhodomelaceae)
(Very scattered)	>0.1%	2	*Caulacanthus ustulatus, Ceramium* spp.	*Caulacanthus ustulatus, Polysiphonia* spp. (or other Rhodomelaceae), *Porphyra* spp./*Pyropia* spp.	*Colpomenia* spp./*Leathesia marina, Ceramium* spp., *Chaetomorpha* spp., Other Ectocarpales/*Sphacelaria* spp., *Polysiphonia* spp. (or other Rhodomelaceae), *Rhodothamniella* spp./Acrochaetiales	*Gelidium pusillum, Petrospongium berkeleyi, Ulva clathrata, Colpomenia* spp./*Leathesia marina,* Other Ectocarpales/*Sphacelaria* spp., *Caulacanthus ustulatus, Ceramium* spp., *Padina pavonica, Asparagopsis armata, Dictyota dichotoma, Hypoglossum hypoglossoides* (or other Delesseriaceae), *Peyssonnelia* spp., *Ulva* spp.	*Cladostephus spongiosus*
(Seldom)	>0.01%	1		*Callithamnion tetricum, Cladophora* spp., *Codium* spp., *Lomentaria articulata*	*Gelidium pusillum, Scytosiphon* spp.	*Rivularia bullata, Hildenbrandia* spp., *Lithophyllum incrustans, Scytosiphon* spp.	*Halopteris scoparia/filicina, Hypnea musciformis, Hypoglossum hypoglossoides* (or other Delesseriaceae), *Rivularia bullata, Cladophora* spp.
(Solitary)	≤0.01%	0		Other Ceramiales	Other Ceramiales		
Taxa richness			9	16	17	27	17

Appendix II.1 (continued)

b) Mid-intertidal

DAFOR (Adapted)	Domin-Krajina Coverage ranges	Domin-Krajina score	Viana do Castelo	Buarcos Bay	Ericeira	Queimado	Arrifes
(Absolute Dominant) Dominant	100%	10					
	>75%	9				*Falkenbergia rufolanosa (Asparagopsis armata)*	
Abundant	>50%	8					
(Highly Frequent) Frequent	>33% >25%	7 6	*Ulva spp.*	*Ulva spp., Chondrus crispus*		*Osmundea hybrida*	*Dictyota dichotoma*
Occasional	>10%	5	*Bifurcaria bifurcata, Chondracanthus acicularis*	*Osmundea pinnatifida, Chondracanthus acicularis*	*Codium* spp., *Dictyota dichotoma, Asparagopsis armata, Bifurcaria bifurcata*	*Dictyota dichotoma, Corallina* spp., *Cystoseira tamariscifolia*	*Halopteris scoparia/filicina, Corallina* spp., *Cystoseira tamariscifolia, Ceramium* spp.
Rare	>5%	4	*Chondracanthus teedei, Halopteris scoparia/filicina, Ceramium* spp., *Osmundea pinnatifida, Sargassum muticum*	*Corallina* spp., *Callithamnion tetricum, Boergeseniella* spp., *Codium* spp.	*Ulva* spp., *Ceramium* spp., *Corallina* spp.	*Halopteris scoparia/filicina, Codium adhaerens, Ceramium* spp.	*Sargassum vulgare, Ulva clathrata, Cladostephus spongiosus*
(Rare scattered)	>1%	3	*Corallina* spp., *Ulva clathrata, Dictyota dichotoma, Chondria coerulescens, Cryptopleura ramosa, Ahnfeltiopsis devoniensis, Codium* spp., *Petrocelis cruenta (Mastocarpus stellatus) Chondrus crispus, Cystoseira tamariscifolia, Cladostephus spongiosus, Laminaria ochroleuca*	*Chondracanthus teedei, Pterosiphonia complanata, Gigartina pistillata, Lomentaria articulata, Gelidium pulchellum, Ahnfeltiopsis devoniensis, Dictyota dichotoma, Chondria coerulescens, Plocamium cartilagineum, Halopteris scoparia/filicina*	*Cystoseira tamariscifolia, Plocamium cartilagineum, Cladophora* spp., *Lithophyllum incrustans, Polysiphonia* spp. (or other Rhodomelaceae), *Colpomenia* spp./*Leathesia marina, Boergeseniella* spp., *Halopteris scoparia/filicina,* Other Ectocarpales/Sphacelaria spp.	*Asparagopsis armata, Cladostephus spongiosus, Lithophyllum incrustans, Colpomenia* spp./*Leathesia marina, Ulva* spp.	*Ulva* spp., *Hypnea musciformis, Pterocladiella capillacea, Plocamium cartilagineum, Lithophyllum incrustans, Padina pavonica, Colpomenia* spp./*Leathesia marina, Pterosiphonia ardreana/pennata, Halopithys incurva*
(Very scattered)	>0.1%	2	*Scinaia furcellata, Gigartina pistillata, Gastroclonium ovatum, Pterosiphonia complanata, Gelidium pulchellum, Mastocarpus stellatus, Cladophora* spp., *Desmarestia ligulata*	*Polysiphonia* spp. (or other Rhodomelaceae), *Ceramium* spp., *Cryptopleura ramosa, Gymnogongrus crenulatus, Lithophyllum incrustans, Grateloupia turuturu, Callblepharis jubata, Gastroclonium ovatum, Cladophora* spp., *Nitophyllum punctatum, Halurus equisetifolius, Ophidocladus simpliciusculus, Callophyllis laciniata, Dictyopteris polypodioides, Cladostephus spongiosus, Gracilaria multipartita, Saccorhiza polyschides*	*Chondracanthus teedei, Osmundea hybrida, Cladostephus spongiosus, Pterosiphonia complanata, Scytosiphon* spp., *Caulacanthus ustulatus*	*Sargassum flavifolium, Padina pavonica, Codium* spp., *Ralfsia verrucosa, Ulva clathrata, Chondracanthus acicularis, Chondria coerulescens, Liagora viscida, Nitophyllum punctatum*	*Polysiphonia* spp. (or other Rhodomelaceae), *Champia parvula, Hildenbrandia* spp., Other Ectocarpales/Sphacelaria spp., *Hypoglossum hypoglossoides* (or other Delesseriaceae), *Dictyopteris polypodioides*
(Seldom)	>0.01%	1	*Callithamnion tetricum, Caulacanthus ustulatus, Colpomenia* spp./*Leathesia marina, Lomentaria articulata, Plocamium cartilagineum, Polysiphonia* spp. (or other Rhodomelaceae), *Porphyra* spp./*Pyropia* spp.	Other Ceramiales, *Hypoglossum hypoglossoides* (or other Delesseriaceae), *Gracilaria gracilis,* Other Ectocarpales/Sphacelaria spp., *Scinaia furcellata*	*Falkenbergia rufolanosa (Asparagopsis armata), Champia parvula, Hypoglossum hypoglossoides* (or other Delesseriaceae), *Peyssonnelia* spp.	*Scytosiphon* spp., *Peyssonnelia* spp., *Champia parvula, Gelidium pusillum, Gigartina pistillata, Ophidocladus simpliciusculus, Plocamium cartilagineum*	*Ralfsia verrucosa, Gastroclonium ovatum, Acrosorium ciliolatum, Amphiroa rigida, Cladophora* spp., *Osmundea hybrida,* Other Ceramiales
(Solitary)	≤0.01%	0		*Acrosorium ciliolatum, Hildenbrandia* spp.	*Cryptopleura ramosa, Jania* spp.		
Taxa richness			37	42	28	29	30

Appendix II.1 (continued)

c) Lower-intertidal

DAFOR (Adapted)	Domin-Krajina Coverage ranges	Domin-Krajina score	Viana do Castelo	Buarcos Bay	Ericeira	Queimado	Arrifes
(Absolute Dominant) Dominant	100%	10					
	>75%	9					
Abundant	>50%	8					Cystoseira
(Highly Frequent)	>33%	7	Laminaria ochroleuca	Saccorhiza polyschides	Cystoseira tamariscifolia	Cystoseira tamariscifolia, Falkenbergia rufolanosa (Asparagopsis armata)	Dictyota dichotoma, tamariscifolia; Sargassum vulgare
Frequent	>25%	6	Chondrus crispus				
Occasional	>10%	5	Ulva spp., Ahnfeltiopsis devoniensis, Osmundea pinnatifida	Pterosiphonia complanata, Osmundea pinnatifida, Boergeseniella spp., Chondrus crispus	Asparagopsis armata, Codium spp.	Asparagopsis armata	
Rare	>5%	4	Gigartina pistillata, Chondracanthus acicularis, Sargassum muticum	Callithamnion tetricum, Gigartina pistillata	Pterosiphonia complanata	Sargassum flavifolium, Dictyota dichotoma	Corallina spp.
(Rare scattered)	>1%	3	Calliblepharis jubata, Corallina spp., Cryptopleura ramosa, Porphyra spp./Pyropia spp., Bifurcaria bifurcata, Halopteris scoparia/filicina, Cystoseira tamariscifolia	Ulva spp., Corallina spp., Gymnogongrus crenulatus, Cryptopleura ramosa, Halurus equisetifolius, Ectocarpales/Sphacelaria spp., Calliblepharis jubata, Chondracanthus teedei, Callophyllis laciniata, Chondria coerulescens, Halopteris scoparia/filicina, Cystoseira baccata	Corallina spp., Hypoglossum hypoglossoides (or other Delesseriaceae), Ulva spp., Plocamium cartilagineum, Boergeseniella spp., Lithophyllum incrustans, Osmundea hybrida, Pterosiphonia ardreana/pennata, Ceramium spp.	Corallina spp., Lithophyllum incrustans, Codium spp./Leathesia marina, Jania spp., Codium adhaerens, Ophidocladus simpliciusculus, Osmundea hybrida, Cladostephus spongiosus	Hypnea musciformis, Halopteris scoparia/filicina, Ceramium spp., Cladostephus spongiosus, Pterosiphonia ardreana/pennata, Ulva spp., Pterocladiella capillacea, Polysiphonia spp. (or other Rhodomelaceae), Halopithys incurva, Lithophyllum incrustans, Codium adhaerens
(Very scattered)	>0.1%	2	Other Ectocarpales/Sphacelaria spp., Callithamnion tetragonum, Callophyllis laciniata, Ulva clathrata, Ceramium spp., Lithophyllum incrustans, Lomentaria articulata, Pterosiphonia complanata, Dictyota dichotoma, Chondracanthus teedei, Callithamnion tetricum, Chondria dasyphylla/scintillans, Halidrys siliquosa, Saccorhiza polyschides	Lithophyllum incrustans, Lomentaria articulata, Dictyota dichotoma, Ophidocladus simpliciusculus, Nitophyllum spp., Plocamium cartilagineum, Cladophora spp., Codium spp., Polysiphonia spp. (or other Rhodomelaceae), Gelidium pulchellum, Gastroclonium ovatum, Ahnfeltiopsis devoniensis, Chondracanthus acicularis, Grateloupia turuturu, Cladostephus spongiosus	Dictyota dichotoma, Polysiphonia spp. (or other Rhodomelaceae), Chondracanthus teedei, Nitophyllum punctatum, Cladostephus spongiosus, Halopteris scoparia/filicina, Padina pavonica, Ophidocladus simpliciusculus, Jania spp., Cystoseira baccata	Mesophyllum lichenoides, Amphiroa rigida, Peyssonnelia spp., Plocamium cartilagineum, Chondria coerulescens, Champia parvula, Ulva spp., Ceramium spp., Halopteris scoparia/filicina, Padina pavonica, Hypoglossum hypoglossoides (or other Delesseriaceae), Gastroclonium ovatum, Gigartina pistillata, Acrosorium ciliolatum	Ulva clathrata, Plocamium cartilagineum, Colpomenia spp./Leathesia marina, Mesophyllum lichenoides, Hypoglossum hypoglossoides (or other Delesseriaceae), Hildenbrandia spp., Padina pavonica, Other Ectocarpales/Sphacelaria spp., Cladophora spp.
(Seldom)	>0.01%	1	Cladostephus spongiosus, equisetifolius, Halurus hypoglossoides (or Hypoglossum other Delesseriaceae)	Other Ceramiales, Dictyopteris polypodioides, Ceramium spp., Hypoglossum hypoglossoides (or other Delesseriaceae), Acrosorium ciliolatum, Bornetia secundiflora, Pterosiphonia ardreana/pennata	Champia parvula, spp./Leathesia marina, ramosa, Peyssonnelia spp., Acrosorium ciliolatum, Mesophyllum lichenoides	Dasya spp., Ulva clathrata, Pterosiphonia ardreana/pennata, Scytosiphon spp., Ahnfeltiopsis devoniensis, Bornetia secundiflora	Champia parvula, Ralfsia verrucosa, Other Ceramiales, Asparagopsis armata, Gigartina pistillata, Taonia atomaria
(Solitary)	≤0.01%	0				Nitophyllum punctatum	Acrosorium ciliolatum, Ahnfeltiopsis devoniensis, Gastroclonium ovatum
Taxa richness			33	42	29	36	33

Appendix II.2

Appendix II.2 (continued)
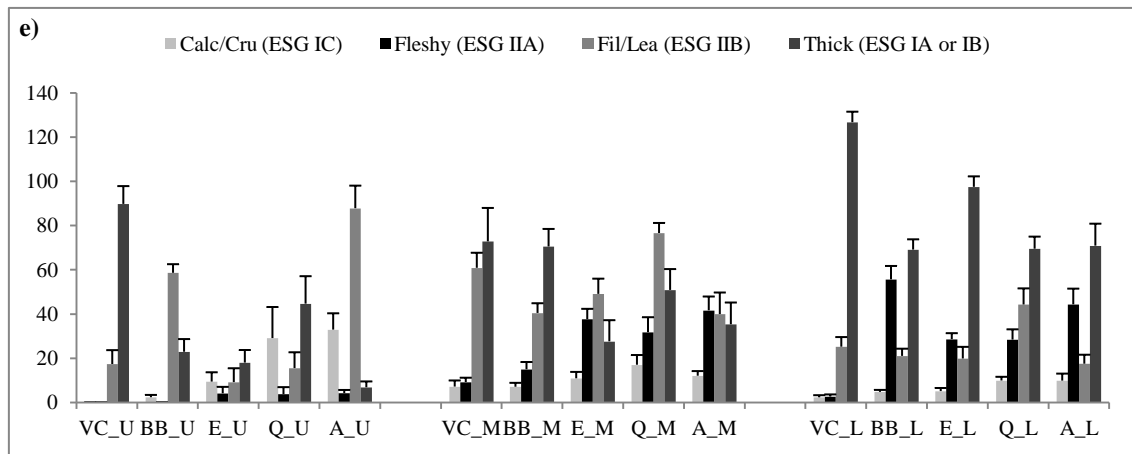
Appendix II.2. Comparison of macroalgal assemblages (% coverage, Mean +SE) along sites' latitudinal gradient (VC = Viana do Castelo, BB = Buarcos Bay, E = Ericeira, Q = Queimado, A = Arrifes) and intertidal zonation patterns (U = Upper-intertidal, M = Mid-intertidal, L = Lower-intertidal).Taxa assignments into functional groups: a) filamentous and leaf-like morphologies (Fil/Leab); b) fleshy morphologies c) Calcareous upright and calcareous and non-calcareous crusts morphologies (Calc/Cru); and d) thick morphologies. And e) thallus morphologies (and corresponding Ecological Status Groups = ESG). Note different scales on the graphics.
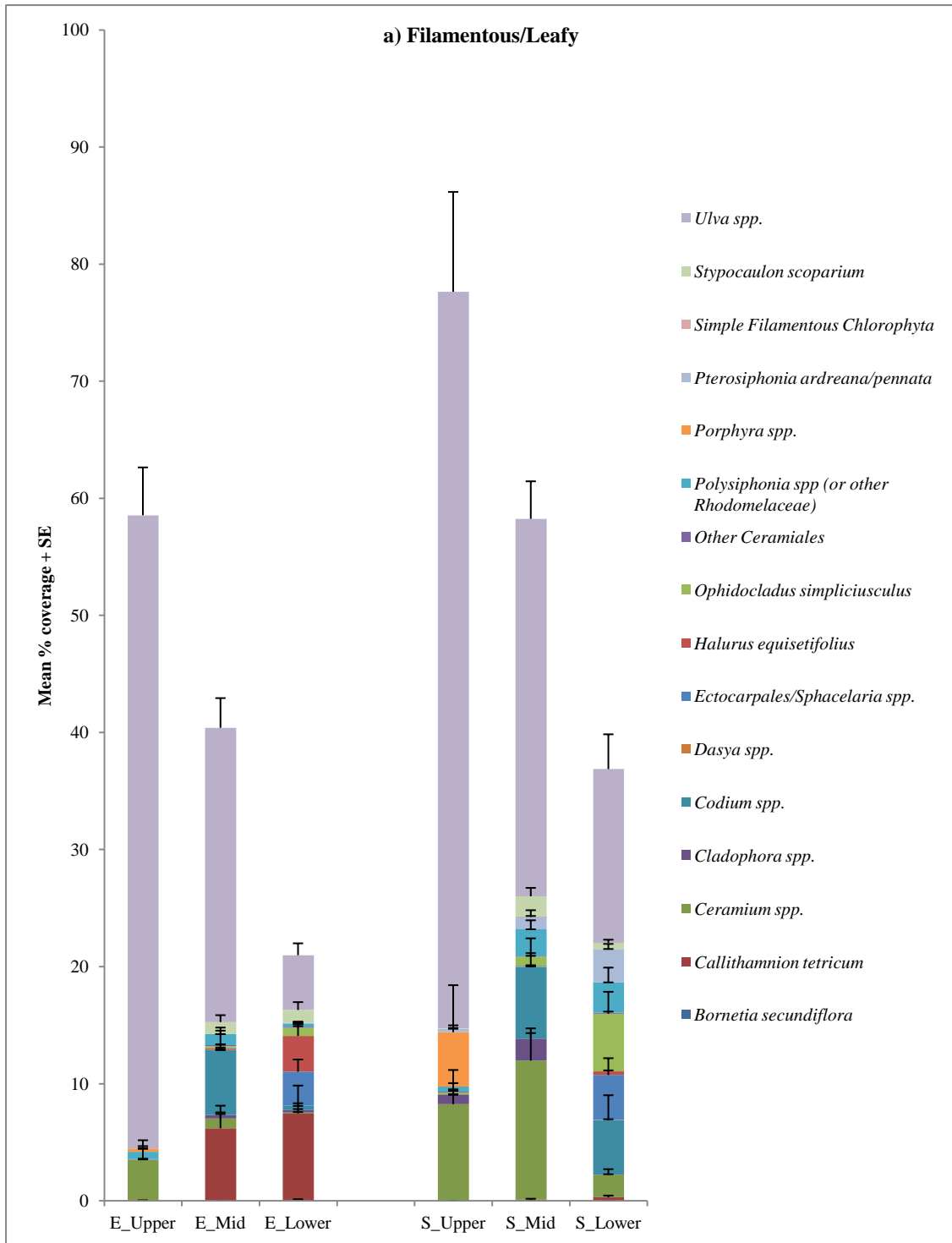
a) Filamentous/Leafy

Appendix III.3 (continued)

Appendix III.3 (continued)
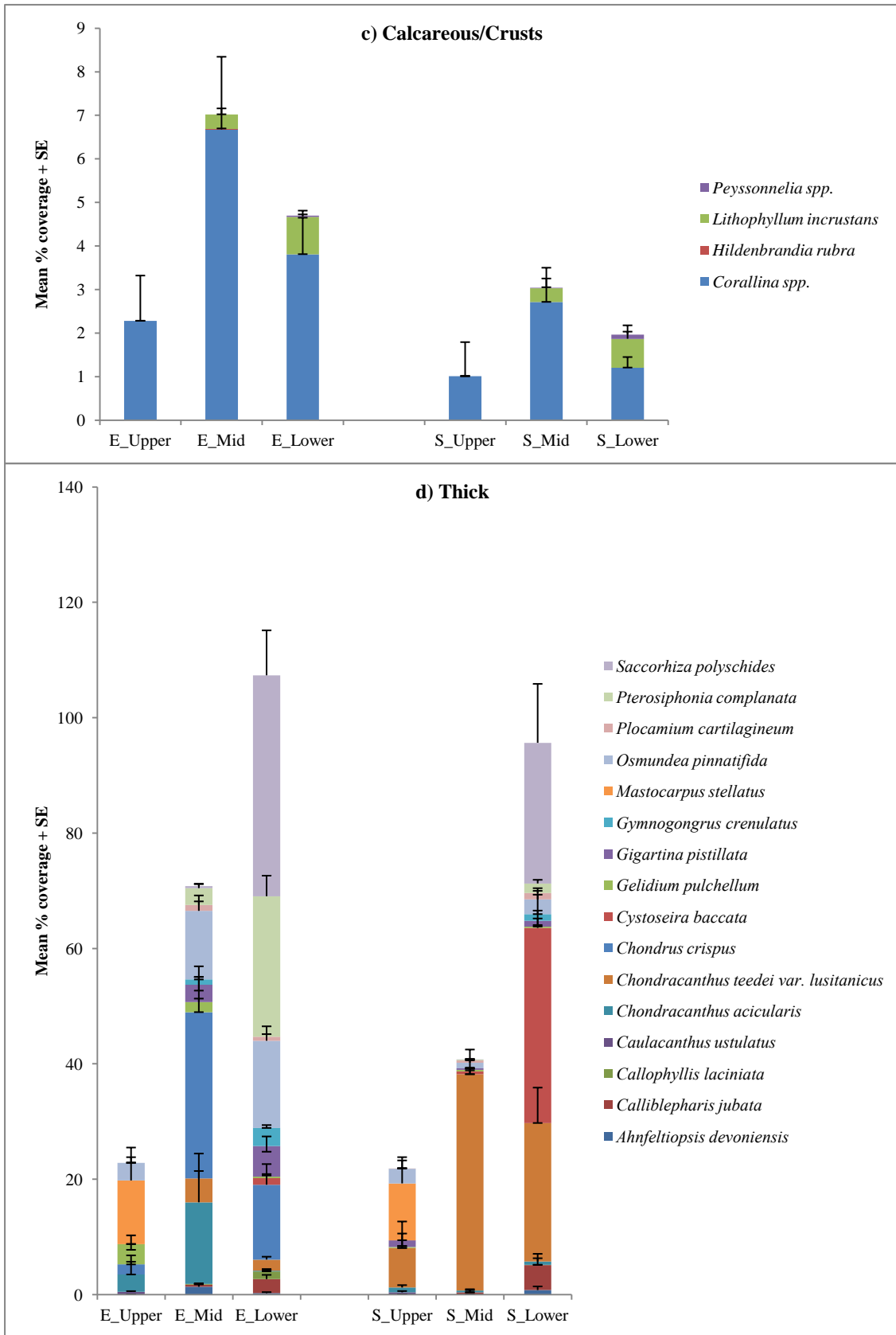
Appendix III.3. Coverage of macroalgae taxa (%, mean + SE) according to each thallus morphology (filamentous/leafy, fleshy, calcareous/crusts, and thick), between wave-exposed with sand-free (E, n= 27) and wave-sheltered with sand-covered (S, n=27) rocky habitats and among Upper- (n=9), Mid- (n=9) and Lower- (n=9) intertidal zones. Note different scales on the graphics.

Appendix III.4. SIMPER tests showing the taxa that mostly contribute to multivariate dissimilarity patterns between (a) wave-exposed with sand-free habitats (E., n=27) and wave-sheltered with sand-covered habitats (S., n=27), and (b) between wave-exposures/sand-depositions per each intertidal zone (n=9 per intertidal zone); without cut off for low contributions.

(a) wave-exposure/sand-deposition

(Average dissimilarity = 73.86)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Ulva spp.	27.97	36.66	11.39	1.23	15.43	15.43
Chondracanthus teedei var. *lusitanicus*	2.01	22.8	8.94	1.17	12.1	27.53
Saccorhiza polyschides	12.88	8.15	6.24	0.73	8.44	35.97
Chondrus crispus	14.52	0	5.65	0.78	7.65	43.62
Cystoseira baccata	0.37	11.43	4.28	0.6	5.8	49.42
Osmundea pinnatifida	10.03	2.04	3.42	1.2	4.64	54.05
Pterosiphonia complanata	9.09	0.6	3.35	0.75	4.53	58.59
Ceramium spp.	1.46	7.35	3.03	0.93	4.1	62.69
Mastocarpus stellatus	3.67	3.28	2.78	0.51	3.77	66.46
Chondria coerulescens	1.09	6.37	2.64	0.75	3.57	70.03
Chondracanthus acicularis	5.77	0.57	2.33	0.4	3.16	73.19
Boergeseniella spp.	5.88	1.97	2.33	0.99	3.15	76.34
Codium spp.	1.98	3.58	1.82	0.77	2.46	78.8
Callithamnion tetricum	4.53	0.13	1.72	0.76	2.33	81.13
Corallina spp.	4.25	1.64	1.55	1.03	2.1	83.23
Gigartina pistillata	2.76	0.81	1.17	0.74	1.58	84.82
Gelidium pulchellum	1.86	0.22	0.82	0.59	1.11	85.93
Calliblepharis jubata	0.94	1.57	0.82	0.59	1.11	87.03
Dictyota dichotoma	0.56	2.01	0.78	0.66	1.06	88.09
Ophidocladus simpliciusculus	0.29	1.97	0.78	0.57	1.05	89.14
Porphyra spp./*Pyropia* spp.	0.09	1.54	0.76	0.23	1.04	90.18
Polysiphonia spp. (or other Rhodomelaceae)	0.61	1.78	0.69	0.78	0.93	91.11
Other *Ectocarpales/Sphacelaria* spp.	0.98	1.29	0.68	0.66	0.92	92.03
Gymnogongrus crenulatus	1.35	0.39	0.57	0.85	0.77	92.8
Pterosiphonia ardreana/pennata	0.02	1.4	0.55	0.83	0.74	93.54
Halopteris scoparia/filicina	0.71	0.76	0.47	0.69	0.64	94.18
Lomentaria articulata	1.15	0.13	0.47	0.45	0.63	94.81
Cryptopleura ramosa	1.11	0.45	0.46	0.87	0.62	95.43
Cladophora spp.	0.18	0.92	0.42	0.71	0.57	96
Halurus equisetifolius	1.06	0.1	0.4	0.53	0.54	96.54
Gracilaria multipartita	0.04	0.9	0.37	0.29	0.5	97.04
Plocamium cartilagineum	0.57	0.51	0.34	0.66	0.46	97.5
Cladostephus spongiosus	0.09	0.71	0.32	0.5	0.44	97.94
Ahnfeltiopsis devoniensis	0.53	0.26	0.28	0.55	0.38	98.32
Lithophyllum incrustans	0.4	0.33	0.21	0.88	0.29	98.61
Callophyllis laciniata	0.48	0.01	0.18	0.54	0.24	98.85

Hypoglossum hypoglossoides (or other Delesseriaceae)	0.05	0.46	0.17	0.53	0.23	99.07
Grateloupia turuturu	0.17	0.19	0.14	0.34	0.19	99.27
Caulacanthus ustulatus	0.16	0.13	0.12	0.58	0.16	99.42
Nitophyllum punctatum	0.19	0.15	0.11	0.54	0.15	99.58
Gastroclonium ovatum	0.15	0.04	0.07	0.52	0.09	99.67
Dictyopteris polypodioides	0.08	0.05	0.05	0.39	0.07	99.73
Chondria dasyphylla/scintillans	0	0.13	0.05	0.24	0.07	99.8
Other Ceramiales	0.07	0.06	0.05	0.54	0.06	99.86
Acrosorium ciliolatum	0.03	0.07	0.03	0.36	0.04	99.91
Peyssonnelia spp.	0.01	0.04	0.02	0.38	0.02	99.93
Dasya spp.	0	0.03	0.01	0.3	0.01	99.94
Bornetia secundiflora	0.03	0	0.01	0.26	0.01	99.96
Gastroclonium reflexum	0	0.02	0.01	0.19	0.01	99.97
Gracilaria gracilis	0.02	0	0.01	0.23	0.01	99.98
Simple Filamentous Chlorophyta	0	0.01	0.01	0.19	0.01	99.99
Scinaia furcellata	0.02	0	0.01	0.19	0.01	100
Hildenbrandia spp.	0	0	0	0.19	0	100

(b) Intertidal zonation

Upper-intertidal (Average dissimilarity = 41.97)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Ulva spp.	54.09	62.91	12.57	1.3	29.95	29.95
Mastocarpus stellatus	11.02	9.85	7.61	1.02	18.14	48.09
Ceramium spp.	3.47	8.25	4.3	1.11	10.24	58.33
Chondracanthus teedei var. *lusitanicus*	0	6.87	3.7	0.53	8.82	67.15
Porphyra spp./ *Pyropia* spp.	0.28	4.63	2.7	0.41	6.43	73.58
Osmundea pinnatifida	3.07	2.56	1.83	1.03	4.35	77.93
Gelidium pulchellum	3.53	0.15	1.82	0.81	4.33	82.27
Chondracanthus acicularis	3.02	0.84	1.58	0.57	3.77	86.04
Corallina spp.	2.28	1.01	1.44	0.87	3.43	89.48
Chondria coerulescens	0	2.35	1.29	0.52	3.07	92.54
Chondrus crispus	1.75	0	0.96	0.39	2.28	94.82
Gigartina pistillata	0	1.18	0.61	0.36	1.45	96.27
Cladophora spp.	0.02	0.84	0.46	0.67	1.09	97.36
Polysiphonia spp. (or other Rhodomelaceae)	0.62	0.44	0.41	0.86	0.97	98.33
Caulacanthus ustulatus	0.47	0.38	0.31	1.26	0.73	99.06
Pterosiphonia ardreana/pennata	0	0.29	0.17	0.35	0.4	99.46
Ophidocladus simpliciusculus	0	0.17	0.09	0.35	0.21	99.67
Other Ceramiales	0.01	0.07	0.04	0.39	0.1	99.77
Plocamium cartilagineum	0	0.04	0.02	0.35	0.06	99.83
Halopteris scoparia/filicina	0	0.03	0.02	0.35	0.04	99.88
Codium spp.	0.03	0	0.02	0.35	0.04	99.92
Hypoglossum hypoglossoides (or other Delesseriaceae)	0	0.02	0.01	0.35	0.03	99.95
Lomentaria articulata	0.02	0	0.01	0.35	0.03	99.98
Callithamnion tetricum	0.02	0	0.01	0.35	0.02	100

Mid-intertidal (Average dissimilarity = 69.23)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Chondracanthus teedei var. *lusitanicus*	4.12	37.52	13.04	2.68	18.84	18.84
Chondrus crispus	28.84	0	11.43	1.26	16.51	35.35

Chondracanthus acicularis	14.16	0.29	5.4	0.58	7.81	43.16
Chondria coerulescens	2.25	14.98	5.11	1.36	7.38	50.54
Ceramium spp.	0.82	11.88	4.41	1.41	6.36	56.9
Osmundea pinnatifida	11.89	1	4.37	1.39	6.31	63.21
Ulva spp.	25.13	32.24	4.2	1.37	6.06	69.27
Codium spp.	5.53	6.11	2.66	1.22	3.83	73.1
Callithamnion tetricum	6.2	0.09	2.49	1.1	3.6	76.7
Boergeseniella spp.	6.16	0.71	2.38	1.02	3.44	80.14
Corallina spp.	6.67	2.71	1.98	1.13	2.86	83
Pterosiphonia complanata	2.92	0.13	1.15	1.31	1.66	84.65
Gigartina pistillata	2.98	0.25	1.14	0.76	1.64	86.3
Lomentaria articulata	2.64	0.01	1.05	0.66	1.52	87.82
Polysiphonia spp. (or other Rhodomelaceae)	0.93	2.3	0.77	1.07	1.11	88.92
Halopteris scoparia/filicina	1.03	1.73	0.75	0.99	1.08	90
Cladophora spp.	0.31	1.89	0.71	1.21	1.03	91.04
Cladostephus spongiosus	0.11	1.65	0.67	0.89	0.96	92
Gelidium pulchellum	1.75	0.26	0.66	1.06	0.95	92.95
Dictyota dichotoma	1.33	0.99	0.54	1.24	0.78	93.73
Ahnfeltiopsis devoniensis	1.35	0.01	0.52	1.14	0.74	94.48
Plocamium cartilagineum	1.02	0.38	0.43	0.65	0.63	95.1
Pterosiphonia ardreana/pennata	0	1.05	0.43	0.63	0.62	95.73
Gracilaria multipartita	0.13	0.98	0.39	0.45	0.56	96.29
Gymnogongrus crenulatus	0.93	0	0.38	0.7	0.55	96.83
Ophidocladus simpliciusculus	0.18	0.79	0.31	1.04	0.44	97.27
Cryptopleura ramosa	0.66	0.17	0.27	0.94	0.39	97.67
Calliblepharis jubata	0.38	0.38	0.26	0.7	0.37	98.04
Lithophyllum incrustans	0.34	0.31	0.19	0.9	0.28	98.32
Cystoseira baccata	0	0.49	0.19	1.01	0.27	98.59
Nitophyllum punctatum	0.35	0.13	0.17	0.55	0.24	98.82
Saccorhiza polyschides	0.35	0.07	0.15	0.41	0.22	99.05
Grateloupia turuturu	0.31	0	0.12	0.69	0.18	99.23
Gastroclonium ovatum	0.26	0.11	0.12	0.77	0.17	99.4
Hypoglossum hypoglossoides (or other Delesseriaceae)	0.1	0.11	0.06	0.76	0.09	99.49
Dictyopteris polypodioides	0.15	0.01	0.06	0.43	0.09	99.58
Halurus equisetifolius	0.12	0	0.05	0.61	0.07	99.65
Other Ceramiales	0.1	0.03	0.05	0.63	0.07	99.72
Callophyllis laciniata	0.11	0	0.04	0.48	0.06	99.78
Other *Ectocarpales/Sphacelaria* spp.	0.03	0.07	0.04	0.54	0.05	99.83
Gracilaria gracilis	0.07	0	0.02	0.43	0.03	99.87
Gastroclonium reflexum	0	0.05	0.02	0.35	0.03	99.9
Scinaia furcellata	0.05	0	0.02	0.35	0.03	99.93
Simple Filamentous Chlorophyta	0	0.04	0.02	0.35	0.03	99.96
Dasya spp.	0	0.03	0.01	0.49	0.02	99.98
Acrosorium ciliolatum	0.01	0	0	0.35	0.01	99.99
Hildenbrandia spp.	0.01	0	0	0.35	0.01	99.99
Peyssonnelia spp.	0	0.01	0	0.35	0.01	100

Appendix **123**

Lower-intertidal (Average dissimilarity = 73.49)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Cystoseira baccata	1.12	33.81	10.92	1.69	14.87	14.87
Saccorhiza polyschides	38.29	24.38	10.23	1.39	13.93	28.79
Pterosiphonia complanata	24.36	1.67	7.57	2.11	10.31	39.1
Chondracanthus teedei var. *lusitanicus*	1.91	24.02	7.5	1.2	10.2	49.3
Chondrus crispus	12.98	0	4.33	1.23	5.9	55.2
Osmundea pinnatifida	15.12	2.55	4.1	1.73	5.58	60.77
Ulva spp.	4.68	14.83	3.52	1.13	4.78	65.56
Boergeseniella spp.	11.48	5.21	2.69	1.49	3.66	69.22
Callithamnion tetricum	7.38	0.29	2.36	1.04	3.21	72.43
Gigartina pistillata	5.31	0.99	1.68	1.13	2.28	74.71
Codium spp.	0.37	4.63	1.63	0.78	2.22	76.92
Ophidocladus simpliciusculus	0.69	4.94	1.58	0.96	2.16	79.08
Calliblepharis jubata	2.44	4.34	1.52	1.04	2.07	81.15
Dictyota dichotoma	0.36	5.06	1.51	1.04	2.06	83.21
Other *Ectocarpales/Sphacelaria* spp.	2.92	3.82	1.21	1.28	1.65	84.86
Halurus equisetifolius	3.06	0.3	0.96	1.09	1.31	86.17
Corallina spp.	3.81	1.2	0.95	1.15	1.29	87.46
Pterosiphonia ardreana/pennata	0.07	2.85	0.91	2.42	1.24	88.7
Gymnogongrus crenulatus	3.12	1.16	0.85	1.74	1.15	89.85
Polysiphonia spp. (or other Rhodomelaceae)	0.27	2.58	0.77	0.72	1.05	90.9
Chondria coerulescens	1.03	1.78	0.72	0.89	0.98	91.88
Cryptopleura ramosa	2.66	1.17	0.66	1.34	0.89	92.78
Gracilaria multipartita	0	1.73	0.61	0.36	0.84	93.61
Ceramium spp.	0.08	1.92	0.6	1.45	0.81	94.42
Callophyllis laciniata	1.33	0.02	0.44	1.17	0.6	95.02
Halopteris scoparia/filicina	1.1	0.53	0.43	0.79	0.58	95.6
Plocamium cartilagineum	0.69	1.1	0.41	1.07	0.56	96.16
Hypoglossum hypoglossoides (or other Delesseriaceae)	0.05	1.25	0.38	0.97	0.51	96.67
Ahnfeltiopsis devoniensis	0.25	0.78	0.32	0.53	0.43	97.1
Lomentaria articulata	0.78	0.37	0.3	0.79	0.41	97.52
Lithophyllum incrustans	0.86	0.66	0.27	1.48	0.37	97.89
Grateloupia turuturu	0.2	0.58	0.27	0.46	0.36	98.25
Chondracanthus acicularis	0.13	0.58	0.24	0.43	0.33	98.58
Cladostephus spongiosus	0.16	0.49	0.22	0.45	0.3	98.87
Gelidium pulchellum	0.29	0.25	0.16	0.62	0.21	99.09
Chondria dasyphylla/scintillans	0	0.39	0.13	0.45	0.18	99.27
Nitophyllum punctatum	0.23	0.31	0.13	0.96	0.17	99.44
Acrosorium ciliolatum	0.07	0.2	0.08	0.63	0.1	99.54
Dictyopteris polypodioides	0.1	0.13	0.07	0.57	0.1	99.64
Cladophora spp.	0.21	0.03	0.07	0.79	0.09	99.73
Gastroclonium ovatum	0.18	0	0.06	0.59	0.08	99.82
Other Ceramiales	0.09	0.09	0.05	0.65	0.07	99.89
Peyssonnelia spp.	0.03	0.1	0.04	0.67	0.05	99.94
Bornetia secundiflora	0.08	0	0.02	0.48	0.03	99.98
Dasya spp.	0	0.04	0.02	0.35	0.02	100

Appendix III.5. SIMPER tests showing the thallus morphologies that mostly contribute to multivariate dissimilarity patterns between (a) wave-exposed with sand-free habitats (E., n=27) and wave-sheltered with sand-covered habitats (S., n=27), and (b) between wave-exposures/sand-depositions per each intertidal zone (n=9 per intertidal zone); without cut off for low contributions.

(a) wave-exposure/sand-deposition

(Average dissimilarity = 36.5)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Thick	67.0	52.8	18.6	1.4	50.9	50.9
filamentous and leaf-like	40.0	57.6	11.2	1.3	30.7	81.6
Fleshy	10.6	13.6	5.1	1.3	13.8	95.5
calcareous upright and calcareous and non-calcareous crusts	4.7	2.0	1.7	1.1	4.5	100.0

(b) Intertidal zonation

Upper-intertidal (Average dissimilarity = 25.7)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
filamentous and leaf-like	58.6	77.6	12.7	1.4	49.2	49.2
Thick	22.9	21.9	10.3	1.5	40.1	89.3
calcareous upright and calcareous and non-calcareous crusts	2.3	1.0	1.4	0.9	5.6	94.9
fleshy	0.0	2.4	1.3	0.5	5.1	100.0

Mid-intertidal (Average dissimilarity = 28.8)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
thick	70.8	40.8	13.4	1.4	46.4	46.4
filamentous and leaf-like	40.4	58.3	8.6	1.5	29.8	76.2
fleshy	14.6	19.9	4.8	1.4	16.6	92.7
calcareous upright and calcareous and non-calcareous crusts	7.0	3.0	2.1	1.1	7.3	100.0

Lower-intertidal (Average dissimilarity = 19.7)	E. Av.Abund	S. Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
thick	107.4	95.7	10.4	1.4	52.7	52.7
filamentous and leaf-like	21.0	36.9	5.8	1.6	29.3	82.0
fleshy	17.3	18.7	2.5	1.2	12.7	94.7
calcareous upright and calcareous and non-calcareous crusts	4.7	2.0	1.1	1.1	5.3	100.0