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GENETIC BACKGROUND, RANGE SHIFTS AND ASSOCIATED MICROBIAL RESPONSES OF CANOPY ALGAE UNDER CHANGING ENVIRONMENT

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“Dove inizia la fine del mare? O addirittura: cosa diciamo quando diciamo: mare? Diciamo l'immenso mostro capace di divorare qualsiasi cosa, o quell'onda che ci schiuma intorno ai piedi? L'acqua che puoi tenere nel cavo della mano o l'abisso che nessuno può vedere? Diciamo tutto in una sola parola o in una sola parola tutto nascondiamo? Sto qui, a un passo dal mare, e neanche riesco a capire, lui, dov'è. Il mare. Il mare.”

– Alessandro Baricco, Oceano Mare

Genetic background, range shifts and associated microbial responses of canopy algae under changing environment

ABSTRACT

Marine forests are a key habitat across temperate rocky shores, increasing dimensional complexity, local biodiversity, and productivity. However, canopy-forming algae are experiencing a general global decline, mostly driven by human pressures on coastal ecosystems and global changes. In contrast with their high ecological relevance, little is known about how their genetic diversity, dispersal and connectivity can be affected by global changes, despite the expected consequences for population resilience. I focused on studying brown macroalgae of the genus *Cystoseira*, one of the leading canopy-forming seaweed genera along European coasts, coupling molecular and ecological approaches to understand several processes that affect these marine forests. I first investigated the present distribution of the closely related *C. amentacea*, *C. tamariscifolia*, and *C. compressa* and projected their distribution under predicted climatic scenarios based on their climatic niche. I coupled this distribution modeling with the genetic characterization of populations to understand the potential consequences of shifts in their distribution ranges for the species gene pools. I found that considerable genetic diversity can be lost during the predicted shifts in distribution, with profound differences between Atlantic and Mediterranean ecosystems. Results suggest that climate change holds an extinction risk for *C. amentacea*. For this latter species, I also modeled the effect of habitat fragmentation on species dispersal and connectivity by simulating stepping-stone dispersal dynamics and comparing with population genetic differentiation. Results revealed that the population genetic structure could be well predicted based on oceanographic transport and dispersal traits of *C. amentacea*, therefore estimating the potential impacts of habitat fragmentation and loss. Finally, hypothesizing a potential role of associated bacteria in macroalgal adaptation and resilience, I explored the association and functional role of the microbial community within *Cystoseira* spp. I characterized the microbial community living in association with *C. compressa* at different locations, to explore its spatial variability. I also compared it with the microbial communities among closely related species of *Cystoseira*. Species-specific seaweed associated bacterial communities could be identified, which are consistent within regions but are differentiated between regions. The structure of bacterial communities associated to *Cystoseira* followed a geographical isolation-by-distance pattern, clearly distinguishable among basins (Atlantic, Mediterranean and Adriatic) possibly driven by population genetic structure of the host. A consistent set of shared bacteria formed the core community of *C. compressa* across its distribution and was characterized by key ecological functions for the holobiont system, for example contributing to the degradation of pollutants at the macroalgal interface. Overall the knowledge acquired in this doctoral thesis is a useful contribution for management actions involving *Cystoseira* populations. Increasing our understanding of the ecology of the species, of its vulnerability to future global changes and the interactions with its bacterial components, can be integrated in informed planning of conservation areas or restoration actions for preserving the ecological role and ecosystem functions of these species that are the structure of important European marine forests.

RESUMO

As florestas marinhas são um habitat-chave das costas rochosas temperadas, aumentando a sua complexidade dimensional, a biodiversidade local e a produtividade. No entanto, as algas de porte arbóreo têm vindo a sofrer um declínio global, decorrente maioritariamente de alterações climáticas globais e, em particular, de pressões humanas sobre os ecossistemas costeiros. Apesar da sua importância ecológica, pouco se sabe de que forma a sua diversidade genética, dispersão e conectividade podem ser afectadas por mudanças climáticas globais, apesar dos efeitos esperados sobre a resiliência das populações. Este estudo focou-se no estudo de macroalgas castanhas do género *Cystoseira*, um dos géneros principais de algas de porte arbóreo, ao longo das costas europeias, e inclui uma abordagem molecular e ecológica de forma a compreender os vários processos que estão a afectar estas florestas marinhas. Em primeiro lugar, investiguei a distribuição geográfica actual de *C. amentacea*, *C. tamariscifolia* e *C. compressa*, espécies mais proximamente relacionadas, e projectei a sua distribuição sob vários cenários climáticos previstos com base no seu nicho climático. Os modelos de distribuição das espécies e a caracterização genética das populações foram analisados em conjunto para tentar compreender quais as consequências de potenciais alterações da distribuição sobre o “pool” genético de cada espécie. Os resultados apontam para uma perda considerável da diversidade genética em resultado de alterações na sua distribuição geográfica, com profundas diferenças entre o Atlântico e o Mediterrâneo. Os modelos sugerem um risco de extinção para *C. amentacea* resultante das mudanças climáticas. Também se modelou o efeito da fragmentação de habitat sobre a dispersão e a conectividade para esta espécie, através da simulação da dinâmica de dispersão “stepping-stone**”**, comparando com a diferenciação genética populacional. Os resultados mostraram que a estrutura genética da população pode ser prevista

com base no transporte oceanográfico e na forma de dispersão, estimando-se assim os potenciais impactos da fragmentação e perda de habitat para *C. amentacea*. Finalmente, assumindo que as comunidades bacterianas associadas têm um papel fundamental na adaptação e resiliência das macroalgas, explorei essa associação, assim como o papel funcional dessas comunidades em *Cystoseira* spp. A comunidade bacteriana que vive em associação com *C. compressa* foi caracterizada em diferentes locais de modo a explorar sua variabilidade espacial. As comunidades bacterianas associadas a espécies de *Cystoseira* filogeneticamente mais próximas, foram igualmente comparadas. Foram identificadas comunidades bacterianas específicas para cada espécie, sendo consistentes dentro de cada região mas diferenciadas entre as várias regiões. A estrutura das comunidades bacterianas associadas a *Cystoseira* seguiu um padrão geográfico de isolamento por distância, claramente diferenciado entre as bacias (Atlântico, Mediterrâneo e Adriático) possivelmente determinado pela estrutura genética populacional do hospedeiro. Um set consistente de bactérias partilhadas formou a comunidade nuclear de *C. compressa* ao longo de toda a sua distribuição e foi caracterizado por funções ecológicas chave para o holobionte, contribuindo, por exemplo, para a degradação de poluentes na interface da alga. De uma forma geral, os conhecimentos adquiridos nesta tese de doutoramento são uma valiosa contribuição para acções de gestão envolvendo populações de *Cystoseira*. Melhorar o conhecimento sobre a ecologia das espécies, a sua vulnerabilidade às futuras alterações climáticas globais e as interações com seus componentes bacterianos, permitirá um planeamento mais informado de potenciais áreas de conservação ou ações de restauro para preservar o papel ecológico e as funções do ecossistema dessas espécies que são a estrutura de importantes florestas marinhas europeias.

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

INTRODUCTION

Global change and the coastal environment

We live in extraordinary times. Human population and technological progress has increased exponentially in the last century and so did the anthropogenic pressures on global ecosystems. Such modifications encompass planetary scale and, after the Rio de Janeiro Earth Summit of 1992, are overall regarded as global changes. Global changes include many different processes, which interact in a complex and nonlinear way. These are generating disproportionately more impacts in coastal regions as most of the global human population lives within 200 km from the coasts (Cohen *et al.* 1997; Airoldi & Beck 2007) and consequently, all the activities including products consumption, waste or transportation, are concentrated along the coastal areas. Coastal marine systems are some of the most ecologically and socio-economically valuable on the planet. They provide numerous ecosystem goods and services such as food, coastal defense (Spalding *et al.* 2014), water purification, marine biodiversity, carbon sequestration and socio-cultural services (Liquete *et al.* 2013).

There is strong scientific consensus that coastal marine ecosystems, along with the goods and services they provide, are particularly threatened by global and climate changes (Leslie 2008; IPCC 2013). Following the human population growth in coastal zones, urbanization and marine infrastructures have sprawled, altering or directly removing the natural habitats (Burke *et al.* 2001). Construction of artificial structures has been adopted to contrast also sea level rise (Fenoglio-Marc & Tel 2010) and erosion (Zhang *et al.* 2004; Cai *et al.* 2009). These processes are in turn driven mainly by climate change, which is expected to produce increases in extreme events, species displacement and ecosystems degradation among other impacts (Harley *et al.* 2006). This creates a positive feedback, as degradation of habitats and species replacement decrease the ecosystem services provided, including coastal defense (Spalding *et al.* 2014), fostering the need for artificial structures. Additionally, population increase, changes in the use of the soil, agricultural activities and alterations in the hydrogeological cycles have amplified the local input of nutrient and sediment load (Rabalais *et al.* 2009). The frequency and extension of these impacts have grown up to a level where their effect can be measured globally, with consequent increase of anoxic and hypoxic zones, algal blooms, species displacement and habitat degradation (Sellner *et al.* 2003; Diaz & Rosenberg 2008). Increase in sea surface temperature, extreme events and ocean acidification driven by global climate change is also causing changes in the species distribution and the loss of entire reef ecosystems (Garrabou *et al.* 2001; Munday *et al.* 2013; Bates *et al.* 2014). Furthermore, overexploitation of marine resources and the introduction of non-native species is decreasing the biodiversity and the resilience of the ecosystems, hampering their ability to buffer the other anthropogenic impacts (Jackson 2001; Worm *et al.* 2006). Understanding the capabilities of species and ecosystems to face those interacting changes is one of the great challenges of the twenty-first century.

Canopy forming algae in Europe: status and trend

Shallow temperate and cold rocky shores are characterized by the presence of macroalgal beds, one of the most productive, diverse and valuable marine habitat (Wahl 2009). Some species of brown algae (mostly Fucales and Laminariales) can fix more than 1 kg Cm^{-2} $year^{-1}$ and even more in kelp forests (Mineur *et al.* 2015). These habitats are dominated by brown perennial algae (Phaeophyceae) which can form dense tri-dimensional structures allowing the coexistence of a rich associated fauna and providing essential ecosystem services (Cheminée *et al.* 2013; Mineur *et al.* 2015). In the Atlantic and Pacific oceans, kelps (Laminariales and Tilopteridales*)* dominates the lower intertidal and subtidal, while Fucales are found in the intertidal zone. In the Mediterranean Sea instead, kelps are virtually absent and the euphotic zone is dominated by Fucales of the genus *Cystoseira* and *Sargassum* (Airoldi & Beck 2007). Quantitative information of the distribution and cover of the macroalgal beds, however, is sparse. Some already compiled information on the distribution exists for oceanic kelps (Merzouk & Johnson 2011; Marzinelli *et al.* 2015, Connor 2004) and fucoids in the Atlantic and Baltic (Margalet *et al.* 1993; Malm & Isaeus 2005). However, no comprehensive and detailed inventory exists for all European countries and the Mediterranean Sea, even if local or regional knowledge can be considerable (Sales & Ballesteros 2009; Macic *et al.* 2010; Thibaut *et al.* 2016). The services that collate digitalized survey data and distributions records, such as GBIF (Gbif 2012) and Algaebase (Guiry & Guiry 2013) made a great step forward making this information available to the public, but they also suffer from bias due to uneven effort of sampling, data storage and mobilization (Beck *et al.* 2014).

A global decline of macroalgal beds has been reported in several studies (Steneck *et al.* 2002; Airoldi & Beck 2007; Mineur *et al.* 2015). Canopy algae under different disturbance and stress regimes tend to shift toward less complex stable state dominated by ephemeral turf forming algae, urchin barrens or mussel beds (Airoldi *et al.* 2015). Depicting the exact cause of this habitat transformation is not straightforward as current knowledge suggests it is driven by a complex of local and global stressors (Strain *et al.* 2014). The decrease in water quality, increase in sediment load, extreme events and altered grazing pressure are among the most important local drivers (Airoldi *et al.* 2015). The shifts from canopies to alternative habitats such as turfs or barrens might even not be reversible once a certain threshold is exceeded (Perkol-Finkel & Airoldi 2010). The profound ecosystem changes that follow the local to regional loss of biogenic habitats translate in a major reduction of spatial diversity, food resources and the many ecosystem services provided (Airoldi *et al.* 2008).

The genus *Cystoseira* as model system

In the Mediterranean Sea, brown algae of the genus *Cystoseira* are the dominant and most widespread canopy forming species. Mediterranean *Cystoseira* spp. comprise various endemisms, and are distributed from the infralittoral down to the upper circalittoral zone, with each species occupying a specific niche defined by depth, water temperature, substrate characteristics, wave exposure and seawater nutrients, competition and effects of herbivores (Ballesteros 1990; Sales & Ballesteros 2009; Giaccone 1994; Vergés *et al.* 2009). As other canopy forming algae, the genus *Cystoseira* has also experienced widespread declines, sometimes leading to local extinction of several species (Soltan *et al.* 2001; Thibaut *et al.* 2005; Serio *et al.* 2006; Perkol-Finkel & Airoldi 2010; THIBAUT *et al.* 2014). After the Barcelona Convention (1992) all the *Cystoseira* species are protected in the Mediterranean except *C. compressa*, which showed more stable populations.

These species present a ramified structure, arising from a holdfast attached to the substrate by a basal disk, and can reach more than 1 m in height (Ribera *et al.* 1992, Gómez Garreta et al. 2001). Some species present aereocystis along the branches, such as *C. compressa* while in others they are absent (e.g. *C. tamariscifolia* and *C. amentacea*). Although individuals are perennial, the canopy is lost after the reproductive season and the species enter a quiescent phase during the colder months. New fronds regrow in spring and reproduction takes place usually during the summer. *Cystoseira* species are hermaphroditic and self-compatible, and they are only known to reproduce by sexual means. Male gametes are released into the water while female gametes are also released in synchrony but remain attached to the conceptacles until few days after fertilization (Robvieux 2013; Engelen et al. 2008). Zygotes are negatively buoyant and tend to quickly settle and develop close to the parent.

Molecular ecology and species resilience

Molecular ecology is a cross-disciplinary science, merging the tools and methods of molecular biology, genomics and bioinformatics with the theory of ecology, evolution, conservation and behavior (Andrew *et al.* 2013). The genetic dimension involves different aspects and tools that are extremely useful in ecology and conservation biology. One of the main focuses is on population genetics, which studies the genotypic and allelic diversity, the genetic structure and their evolution in natural populations (Hamilton *et al.* 2009). Genetic diversity is essential for the long-term persistence of populations exposed to environmental changes (Bakker et al. 2010). High genetic variability increases the probability to have some individuals in the population pool with the best genotype for a certain environmental condition and therefore allows populations to adapt to changing conditions. On the contrary, low genetic diversity has been related to reduced fitness and eventually to a higher risk of extinction (Frankham 2005). Genetic diversity can have important ecological consequences at each level of species organization, from population to ecosystem levels, and it can also be linked to species diversity (Vellend & Geber 2005; Hughes *et al.* 2008). Preservation of the genetic diversity is, therefore, a major objective of management and conservation of species.

The molecular tools allow also quantifying gene flow among populations. The study of gene flow gives indications about the realized connectivity and migrations rates in a metapopulation context. It also allows identifying barriers and limitations to the genetic exchange among populations. In the framework of landscape genetics, knowledge of how landscape characteristics influence population genetic structure enables us to understand the processes and patterns of gene flow and local adaptation. This is essential, not only for improving ecological knowledge but also for managing the genetic diversity of vulnerable and endangered populations (Manel *et al.* 2003; Storfer *et al.* 2007; Manel & Holderegger 2013). Overall these aspects of molecular ecology give fundamental information to identify management units, and help understanding metapopulation resilience and designing marine protected areas or restoration actions.

Macroalgal holobiont theory

Bacteria tend to form complex associations with macroalgae. They can aggregate in biofilms on the surface, colonize the interstitial spaces between cell walls or even occur as endosymbionts (Delbridge *et al.* 2003; Wahl *et al.* 2012; Hollants *et al.* 2013; Aires *et al.* 2015). These relationships are mediated by complex chemical interactions (Goecke *et al.* 2010) which contribute to different essential functions of the algal host, such as morphological development, growth and nutrient acquisition, spore release and settlement, and protection from fouling (Egan *et al.* 2012; Aires *et al.* 2013). Evidence suggests that the microbial-macroalgae association tend to be specific to species or phylogenetically related groups (Hollants *et al.* 2013; Aires *et* *al.* 2015, 2016). For this reason, it has been suggested that the macroalgae and their microbiome can be considered as holobionts, where the hosts and their specific associated microbiota behave as a singular entity (Wahl *et al.* 2012; Barott *et al.* 2012; Egan *et al.* 2012). The study of associated bacterial communities can be central in understating the future of canopy algae under a changing environment. Alteration of the associated microbial community might concur to the detrimental effects of stressors observed in canopy algae, facilitating the colonization by pathogens and epiphytes or losing some key function for the holobiont (Wahl et al. 2012). Furthermore, associated bacteriomes can have a potential role in macroalgal adaptation and resilience to a changing environment. For instance, adaptation of the holobiont can be accomplished by adjustments in the microbiome, enabling the holobiont to persist without or with minor adaptation of the main host.

Aims, general approach and structure of the thesis

In this thesis, I focused on the *Cystoseira* species that are most common along the coasts of the Mediterranean and North-West Atlantic, with the aim of understanding whether these canopy species (and consequently the ecosystems they generate) will persist and adapt to future global change scenarios. First, I investigated the present distribution of *C. amentacea*, *C. tamariscifolia* and *C. compressa* and projected their distribution under predicted climatic scenarios. I coupled the distribution modeling with genetic characterization of the populations to understand the potential effects of shifts in their distribution ranges. To enable the genetic approach, I developed new microsatellite markers for *C. compressa*. Then, I modeled the effect of habitat fragmentation on species dispersal and connectivity on the species that resulted to be the most exposed to risks of genetic erosion and local extinction, specifically *C. amentacea*. Finally, I characterized the microbial community living in association with *Cystoseira compressa,* the most common and widespread species across Mediterranean and close Atlantic coasts, characterizing its spatial and inter-specific variability and its potential functional roles as part of the *Cystoseira* holobiont. The thesis is structured in 4 chapters, each corresponding to a stand-alone manuscript for publication.

Chapter one: While microsatellite markers for two of the studied species were already available, genetic markers for *C. compressa* were missing. Therefore, we developed 12 microsatellite markers for *Cystoseira compressa*. The microsatellite markers allowed differentiating relatively geographically close populations. Therefore, they provide useful genetic tools for population genetics studies. These markers were used in the subsequent chapters two and four to characterize the population genetic structure of *C. compressa* and to predict the consequences of a changing climate on this species.

Chapter two: In this chapter I inferred the genetic structure of three different *Cystoseira* species (*C. amentacea, C. tamariscifolia* and *C. compressa)* along their distribution ranges and used a correlative approach to develop a model of the present distribution and to predict future changes in distribution and in population genetic structure of according to different climatic scenarios. The aim was to predict the distribution of these key habitat forming species according to their climatic niche to identify future risks and possible conservation strategies. *C. amentacea* and *C. compressa* were predicted to lose most of their suitable habitat in the Mediterranean region in both climatic scenarios used, while *C. tamariscifolia* showed in future simulations a profound regression at its trailing edge of distribution in the worst-case scenario. According to the inferred population genetic diversity and differentiation of populations of those species, we predicted a consequent loss of genetically distinct clusters and depletion of an important part of each species' genetic diversity. Results from this study show also profound differences in the consequences of climate change between Atlantic and Mediterranean habitat and highlighted a particularly high extinction risk for *C. amentacea*.

Chapter three: In this thesis chapter, I analyzed the genetic structure and estimated the connectivity of populations along discontinuous rocky habitat patches in southern Italy, using microsatellite markers at multiple scales. We used *C. amentacea* as a model species, also because in chapter two it was the species most exposed to genetic erosion and risk of extinction by changes in habitat suitability due to its fragmented distribution and isolated populations. In addition, I modeled the effect of rocky habitat continuity and ocean circulation on gene flow by simulating Lagrangian particle dispersal based on ocean surface currents allowing multigenerational stepping-stone dynamics. The results highlighted the importance of spatially explicit modeling of stepping stone dynamics and oceanographic directional transport coupled with habitat suitability, to better describe and predict marine population structure and differentiation.

Chapter four: In the last section, I analyzed bacteria communities associated with *Cystoseira compressa* and closely related macroalgal species (*C. tamariscifolia, C. amentacea* and *Sargassum vulgare*) across North-East Atlantic, central Mediterranean and Adriatic basins using a next generation sequencing approach (16S amplicon sequencing). The aim was to explore the specificity and spatial variability of the *Cystoseira* holobiont along geographical and phylogenetic gradients. Furthermore, I identified the shared bacteria among geographical regions for the most widespread species *C. compressa.* These shared bacteria can describe the bacterial core community of the holobiont of this species, therefore I further explored its potential functional role by predicting metagenome functional content from marker gene in a bioinformatics approach. Results showed large spatial differences in the composition and structure of the microbial communities, but with consistent differences among species at regional scales. The community structures of these species-specific bacterial communities are locally shaped by geographical and environmental local conditions. Additionally, the bacterial structure of *C. compressa* followed a geographical isolation-by-distance pattern, clearly distinguishable among basins and possibly driven by population genetic structure of the host. However, across its distribution, *C. compressa* has a small set of shared bacteria forming the core community of its holobiont system. This core comprised bacteria with different functional roles, including defense from xenobiotics pollutants, contributing to the structure and stability of the holobiont system.

References

Aires T, Moalic Y, Serrao EA, Arnaud-Haond S (2015) Hologenome theory supported by cooccurrence networks of Species-specific bacterial communities in siphonous algae (*Caulerpa*). *FEMS Microbiology Ecology,* **91**.

Aires T, Serrão EA, Engelen AH (2016) Host and Environmental Specificity in Bacterial Communities Associated to Two Highly Invasive Marine Species (Genus *Asparagopsis*). *Frontiers in microbiology,* **7**, 559.

Aires T, Serrão EA, Kendrick G, Duarte CM, Arnaud-Haond S (2013) Invasion Is a Community Affair: Clandestine Followers in the Bacterial Community Associated to Green Algae, Caulerpa racemosa, Track the Invasion Source. *PLoS ONE,* **8**, e68429.

Airoldi L, Balata D, Beck MW (2008) The Gray Zone: Relationships between habitat loss and marine diversity and their applications in conservation. *Journal of Experimental Marine Biology and Ecology*, **366**, 8–15.

Airoldi L, Ballesteros E, Buonomo R *et al.* (2015) Marine forests at risk: solutions to halt the loss and promote the recovery of mediterranean canopy-forming seaweeds. *5th Mediterranean Symposium on Marine Vegetation*, 28–33.

Airoldi L, Beck MW (2007) Loss, Status and Trends for Coastal Marine Habitats of Europe. *Oceanography and Marine Biology: An Annual Review*, 345–405.

Andrew RL, Bernatchez L, Bonin A *et al.* (2013) A road map for molecular ecology. *Molecular Ecology,* **22**, 2605–2626.

Ballesteros E (1990) Structure and dynamics of the community of *Cystoseira zosteroides* (Turner) C. Agardh (Fucales, Phaeophyceae) in the northwestern Mediterranean. *Scientia Marina,* **3**, 217–299.

Barott KL, Rodriguez-Mueller B, Youle M *et al.* (2012) Microbial to reef scale interactions between the reef-building coral *Montastraea annularis* and benthic algae. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1655–1664.

Bates AE, Pecl GT, Frusher S *et al.* (2014) Defining and observing stages of climate-mediated range shifts in marine systems. *Global Environmental Change*, **26**, 27–38.

Bakker J, Rijswijk MEC van, Weissing FJ, Bijlsma R (2010) Consequences of fragmentation for the ability to adapt to novel environments in experimental *Drosophila* metapopulations. Conservation Genetics, 11, 435–448.

Beck J, Böller M, Erhardt A, Schwanghart W (2014) Spatial bias in the GBIF database and its effect on modeling species’ geographic distributions. *Ecological Informatics,* **19**, 10–15.

Burke L, Kura Y, Kassem K *et al.* (2001) *Coastal Zone: Extent and Change*.

Cai F, Su X, Liu J, Li B, Lei G (2009) Coastal erosion in China under the condition of global climate change and measures for its prevention. *Progress in Natural Science*, **19**, 415–426.

Cheminée A, Sala E, Pastor J *et al.* (2013) Nursery value of *Cystoseira* forests for Mediterranean rocky reef fishes. *Journal of Experimental Marine Biology and Ecology*, **442**, 70–79.

Cohen JE, Small C, Mellinger A, Gallup J, Sachs J (1997) Estimates of Coastal Populations. *Science,* **278**.

Connor, D.W., Allen, J.H., Golding, N., Howell, K.L., Lieberknecht, L.M., Northen, K.O. & Beker, J.B. 2004. The Marine Habitat Classification for Britain and Ireland. Version 04.05. Peterborough: JNCC. Online. Available HTTP: http://www.jncc.gov.uk/MarineHabitatClassification (accessed 8 June 2006).

Delbridge L, Coulburn J, Fagerberg W, Tisa LS (2003) Community profiles of bacterial endosymbionts in four species of *Caulerpa*. *4th International Symbiosis Congress*, 335–344.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science*, **321**, 926–929.

Egan S, Harder T, Burke C *et al.* (2012) The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS microbiology reviews*, **37**, 462–76.

Engelen A, Espirito-Santo C, Simões T, Monteiro C, Serrão EA, Pearson GA, Santos R. (2008) Periodicity of propagule expulsion and settlement in the competing native and invasive brown seaweeds, *Cystoseira humilis* and *Sargassum muticum* (Phaeophyta). *Eur J Phycol*, **43**, 275-282.

Fenoglio-Marc L, Tel E (2010) Coastal and global sea level change. *Journal of Geodynamics*, **49**, 151–160.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

Garrabou J, Perez T, Sartoretto S, Harmelin JG (2001) Mass mortality event in red coral Corallium rubrum populations in the Provence region (France, NW Mediterranean). *Marine Ecology Progress Series*, **217**, 263–272.

Gbif (2012) Global Biodiversity Information Facility (GBIF). *Natural History*, **29**, 1–2.

Giaccone, G., Alongi, G., Pizzuto, F., and Cossu, A. V. L. (1994). La Vegetazione marina bentonica fotofila del Mediterraneo: 2.: Infralitorale e Circalitorale: proposte di aggiornamento. *Boll. dell'Accademia Gioenia di Sci. Nat.* **27**, 111–157.

Goecke F, Labes a, Wiese J, Imhoff J (2010) Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, **409**, 267–299.

Gómez Garreta, A., Barceló, M. C., Gallardo, T., Pérez-Ruzafa, I., Ribera, M. A., & Rull, J. (2001). Flora Phycologica Iberica Vol. 1. Fucales. *Universidad de Murcia*.

Guiry MD, Guiry GM (2013) AlgaeBase. *National University of Ireland*.

Hamilton MB (2009) *Population genetics.* Wiley-Blackwell.

Harley CDG, Randall Hughes A, Hultgren KM *et al.* (2006) The impacts of climate change in coastal marine systems. *Ecology Letters*, **9**, 228–241.

Hollants J, Leliaert F, De Clerck O, Willems A (2013) What we can learn from sushi: a review on seaweed-bacterial associations. *FEMS microbiology ecology*, **83**, 1–16.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.

IPCC (2013) IPCC Fifth Assessment Report (AR5). *IPCC.*

Jackson JBC (2001) Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *Science*, **293**, 629–637.

Leslie HM (2008) Global Coastal Change. *Quarterly Review of Biology*, **83**, 136–137.

Liquete C, Piroddi C, Drakou EG *et al.* (2013) Current Status and Future Prospects for the Assessment of Marine and Coastal Ecosystem Services: A Systematic Review. *PLoS ONE*, **8**.

Macic V, Antolic B, Thibaut T, Svircev Z (2010) Distibution of the most common *Cystoseira* c . Agardh species (heterokontophyta , fucales) on the coast of montenegro (south-east adriatic sea). *Fresenius Environmental Bulletin*, **19**, 1191–1198.

Malm T, Isaeus M (2005) Distribution of macroalgal communities in the central Baltic Sea. *Annales Botanici Fennici*, **42**, 257–266.

Manel S, Holderegger R (2013) Ten years of landscape genetics. *Trends in ecology & evolution*. **28**, 614-621

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**, 189–197.

Margalet JL, Almaraz T, Navarro MJ, Pérez-Ruzafa IM (1993) Mapas de distribución de algas marinas de la Península Ibérica. III. *Fucus ceranoides* L., *E. serratus* L., *E. spiralis* L. y *E. vesiculosus* L. (Fucales, Fucophyceae)*. *Botanica Complutensis*, **18**, 267–290.

Marzinelli EM, Williams SB, Babcock RC *et al.* (2015) Large-scale geographic variation in distribution and abundance of Australian deep-water kelp forests. *PLoS ONE*, **10**.

Merzouk A, Johnson LE (2011) Kelp distribution in the northwest Atlantic Ocean under a changing climate. *Journal of Experimental Marine Biology and Ecology*, **400**, 90–98.

Mineur F, Arenas F, Assis J *et al.* (2015) European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of Sea Research*, **98**, 91–108.

Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ (2013) Predicting evolutionary responses to climate change in the sea. *Ecology letters*, **16**, 1488–500.

Perkol-Finkel S, Airoldi L (2010) Loss and recovery potential of marine habitats: an experimental study of factors maintaining resilience in subtidal algal forests at the Adriatic Sea. *PLoS ONE*, **5**, e10791.

Rabalais NN, Turner RE, Díaz RJ, Justić D (2009) Global change and eutrophication of coastal waters. *ICES Journal of Marine Science*, **66**, 1528–1537.

Ribera M a., Gomez Garreta a., Gallardo T *et al.* (1992) Check-list of Mediterranean Seaweeds. I. Fucophyceae (Warming, 1884). *Botanica Marina*, **35**, 109–130.

Robvieux P (2013) Conservation des populations de Cystoseira en régions Provence-Alpes-Côte-d’Azur et Corse. *Université de Nice - Sophia Antipolis - UFR Sciences*, *Version 1*, 302. Retrieved from http://tel.archives-ouvertes.fr/tel-00876899/

Sales M, Ballesteros E (2009) Shallow *Cystoseira* (Fucales: Ochrophyta) assemblages thriving in sheltered areas from Menorca (NW Mediterranean): Relationships with environmental factors and anthropogenic pressures. *Estuarine, Coastal and Shelf Science*, **84**, 476–482.

Serio D, Alongi G, Catra M, Cormaci M, Furnari G (2006) Changes in the benthic algal flora of Linosa Island (Straits of Sicily, Mediterranean Sea). *Botanica Marina*, **49**, 135–144.

Sellner KG, Doucette GJ, Kirkpatrick GJ (2003) Harmful algal blooms: Causes, impacts and detection. *Journal of Industrial Microbiology and Biotechnology*, **30**, 383–406.

Soltan D, Verlaque M, François Boudouresque C, Francour P (2001) Changes in Macroalgal Communities in the Vicinity of a Mediterranean Sewage Outfall After the Setting Up of a Treatment Plant. *Marine Pollution Bulletin*, **42**, 59–70.

Spalding MD, Ruffo S, Lacambra C *et al.* (2014) The role of ecosystems in coastal protection: Adapting to climate change and coastal hazards. *Ocean and Coastal Management*, **90**, 50–57.

Steneck RS, Graham MH, Bourque BJ *et al.* (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, **29**, 436–459.

Storfer a, Murphy M a, Evans JS *et al.* (2007) Putting the “landscape” in landscape genetics. *Heredity*, **98**, 128–42.

Strain EMA, Thomson RJ, Micheli F, Mancuso FP, Airoldi L (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology*, **20**, 3300–3312.

Thibaut T, Blanfuné A, Boudouresque C-F *et al.* (2016) Unexpected Temporal Stability of *Cystoseira* and *Sargassum* Forests in Port-Cros, one of the Oldest Mediterranean Marine National Parks. *Cryptogamie, Algologie*, **37**, 61–90.

Thibaut T, Blanfune A, Boudouresque C-F, Verlaque M (2014) Decline and local extinction of Fucales in French Riviera: the harbinger of future extinctions? *Mediterranean Marine Science*, **16**, 206–224.

Thibaut T, Pinedo S, Torras X, Ballesteros E (2005) Long-term decline of the populations of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Albères coast (France, North-western Mediterranean). *Marine Pollution Bulletin*, **50**, 1472–89.

Vellend M, Geber MA (2005) Connections between species diversity and genetic diversity. *Ecology Letters*, **8**, 767–781.

Vergés A, Alcoverro T, Ballesteros E (2009) Role of fish herbivory in structuring the vertical distribution of canopy algae Cystoseira spp. in the Mediterranean Sea. *Marine Ecology Progress Series*, **375**, 1–11.

Wahl M (2009) *Marine Hard Bottom Communities* (M Wahl, Ed,). Springer Berlin Heidelberg, Berlin, Heidelberg.

Wahl M, Goecke F, Labes A, Dobretsov S, Weinberger F (2012) The second skin: Ecological role of epibiotic biofilms on marine organisms. *Frontiers in Microbiology*, **3**. 292

Worm B, Barbier EB, Beaumont N *et al.* (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science (New York, N.Y.)*, **314**, 787–90.

Zhang KQ, Douglas BC, Leatherman SP (2004) Global warming and coastal erosion. *Climatic Change*, **64**, 41–58.

CHAPTER 1

Microsatellite markers for the fucoid macroalgae *Cystoseira compressa* (Esper) Gerloff & Nizamuddin.

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Abstract

Cystoseira compressa is a brown macroalga widespread in the Mediterranean Sea and adjacent North East Atlantic waters. Rising seawater temperature and increasing frequency of extreme events are threatening this ecologically valuable foundation species. Understanding the species effective dispersal, connectivity and general populations structuring, which are currently unknown, is a priority for its effective conservation. We developed 12 microsatellite markers, in order to provide genetic tools for restoration and management and improve the understanding of the consequences of changing climate. The loci were validated on two populations of southern Italian coasts about 400 km apart. Allelic richness within population ranged between 2 and 6 and mean gene diversity (H_E) between 0.033 and 0.65. Distinct genotypes were associated to single individuals and the two populations were clearly differentiated (F_{ST}= 0.373). These results confirm the usefulness of these markers for future studies of population genetics and management of the species.

Keywords microsatellites; genetic diversity; brown macroalgae; *Cystoseira compressa*

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Introduction

Fucoid algae of the genus *Cystoseira* are ecologically important habitat forming species, contributing to the complexity and diversity of coastal ecosystems. However, various species of the group are declining across their distribution range, especially in the Mediterranean Sea, where there is a high level of endemism (Airoldi and Beck 2007). These macroalgae are endangered mainly by local scale impacts (eutrophication, coastal infrastructure, sediment load) and by natural events connected to climate change (heat waves, increase storminess) (Perkol-Finkel and Airoldi 2010; Strain et al. 2014; Airoldi et al. 2015). *Cystoseira compressa* is one of the most widespread species of the genus and can be found all across the Mediterranean Sea and in the adjacent North East Atlantic Ocean, along Moroccan and Portuguese coasts. It occupies the shallow subtidal and intertidal zone in rocky shores, which makes it more exposed to anthropic pressures. Although *C. compressa* is more resilient to local stressors than other comparable species (Mangialajo et al. 2008b; Mangialajo et al. 2008a), the ability of the species to cope with global climate change is unknown. Most late succession brown algae are characterized by low dispersal, which limits their ability to colonize new areas and to shift their distributional ranges to track the temperature change (Nicastro et al. 2013; Neiva et al. 2015). High-resolution molecular markers enable the assessment of the population genetic structure and connectivity of *C. compressa* and therefore provide insights into the evolutionary biology, ecology and conservation of the speceis (Balloux and Lugon-Moulin 2002). This information provides a fundamental step to improve the management of the species and to understand the consequences of ongoing climatic and environmental changes.

Material and methods

Genomic DNA was extracted from 12 individuals of *C. compressa* originating from two locations in the Iberian Peninsula (2 individuals from Mallorca in Spain and Algarve in Portugal) and three locations from Italian coasts: Ancona (2 individuals), Livorno (3 individuals) and Crotone (3 individuals). From each individual a total of 100 mg of silica dried tissue were used for genomic DNA extraction following the CTAB protocol (Doyle and Doyle 1990) and the pooled DNA was sequenced using 454 DNA shotgun pyrosequencing (Biocant - Cantanhede, Portugal), producing 141780 individual sequences, ranging from 44 bp to 612 bp. Microsatellite loci from di- to hexanucleotide motifs were searched among the sequences obtained, using WebSat (Martins et al. 2009). Based on a high number of repeats, the presence of anchoring sites and the melting temperature of primers, 24 potential microsatellites were selected and tested using labeled primers. According to their amplification success, the possibility of multiplex reaction and polymorphism levels, 12 out of the 24 microsatellites were selected. These microsatellite loci amplified consistently and proved to be polymorphic across 60 specimens coming from two populations of Sicily island, Italy (Portopalo di Capopassero – SR, 36.681627 N 15.137066 E, and Sant'Elia – PA, 38.096884 N, 13.540001 E) (Table 1).

For genotyping, 10 ng of DNA and fluorescent-dye labeled primers were used to produce three different multiplex reactions (Table 1) using the QIAGEN multiplex kit (Cat No./ID:206143) according to the manufacture protocol. All polymerase chain reactions (PCR) were performed in a GeneAmp 9700 thermocycler (PE, Applied Biosystems) using the following conditions: 15 min at 95°C denaturation phase followed by 35 cycles of 30 s at 94°C, 1 min 30 s at 58°C annealing step and 1 min at 72°C. Final elongation step was set at 30 min at 60°C. Genotyping was performed on an ABI Prism 3130xl automated DNA sequencer with GeneScan 500 Liz size standard (Applied Biosystems) at CCMAR (Faro, Portugal).

Allele sizes were scored using STRand v.2.4.59 (http://www.vgl.ucdavis.edu/STRand). The number of alleles per locus, observed (H_O), expected (H_E) heterozygosity and genetic distance matrix among samples were estimated using Genalex (Peakall and Smouse 2006). Linkage disequilibrium and deviations from Hardy-Weinberg equilibrium (HWE) as F_{IS} were tested in GENEPOP v. 4.2.2 (Rousset 2008). Null allele presence was detected with Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004).

Results and Discussion

The number of alleles per locus ranges from 2 to 6 alleles per locus per population with a mean value of 3.25 and 3.50 for the two populations of Porto Palo and Sant'Elia respectively. Observed heterozygosity ranged from 0.033 to 0.300 while expected heterozygosity from 0.033 to 0.656. The allelic richness found is comparable to what was observed in other Fucales (Engel et al. 2003) but lower than that shown by recently developed microsatellite markers for *Cystoseira tamariscifolia* and *Cystoseira amentacea* (Robvieux et al. 2012; Engelen et al. 2016).

Heterozygosity deficit and deviation from Hardy-Weinberg equilibrium were detected at most of the loci and Microchecker reported evidence of null alleles with the exception of loci Cc22, Cc04, Cc06. This deficit was mostly consistent across populations and may be due to inbreeding among relatives or Wahlund effect. Linkage disequilibrium after Bonferroni correction was significant only for the loci pair Cc9 – Cc19.

Each specimen was identified by a unique multi locus genotype and allele polymorphism was sufficient to clearly identify differentiation between population clusters ($F_{ST} = 0.373$; $p < 0.01$) (Fig. 1). This preliminary assessment demonstrated the efficacy of the 12 markers in populations genetic studies, even at relatively small spatial scale. *Cystoseira compressa* is a widespread species in the Mediterranean and adjacent Atlantic Ocean and is a key habitat forming species. The use of these markers can help provide essential insight for the development of management and restoration strategies for this important species. For instannce they can improve our understanding of populations connectivity among marine protected areas or assess the effectiveness of artificial structures in fostering habitat restoration.

References

Airoldi L, Ballesteros E, Buonomo R, Van Belzen J, Bouma TJ, Cebrian E, De Clerk O, Engelen AH, Ferrario F, Fraschetti S, Gianni F, Guidetti P, Ivesa L, Mancuso FP, Micheli F, Perkol-Finkel S, Serrao EA, Strain EM, Mangialajo L (2015) Marine forests at risk: solutions to halt the loss and promote the recovery of mediterranean canopy-forming seaweeds. *Proceedings of the 5th Mediterranean Symposium on Marine vegetation.* Portoroz, Slovenia. 2014

Airoldi L, Beck MW (2007) Loss, status and trends for coastal marine habitats of europe. *Oceanogr Mar Biol An Annu Rev,* ***45****,* 345–405.

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular ecology,* **11**, 155–65.

Doyle, J. J. and J. L. Doyle. 1990. A rapid total DNA preparation procedure for fresh plant tissue. *Focus* **12**, 13-15.

Engel CR, Brawley S, Edwards KJ, Serrão E (2003) Isolation and cross-species amplification of microsatellite loci from the fucoid seaweeds *Fucus vesiculosus*, *F. serratus*, and *Ascophyllum nodosum* (Heterokontophyta, Fucaceae). *Mol Ecol Notes,* **3**, 180–2.

Engelen, A. H., Costa, J., Bermejo, R., Marba, N., Duarte, C. M., & Serrão, E. A. (2016) A population genetics toolbox for the threatened canopy-forming brown seaweeds *Cystoseira tamariscifolia* and *C. amentacea* (Fucales, Sargassaceae). *Journal of Applied Phycology,* 1-3, in press.

Mangialajo L, Chiantore M, Cattaneo-Vietti R (2008a) Loss of fucoid algae along a gradient of urbanisation, and structure of benthic assemblages. *Mar Ecol Prog Ser.* **358,** 63–74. doi: 10.3354/meps07400

Mangialajo L, Chiantore M, Cattaneo-Vietti R (2008b) Changes in marine macroalgal assemblages due to human impacts: general trends and applications in coastal management. *Biol Mar Mediterr,* ***15****,* 115–117.

Martins WS, Lucas DCS, Neves KFDS, Bertioli DJ (2009) WebSat - a web software for microsatellite marker development. *Bioinformation*, **3**, 282–283

Neiva J, Assis J, Coelho NC, Fernandes F, Pearson GA, Serrão EA (2015) Genes Left Behind: Climate Change Threatens Cryptic Genetic Diversity in the Canopy-Forming Seaweed *Bifurcaria bifurcata*. *PLoS One,* doi: 10.1371/journal.pone.0131530

Nicastro KR, Zardi GI, Teixeira S, Neiva J, Serrão EA, Pearson GA (2013) Shift happens: trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga *Fucus vesiculosus. BMC Biol* **11,**.doi: 10.1186/1741-7007-11-6

Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* **6**, 288–295. doi: 10.1111/j.1471-8286.2005.01155.x

Perkol-Finkel S, Airoldi L (2010) Loss and recovery potential of marine habitats: an experimental study of factors maintaining resilience in subtidal algal forests at the Adriatic Sea. *PLoS One* **5**, e10791.

Robvieux P, Videment J, Ribout C, et al (2012) First characterization of eight polymorphic microsatellites for *Cystoseira amentacea* var. *stricta* (Fucales, Sargassaceae). *Conserv Genet Resour,* **4**, 923–925. doi: 10.1007/s12686-012-9674-x.

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103-106.

Strain EMA, Thomson RJ, Micheli F, et al (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Glob Chang Biol,* **20**, 3300–3312. doi: 10.1111/gcb.12619.

Van Oosterhout C, Hutchinson WF, Wills P, Shipley P (2004) Micro- checker: software for identifying and correcting genotyping errors in microsatellites data. *Mol Ecol Notes,* **4**, 535–538.

Figures and tables

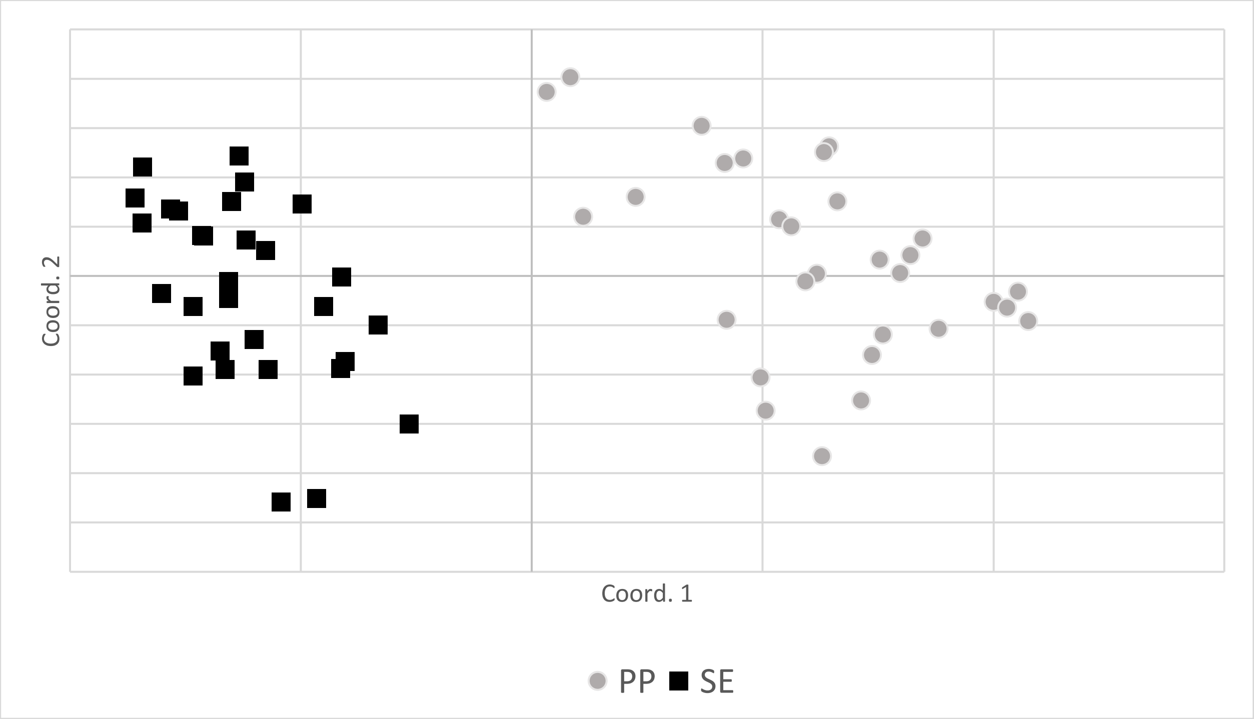

Figure. 1 -. Principal Coordinate Analysis based on genetic distance among samples. PP: Portopalo; SE: Sant'Elia populations.

Table 1 - Characterization of 12 polymorphic microsatellite loci in C. compressa

							Portopalo				*Sant'Elia*			
Multiplex mix	**Locus**	**Genebank code**	**Motif**	**Primer Sequence**	**Ta (ºC)**	**Size Range (bp)**	***Na***	H_e	H_o	F_{is}	***Na***	H_e	H_o	F_{is}
1	Cc01	KY820873	$(TA)_{10}$	F: GCTGGCGTTCTTTGTAGGTACT R: AAGATGGAAGCGAGTCAGTCA	58	235-254	5	0.538	0.233	0.566*	4	0.488	0.167	0.659*
	Cc06	KY820875	$(TAC)_{10}$	F: CACCTTCTGATCTGCTGGAGT R: TCAAGTATGAAACACCAAAGGG	58	288-297	3	0.513	0.133	0.740*	2	0.375	0.033	0.911*
	Cc04	KY820874	$(TAGCTG)_6$	F: GGGTTTACAGGACGTTAATGGA R: ATCGACAGCTATTCCTCAAAGC	58	393-411	2	0.180	0.067	0.630*	2	0.033	0.033	-0.017
	Cc11	KY820876	$(ACT)_{11}$	F: GGTAAGATTTATTGGGGAAGGC R: TCAGCGAGAAACAACGAAGATA	58	299-311	3	0.347	0.069	0.801*	4	0.332	0.167	0.497*
	Cc07	KY820877	$(ACT)_{11}$	F: AAGCTATCGGCGCATTAAAA R: CCTTCTTATTGTCGAGTCCCAG	58	204-227	3	0.509	0.100	0.804*	2	0.206	0.033	0.838*
2	Cc02	KY820878	$(AT)_{14}$	F: CAGCATAGAACGGTGACGAA R: ACTATACACACTTTCCCCAGGC	58	229-233	3	0.291	0.067	0.771*	2	0.124	0.133	-0.071
	Cc05	KY820879	$(TA)_{11}$	F: AAGATTAGTATGACAGGGCCGA R: GACTCAAGTAAGCACATGGCAG	58	263-279	5	0.467	0.067	0.857*	6	0.591	0.200	0.661*
	Cc09	KY820880	$(AGGC)_{10}$	F: CCGAGTTTATCAGGTGTCATCA R: AGAGAGTCCTCCTCCTCCCTT	58	273-334	3	0.557	0.034	0.938*	6	0.656	0.100	0.848*
3	Cc14	KY820881	$(AT)_9$	F: CATACACGTAGGGTGCAGGTAA R: TCAGTTTTCAAGGGGCTCTCTA	58	184-203	3	0.539	0.167	0.691*	6	0.543	0.233	0.570*
	Cc19	KY820882	$(AT)_9$	F: CTAACAATGCCCTCCTCAAAAC R: ATGTTTCACCTGTGTCCCTTTC	58	157-165	4	0.507	0.133	0.737*	4	0.545	0.200	0.633*
	Cc22	KY820883	$(CGTCAA)_6$	F: ACGGTTACGCCAGTAGAAATGT R: AGCTACCCGATGCACGAT	58	194-209	2	0.033	0.033	-0.017	2	0.033	0.033	-0.017
	Cc24	KY820884	$(GTA)_{12}$	F: AATGAATGGACAGATGTTGCC R: CTCGACAATATGACACGTCCC	58	310-319	3	0.512	0.300	0.414*	2	0.420	0.200	0.524*

* significant deviation from HWE (by Markov chain method).

CHAPTER 2

Predicted extinction of unique genetic diversity in marine forests of *Cystoseira* spp

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Abstract

Climate change is inducing shifts in the range of species distribution across the globe. Range shifts can affect the genetic pools of species, including loss of genetic variability and evolutionary potential. Species with long generation time, low dispersal and connectivity, such as brown algae, are more prone to genetic erosion. In this study, we focus on three habitat-forming seaweed species throughout their entire ranges in the Atlantic and Mediterranean regions, *Cystoseira tamariscifolia*, *C. amentacea* and *C. compressa*. We aim to 1) describe their population genetic structure and diversity, 2) model the present and predict the future distribution and 3) assess the consequences of range shifts for their population genetic structure, according to an optimistic and a pessimistic climatic scenario. Our results produced models that were accurate, according to performance statistics, in describing current distribution. A net loss of suitable areas was predicted in both climatic scenarios across the range of distribution of the three species. This loss was particularly severe for *C. amentacea* in the Mediterranean Sea (-90% in the worst climatic scenario), suggesting that the species might become potentially at extinction risk. In all species, genetic data showed very differentiated populations, indicating low inter-population connectivity, and high and distinct genetic diversity in areas that were predicted to become lost, causing erosion of unique evolutionary lineages. Our results indicated that the Mediterranean Sea is the most threatened region, where future suitable C*ystoseira* habitats will become more limited and species with the same ecological niche and functional role are absent. The projected accelerated loss of already fragmented and disturbed populations and the long-term genetic effects highlight the urge for local scale management strategies that sustain the capacity of these habitat forming species to cope with and adapt to climatic impacts while waiting for global emission reductions.

Introduction

The distribution of species is predicted to be increasingly affected by anthropogenic climate warming (Sunday *et al.* 2012). Geographic range shifts caused by recent temperature changes have been described for many marine species, including sessile ecosystem structuring canopies (e.g., Smale & Wernberg 2013; Nicastro et al. 2013; Lourenço et al. 2016). In sessile species, range shifts happen by local extinction at the trailing edge and dispersal and colonization of new areas at the expanding edge. Such changes are a product of a complex interaction of factors: abiotic conditions, biotic interactions, dispersal potential and evolutionary capacity (Soberon 2005), which complicate predictions of future distributions. However, mechanistic understanding of these processes at the community level is commonly limited and predictions can be based solely on climatic data (Bates *et al.* 2014), for which future predicted values are available and have successfully been applied (e.g., Assis et al. 2014; Neiva et al. 2015). Latitudinal range contractions and expansions of marine organisms are more predictable because their current distribution ranges tend to follow their thermal limits closely (Sunday *et al.* 2012). Besides, assuming that correlative species distribution modeling based on environmental data approximates the fundamental "climatic" niche of a species, these models may be suitable for large-scale range shift predictions (Peterson 2006; Kearney & Porter 2009).

When species are subject to rapid changes in their distribution they can be exposed to genetic erosion and loss of genetic diversity (Hampe & Petit 2005; Nicastro *et al.* 2013; Neiva *et al.* 2015). Taking into account the genetic consequences of species distribution range shifts is still relatively recent (Pfenninger *et al.* 2012), despite its importance for the future evolutionary potential and resilience of the species and the ecosystem they support (O'Leary et al 2017).

Species with longer generation times, limited population connectivity, and restricted dispersal are more prone to local extinction and genetic erosion due to changes in climate conditions (Bates *et al.* 2014). Key fundamental marine organisms that fit these criteria are late succession brown algae. For various representatives of this group local to regional extinctions are increasingly reported, such as for *Scytothalia dorycarpa* (Smale & Wernberg 2013), *Fucus vesiculosus* (Nicastro *et al.* 2013; Assis et al. 2014), *Bifurcaria bifurcata* (Neiva *et al.* 2015), *Fucus guiryi* (Lourenço et al. 2016). Understanding the evolution of distributional ranges is therefore important for conservation and management of this ecologically important group.

Here, we aim to use closely related species with distinct distribution ranges to 1) estimate the genetic structure and diversity of three habitat-forming seaweed species, *Cystoseira* *tamariscifolia*, *C. amentacea* and *C. compressa*, throughout their entire distributions in the Atlantic and Mediterranean, 2) model their present and predict their future distributions and 3) assess the consequences of range shifts for their population genetic structure, according to different climatic scenarios. Our comparative approach allowed us to infer future conservation challenges for key structural species of the Mediterranean and Atlantic, and highlighted particularly high threat levels predicted for Mediterranean endemic biodiversity.

Methods

Biological data and study area

We selected three representative species of the genus *Cystoseira* (C. *tamariscifolia, C. amentacea* and *C. compressa*). The species *C. tamariscifolia* occurs exclusively in the Atlantic and the entrance to the Mediterranean, *C. amentacea* is a Mediterranean endemic, and *C. compressa* occurs throughout the Mediterranean but also in the Atlantic transition zone and in the Azores. We compiled georeferenced presence records from field observations, available literature and online databases. We found 52 publications (Table S1) with a useful description of species location and used two available databases: Algaebase (Guiry & Guiry 2013) and the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/). All the records were double-checked for referencing errors and misplacement.

We used a resolution of 5 arc minutes (~ 9.2 km) for georeferencing species data as well as the climatic variables. We obtained 259 occurrence records for *C. amentacea* (171 cells), 475 for *C. compressa* (282 cells), and 580 for *C. tamariscifolia* (315 cells).

The study area covered the geographic distribution of the three species, comprising the Mediterranean and the Black Sea and the East coast of the North Atlantic Ocean, from Cape Verde islands to the Baltic Sea. As these species occur in shallow subtidal or intertidal zones, we delimited a 20 m depth threshold along the coastline from the 30 arc-seconds resolution GEBCO's gridded bathymetric data set (http://www.gebco.net/).

Environmental data

We selected the most relevant ocean variables, available for both current and future climatic conditions, according to previous literature on niche models affecting population genetic diversity of brown algae (Assis et al. 2014, 2016a,b; Neiva et al. 2014, 2015). Maximum, mean, minimum and range values of sea surface temperature (SST), surface air temperature (SAT) (because the upper limits of the species are in the intertidal zone), and mean salinity (particularly relevant in the Baltic) were used. Data were obtained from Bio-ORACLE dataset (Tyberghein *et al*. 2012) and tested by Pearson's correlation coefficient < |0.7| in our study area. We also produced a multi-model ensemble using five Ocean General Circulation Models (CNRM-CM5, HadGEM2-ES, IPSL-CM5A-LR, MPI-ESM-MR and NASA/GISS) pertaining to the Coupled Model Intercomparison Project Phase 5 (CMIP5; http://cmip-pcmdi.llnl.gov/cmip5/). We averaged 20 years of monthly data for 2050 (from 2030 to 2050) and 2100 (from 2080 to 2100) pertaining to two contrasting future scenarios : the high (RCP 8.5) and low (RCP 2.6) emissions scenarios of the IPCC Representative Concentration Pathways projections (Moss *et al.* 2010; van Vuuren *et al.* 2011). These scenarios allow projecting future environmental data according to different trajectories of socio-economic development and associated greenhouse gas emissions (van Vuuren *et al.* 2011).

Current and future SDMs

We implemented an ensemble approach to obtain the current and the two future climate scenarios predictions for the three *Cystoseira* species. We used the “biomod2” package (Thuiller *et al.* 2014) to perform six presence-absence algorithms: flexible discriminant analysis (FDA), generalized additive model (GAM), generalized boosting model (GBM), generalized linear model (GLM), multiple adaptive regression splines (MARS), and randomForest (RF). We used two sets of pseudo-absences extracted at random in a proportion of ten pseudo-absences per presence cell as previously done in Chefaoui et al. (2008 and 2016). Data were split into a calibration (70 %) and a validation set (30 %) in each of the 10 iterations performed for each model and set of pseudo-absences. Thus, 120 models were computed for each species (2 pseudo-absence sets x 6 modeling techniques x 10 iterations). We evaluated model performance using the true skill statistic (TSS; Allouche et al. 2006), the area under the receiver operating characteristic (ROC) curve (AUC), and ROC-derived sensitivity (presences correctly predicted) and specificity (absences correctly predicted) measures (Fielding and Bell 1997), with the threshold that optimized ROC and TSS scores (Thuiller et al. 2014). The consensus prediction under current conditions for each species was an ensemble computed through the average of binary predictions (committee averaging ensemble), an approach which obtained the best performance predicting other coastal species (Chefaoui et al. 2016). To produce the ensemble, we strictly used just the models with TSS > 0.7. Ensembles were projected to the RCP 2.6 and RCP 8.5 scenarios to obtain the predictions for the future distribution. To assess the uncertainty in future projections related to the different values of each variable between the training range and the novel scenarios we computed a clamping mask. We estimated the importance of each variable following a procedure similar to "randomForest" (Liaw and Wiener 2002). The correlation between the full model and a model rearranged without one of the variables using three iterations was calculated to obtain an importance value from 0 to 1 (highest importance) (Thuiller et al. 2013). Analyses were performed in R (R Core Team 2015) using a computational cluster facility.

Genetic analysis

Genomic DNA was extracted from algal tissue using the cetyl trimethyl ammonium bromide (CTAB) method optimized for brown algae (Hoarau et al. 2007). For *C. amentacea*, a selection of 8 microsatellites previously developed for *C. tamariscifolia* were used (Engelen et al. 2016, Chapter 3). For *C. tamariscifolia* and *C. compressa*, specifically developed microsatellite markers were used (respectively 6 and 10) (Chapter 1). Fragment lengths were measured on an ABI PRISM 3130 DNA analyzer (Applied Biosystems) with the LIZ 500 (GeneScan) standard at the CCMAR sequencing platform. Alleles were scored using the free software STRand (Toonen & Hughes 2001) and further binned into size classes with the msatAllele R package (Alberto 2013). Departures from Hardy-Weinberg equilibrium (null hypothesis H_0: F_{IS}=0) were tested with permutations implemented in GENETIX (Belkhir et al. 1996-2004) and confidence intervals were estimated by bootstrapping (number of replicates =1000) within the DiveRsity R package (Keenan et al. 2013). Allelic richness and confidence intervals were determined after 999 resampling in DiveRsity. We used the STRUCTURE software to infer population structure (Pritchard *et al.* 2000). This analysis was replicated ten times and the mean of the permuted Q-matrices across replicates was obtained in CLUMPP (Jakobsson and Rosenberg 2007). The resulting output was plotted with DISTRUCT (Rosenberg 2002; http://www.cmb.usc.edu/noahr/distruct.html).

Results

Species distribution models

After correlation analysis, three variables were used for the modeling approach: maximum SST, minimum SST and the range of SAT. Overall validation scores were high for the models of the three *Cystoseira* species (Table 1). GBM, RF and GLM obtained more accurate results, though no model performed consistently better across all species. FDA and MARS models for *C.*

tamariscifolia and GLM for *C. compressa* produced lower validation scores in TSS than the threshold (TSS<0.7) and were excluded from the ensemble model. Overall, *Cystoseira amentacea* obtained the highest validation scores in the ensemble model (TSS = 0.809) compared to *C. tamariscifolia* and *C. compressa* (TSS = 0.781 and 0.771 respectively)

Variable importance scores revealed differences depending on the species, although the highest relevance was always achieved by SST (Figure 1). Minimum SST was the variable with greater relative importance for the models of two mainly mediterranean species: *C. amentacea* and *C. compressa,* suggesting that the lower SST of the Atlantic waters adjacent to the Mediterranean might be limiting their current distribution. For the Atlantic species, *C. tamariscifolia*, maximum SST obtained the higher scores in the models.

Ensemble models for current conditions correctly matched the present known distribution of the three species (figure 2). *C. compressa* showed the wider longitudinal range, encompassing the eastern Mediterranean Sea from Greece and Turkey to the Azores islands in the Atlantic Ocean. In the Atlantic coast, prediction for this species extended continuously from Morocco to the South of Portugal. Suitable climatic niche for the species was also found in the Cantabrian Sea but we could not find records of *C. compressa* in this region, either because of discontinuity in the suitable habitat along the Portuguese coast or because of under-sampling efforts in the region. Suitable habitats for *C. tamariscifolia* are distributed mainly on the Atlantic coast, from Western Sahara to the north of England, with an intrusion in the Mediterranean coasts of southern Spain, Morocco and Algeria. According to its observed distribution and our models, *C. amentacea* is essentially a Mediterranean endemic species, with some potential suitable area in southern Portugal not supported by direct observational records, and low presence probability on the west coast of Portugal.

By 2050 our ensemble models predicted already considerable changes in probabilities of occurrence, particularly for the species *C. amentacea* and *C. compressa* (Fig. S1). For those species, only between 30% and 43% (depending on the RCP scenario) of the current distribution range was predicted to maintain a high probability of occurrence (Fig. 3). In contrast, in both scenarios, *C. tamariscifolia* showed much less loss of suitable area, conserving between 75% and the 81% of present distributional area in the optimistic and pessimistic scenarios respectively. According to our model, by 2100, the present known distribution will largely retract due to decrease in the probability of occurrence or local populations extinctions under both climate scenarios considered (Fig. 2). However, some considerable differences could be found among species. For *C. compressa* the model predicted a 52% loss of original distributional area (medium to high suitable areas with probability of occurrence > 30%) in the optimistic scenario (Fig. 3). In the pessimistic scenario, a substantial increase of new suitable area was also predicted (30% of original area of distribution), mostly along the Atlantic coasts of Portugal and France but the area lost in the Mediterranean Sea increased to 89%. The areas forecasted to become largely unsuitable for *compressa* are the central and eastern Mediterranean Sea ($P < 0.3$), especially Italian and Greek coasts where a large decrease in habitat suitability was predicted.

The ensemble model predicted a large range shift for *C. tamariscifolia* in the high emission scenario, in which the southern trailing edge would move about 2000 km north, from 20°N latitude to 33°N. However, this would not be compensated by an equal expansion of the leading edge further into the North Sea. The projected distributional range shift was much more conserved in the RCP 2.6 low emission scenario, in which the species would balance the net losses of suitable habitat with the expansion of new area (up to 10% of habitat with increased probability of occurrence, against 12% decrease in area).

The suitable habitat for *C. amentacea* was predicted to contract considerably in the optimistic scenario and even more in the RCP 8.5 scenario, with habitat loss of 48% and 90% respectively. New suitable areas would only marginally compensate this habitat loss, as they accounted for only 11% in optimistic scenario and 16% in the pessimistic one. Furthermore, the overall probability of occurrence would be lower than current levels.

Uncertainty in future predictions using the RCP 8.5 scenario was higher than for RCP 2.6 in both 2050 and 2100 projections (Supporting material S2). However, the areas with a higher uncertainty due to extreme values outside the range of the current conditions shared by both scenarios were limited to the Red Sea and some marginal areas of the Baltic Sea.. Uncertainty areas were mostly limited to habitat with no presence or low probability of occurrence for all the species and did not affect the predictions substantially.

Population structure

The ten microsatellite markers used for *C. compressa* showed 3 to 19 alleles per locus (110 in total). Seven populations out of 8 exhibited a significant positive F_{IS} indicating heterozygote deficiency (Table 2). Higher genetic diversity was found in Iberian populations (supporting material S3) compared to Italian and French populations, with the latter showing the lowest allelic richness (2.17) and H_E (0.320). The best number of clusters (K=8) matches the number of populations of *C. compressa* analyzed. Populations were differentiated but some degree of admixture exists, particularly among the western Mediterranean populations.

C. amentacea exhibited highly variable markers with 15 to 33 alleles per locus. Half of the populations presented significant heterozygote deficiency. Population structure analysis found the best number of clusters at K=10. Populations were highly differentiated across the basin and subdivision largely reflected geographic distance among populations. The Spanish population of Santa Pola was the only exception, which was assigned mainly to the same group of the population of Marseille (supporting material S4). When East (Italian) and West (France and Spain) Mediterranean populations were compared, eastern ones showed lower allelic richness, H_E and number of private alleles (Western: H_E: 0.77 ± 0.04, Ar: 16.50 ± 2.25, priv.all: 9.33; Eastern H_E: 0.64 ± 0.12, Ar: 13.50 ± 2.88, priv.all: 6.33), however these differences were non significant.

C. tamariscifolia showed an average of 24 alleles/locus for six microsatellite markers. Allelic richness, as well as H_E, significantly (Ar: R^2=0.30, p=0.05; H_E: R^2=0.37, p=0.03) decreased with increasing latitude (figure 4). A major genetic subdivision could be obtained already at K=2, dividing the distribution into a northern and southern clade of 7 and 6 populations respectively. The highest levels of genetic diversity were found in the southern Portugal populations of Sagres and Albufeira. Northern populations showed much lower number of private alleles, allelic richness and H_E when compared with the southern cluster.

Discussion

Our models revealed that populations are genetically distinct with low connectivity and predicted extensive modifications of the distribution of suitable habitats with loss of genetic diversity for the three species of *Cystoseira*, according to future climate change scenarios.. A remarkable decrease in available habitat is predicted to occur already in the best-case scenario of reduced greenhouse gases (GHG) concentration. The expansion of the distributional range would only partially compensate the loss of habitat, whereas the Mediterranean Sea would suffer considerable habitat retractions and erosion of evolutionary lineages.

Both scenarios used for the projections resulted in important changes in predicted distribution of the species, and most of the habitat loss was observed already in the best-case scenario (RCP 2.6). Even in the low GHG simulation, important areas of the Mediterranean Sea would become partially or entirely unsuitable for *C. compressa* and *C. amentacea* species. The Atlantic species, *C. tamariscifolia,* which could benefit more from the availability of new favourable habitat, would be affected by heavy losses at its trailing edge in the worst-case scenario, with great impact on the global gene pool of the species which is much richer in the southern range. This loss of suitable climatic conditions at the trailing edges is expected to drive local extinction, as local adaptation processes might hypothetically not be able to cope with the rate of change (e.g., Hu & Fraser 2016). The loss of local populations can also have cascading effects on coastal ecosystem functioning (Mineur *et al.* 2015) but, what we show here is that it can be a major cause of intra-specific genetic diversity loss.

We found high and unique diversity in the regions most vulnerable to climate change according to the projections, especially in the case of *C. tamariscifolia* and partially for *C. amentacea.* High and unique diversity is harboured on the African coast and Gibraltar region. These probably acted as climatic refugia during the Last Glacial Maximum and as origin of secondary postglacial colonization (Assis et al. 2014, 2016; Lourenço et al. 2016). According to our model, this region corresponds to the expected trailing edge of the shifting distributional range for *C. tamariscifolia* and therefore has the highest probability to be lost in the future. The Mediterranean species showed a less clear pattern of a diversity richness gradient across the distribution range. However, every population of *C. amentacea* and *C. compressa* showed high level of distinctiveness and the loss of any population might be linked to the permanent loss of genetic variability out of the genetic pool of the species (Hampe & Petit 2005) and possibly of locally adapted pools. The presence of distinct genetic lineages was less evident but still detectable for *C. compressa* and no strong West-East pattern of genetic diversity across the distribution could be found. This lack could be linked to the buoyancy properties of *C. compressa,* which differently from other *Cystoseira* species has aerocysts on the apical branches, which allow for greater dispersal capability (Falace *et al.* 2005). Also this species has a less fragmented presence across its range of distribution which could have maintained a higher gene flow.

The predicted habitat shift of the expanding edge should allow for colonization of new areas, particularly along the North Atlantic and the North Sea coasts. *C. compressa* was predicted to thrive well mostly in its Atlantic distribution and extend further north like *C. tamariscifolia.* However, only for the latter species the gained areas could quantitatively compensate the area lost at the trailing edge and only in the best-case scenario. Also, the actual spatial tracking of the environmental niche can lag behind the increase in suitable areas (Urban *et al.* 2012). Low dispersal traits can increase the time needed for effective colonization of new areas (Urban *et* *al.* 2012; Knutsen *et al.* 2013). This is especially the case if habitat is fragmented (Opdam & Wascher 2004), and biotic interactions might limit settlement or development (Urban *et al.* 2012; Cahill *et al.* 2013). Geographic range expansions will occur starting from expanding marginal populations of the present distributional range that are, at least for *C. tamariscifolia,* already low in genetic diversity. This is the pattern expected and well documented for expansions into pre-glaciated regions (e.g., Assis et al. 2016). The expected range shift is likely to reduce further the heritable variability of marginal population and compromise the ability to respond to selection under environmental changes (Pujol & Pannell 2008). In contrast, the expanding edge for *C. compressa* would correspond to the Gibraltar Strait region, which does not present depleted genetic diversity compared to the genetic pool of the populations. Considering this species' higher dispersal capabilities, it might have better opportunities to succeed in colonizing new areas. *C. amentacea* was also predicted to find increasing suitable habitat towards the North Atlantic coasts, however, its fragmented distribution and lack of floating vescicles could drastically reduce the likelihood of dispersal, as demonstrated by low levels of genetic exchanges among populations.

Mediterranean vs. Atlantic ecosystems

Our results suggest that the Mediterranean region will probably face substantially different ecological problems than the North Atlantic coasts. Atlantic populations of *Cystoseira* species analyzed here were predicted to be subjected to important changes in their distribution, but suitable climatic conditions would persist and eventually expand along most of the Atlantic range. Although the global area is not as reduced as in the more Mediterranean species, the evolutionary genetic consequences would however be very large in this Atlantic species, with great empoverishment of the species gene pool as the southern populations become lost. This is due to the sharp gradient of variation on genetic diversity from south to north along Atlantic coasts that comprise a large gradient of latitudes and reach previously glaciated areas which could only recently have been colonized by the present populations. From an ecological stand point, the northwards expansion and colonization of new habitats by *Cystoseira tamariscifolia* in the Atlantic could result in unpredictable changes in ecosystems as the shallow subtidal and intertidal rocky shore is often shared with other canopy-forming species, such as *Laminaria spp.* and *Fucus spp.,* which contribute to structural complexity of the ecosystem and have a partially overlapping functional role (Airoldi & Beck 2007).

In contrast with the Atlantic populations, Mediterranean ones are expected to suffer major changes in climatic conditions which, consequently, reduce their probability of presence. The Eastern Mediterranean is the region that will be more affected by the increase in SST with large effects on the habitat distribution of *C. compressa* and *C. amentacea*, already in the best-case scenario. Climatic conditions in this area were predicted to become largely unsuitable for the latter species with a potential risk of extinction in the whole basin under the business-as-usual scenario.

These canopy-forming macroalgae characterize specific communities (EUNIS A3.132 and A3.333) (Davies *et al.* 2004). In the Mediterranean Sea, they represent the most complex and stable communities that can dominate the shallow euphotic fringe and essentially have no functional equivalent (Benedetti-Cecchi *et al.* 2001), particularly in wave exposed areas where *C. amentacea* dominates (Bulleri *et al.* 2002). It has been demonstrated that multiple anthropogenic stressors can drive, in some cases irreversibly, the ecosystem toward another stable state (Airoldi *et al.* 2015) with cascading effects on the whole ecosystem (Thrush *et al.* 2011; Araujo *et al.* 2013). Those alternative stable states can include urchin barrens, mussels beds and turf algae (Lemée *et al.* 2012; Connell *et al.* 2014). Additionally, stressors can interact in a non-additive way (Strain *et al.* 2014), amplifying the negative effects of the single factors. For instance, *Cystoseira* resilience to temperature stress can further decrease when exposed to eutrophication (Mancuso *et al.* 2016) or high sediment load (Strain *et al.* 2015).The co-occurrence of multiple stressors makes it difficult to quantify the importance of climate change alone on the loss of macroalgal forest biodiversitys. However, this study can give now an indication of what is the relative impact that we can expect solely based on the climatic niche tracking of the different species. It is necessary to consider that climatic niche models do not incorporate evolutionary processes, which could select for more tolerant phenotypes and help the populations to adapt to the environmental changes (Somero 2012). These models also do not incorporate the negative effects of other anthropogenic local stressors or variables for which no simulated data is available. Therefore, we expect that the predicted probability of occurrence of the species as well as its effect on the diversity does not describe the upper threshold estimation of the future distribution of these essential habitats inevitably.

Conclusions and recommendations

Overall, the study underlines the importance of complying with the lower emissions objective and of the management of local stressors to build up resilience of key coastal ecosystems. This study stresses the high dependence on compliance with the global challenge of greenhouse gas reduction at its lower objective recently set in the COP21 of the United Nations framework convention on climate change (UNFCCC). The environmental changes under this objective of climate mitigation would still affect the *Cystoseira* macroalgae distribution, but range shifts would be limited mostly to Eastern Mediterranean area. On the contrary, the highest representative concentration pathway used in the simulation (RCP 8.5) would expose coastal ecosytem to profound changes, including extinction risk and genetic erosion in both Mediterranean and North Atlantic ecosystems. COP21 committed to goals of GHG reduction that are ambitious and perhaps hard to archive (Tomlinson 2015). However, considering the interplay of global with local stressors, we suggest that acting on local pressures should be a priority. This would be a relatively easier and faster solution compared to climate mitigation that could help increase resilience on global stressors and minimize the habitat loss, while allowing more time for the species to adapt, especially in the Mediterranean region.

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References

Airoldi L, Ballesteros E, Buonomo R et al. (2015) Marine forests at risk: solutions to halt the loss and promote the recovery of mediterranean canopy-forming seaweeds. *5th Mediterranean Symposium on Marine Vegetation.*

Airoldi L, Beck MW (2007) Loss, Status and Trends for Coastal Marine Habitats of Europe. *Oceanography and Marine Biology: An Annual Review*, **45**, 345–405.

Allouche, O., A. Tsoar, and R. Kadmon. 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, **43**, 1223–1232.

Araujo RM, Bartsch I, Bekkby T, Erzini K, Sousa-Pinto I (2013) What is the impact of kelp forest density and/or area on fisheries? *Environmental Evidence*, **2**, 15.

Assis J, Serrão EA, Claro B, Perrin C, Pearson GA (2014). Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. *Molecular Ecology* **23**, 2797-2810

Assis, J., Lucas, A. V., Bárbara, I., & Serrão, E. Á. (2016). Future climate change is predicted to shift long-term persistence zones in the cold-temperate kelp Laminaria hyperborea. *Marine environmental research*, **113**, 174-182.

Bates AE, Pecl GT, Frusher S *et al.* (2014) Defining and observing stages of climate-mediated range shifts in marine systems. *Global Environmental Change*, **26**, 27–38.

Benedetti-Cecchi L, Pannacciulli F, Bulleri F *et al.* (2001) Predicting the consequences of anthropogenic disturbance: large-scale effects of loss of canopy algae on rocky shores. *Marine Ecology Progress Series*, **214**, 137–150.

Bulleri F, Benedetti-cecchi L, Acunto S, Cinelli F, Hawkins SJ (2002) The influence of canopy algae on vertical patterns of distribution of low-shore assemblages on rocky coasts in the northwest Mediterranean. *Journal of Experimental Marine Biology and Ecology*, **267**, 89–106.

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC *et al.* (2013) How does climate change cause extinction? *Proceedings. Biological sciences / The Royal Society*, **280**, 20121890.

Chefaoui, R. M., & Lobo, J. M. (2008). Assessing the effects of pseudo-absences on predictive distribution model performance. *Ecological modelling*, ***210***, 478-486.

Chefaoui, R. M., Assis, J., Duarte, C. M., & Serrão, E. A. (2015). Large-Scale Prediction of Seagrass Distribution Integrating Landscape Metrics and Environmental Factors: The Case of Cymodocea nodosa (Mediterranean–Atlantic). *Estuaries and Coasts*, 1-15.

Connell S, Foster M, Airoldi L (2014) What are algal turfs? Towards a better description of turfs. *Mar Ecol Prog Ser*, **495**. 299-307.

Davies C, Moss D, Hill M (2004) EUNIS habitat classification revised 2004. Report to: European Environment Agency and European Topic Centre on Nature Protection and Biodiversity. October 2004. Online. Revised description (2012) available at: http://www.eea.europa.eu/themes/biodiversity/eunis/eunis-habitat-classification.

Falace A, Zanelli E, Bressan G (2005) Morphological and reproductive phenology of Cystoseira compressa (Esper) Gerloff & Nizamuddin (Fucales, Fucophyceae) in the Gulf of Trieste (North Adriatic. *Annales. Series Historia Naturalis*, **5**, 5–12.

Fielding, A.H., and J.F. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, **24**, 38–49.

Guiry MD, Guiry GM (2013) AlgaeBase. *National University of Ireland*.

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, **8**, 461–467.Hu Z, Fraser C (2016) *Seaweed Phylogeography* (Z-M Hu, C Fraser, Eds,). Springer. Netherlands, Dordrecht.

Jakobsson, Mattias, and Noah A. Rosenberg. "CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure." *Bioinformatics*, **23**, 1801-1806

Kearney M, Porter W (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology letters*, **12**, 334–50.

Knutsen H, Jorde PE, Gonzalez EB *et al.* (2013) Climate Change and Genetic Structure of Leading Edge and Rear End Populations in a Northwards Shifting Marine Fish Species, the Corkwing Wrasse (Symphodus melops). *PloS one*, **8**, e67492.

Lemée R, Mangialajo L, Cohu S (2012) Interactions between scientists, managers and policy makers in the framework of the French MediOs project on Ostreopsis (2008-2010). *Cryptogamie Algologie*, **33**, 137–142.

Liaw, A., and M. Wiener. 2002. Classification and regression by randomForest. *R News*, **2**, 18–22.

Lourenço CR, Zardi GI, McQuaid CD, Serrao EA, Pearson GA, Jacinto R, Nicastro KR (2016) Upwelling areas as climate change refugia for the distribution and genetic diversity of a marine macroalga. *Journal of Biogeography* 10.1111/jbi.12744

Mancuso (2016). Effects of local and global stressors on the status and future persistence of intertidal canopy-forming algae. PhD thesis, *Università di Bologna.*

Mineur F, Arenas F, Assis J *et al.* (2015) European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of Sea Research*, **98**, 91–108.

Moss RH, Edmonds JA, Hibbard KA et al. (2010) The next generation of scenarios for climate change research and assessment. Nature, 463, 747–56.

Neiva J, Assis J, Coelho NC *et al.* (2015) Genes Left Behind: Climate Change Threatens Cryptic Genetic Diversity in the Canopy-Forming Seaweed *Bifurcaria bifurcata* (S Lin, Ed,). *PLOS ONE*, **10**, e0131530.

Nicastro KR, Zardi GI, Teixeira S *et al.* (2013) Shift happens: trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga *Fucus vesiculosus*. *BMC Biology*, **11**, 6.

O'Leary JK, Micheli F, Airoldi L, Boch C, Leo G De, Elahi R, Ferretti F, Graham NAJ, Litvin SY, Low NH, Lummis S, Nickols KJ, Wong J (2017) The Resilience of Marine Ecosystems to Climatic Disturbances. Bioscience **76**,1–13

Opdam P, Wascher D (2004) Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation*, **117**, 285–297.

Peterson A (2006) Uses and requirements of ecological niche models and related distributional models. *Biodiversity Informatics*, **3**, 59-72.

Pfenninger M, Bálint M, Pauls SU (2012) Methodological framework for projecting the potential loss of intraspecific genetic diversity due to global climate change. *BMC Evolutionary Biology*, **12**, 224.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Provan J (2013) The effects of past, present and future climate change on range-wide genetic diversity in northern North Atlantic marine species. *Frontiers of Biogeography*, **5**, 60-66.

Pujol B, Pannell JR (2008) Reduced Responses to Selection After Species Range Expansion. *Science*, **321**, 96–96.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/).

Rosenberg, N. A., 2002 Distruct:A Program for the Graphical Display of Structure Results (http://www.cmb.usc.edu/noahr/distruct.html).

Smale DA, Wernberg T (2013) Extreme climatic event drives range contraction of a habitat-forming species. *Proceedings. Biological sciences / The Royal Society*, **280**, 20122829.

Soberon J (2005) Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*, **2**, 1–10.

Somero GN (2012) The Physiology of Global Change: Linking Patterns to Mechanisms. *Annual Review of Marine Science*, **4**, 39–61.

Strain EMA, van Belzen J, van Dalen J, Bouma TJ, Airoldi L (2015) Management of local stressors can improve the resilience of marine canopy algae to global stressors. *PloS one*, **10**, e0120837.

Strain EMA, Thomson RJ, Micheli F, Mancuso FP, Airoldi L (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology*, **20**, 3300–3312.

Sunday J, Bates A, Dulvy N (2012) Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, **2**, 686-690.

Thrush S, Chiantore M, Asnagi V *et al.* (2011) Habitat–diversity relationships in rocky shore algal turf infaunal communities. *Marine Ecology Progress Series*, **424**, 119–132.

Thuiller,W., Georges, D., and Engler, R. (2014). biomod2: Ensemble platform for species distribution modeling. R package version 3.1-64. http://CRAN.R-project.org/package=biomod2

Tomlinson L (2015) *Procedural Justice in the United Nations Framework Convention on Climate Change*. Springer International Publishing, Cham.

Tyberghein, L., Verbruggen, H., Pauly, K., Troupin, C., Mineur, F., & De Clerck, O. (2012). Bio-ORACLE: a global environmental dataset for marine species distribution modelling. *Global Ecology and Biogeography*, **21**, 272-281.

Urban MC, Tewksbury JJ, Sheldon KS (2012) On a collision course: competition and dispersal differences create no-analogue communities and cause extinctions during climate change. *Proceedings. Biological sciences / The Royal Society*, **279**, 2072–80.van Vuuren DP, Edmonds JA, Kainuma M, Riahi K, Weyant J (2011) A special issue on the RCPs. *Climatic Change*, **109**, 1–4.

Figures and Tables

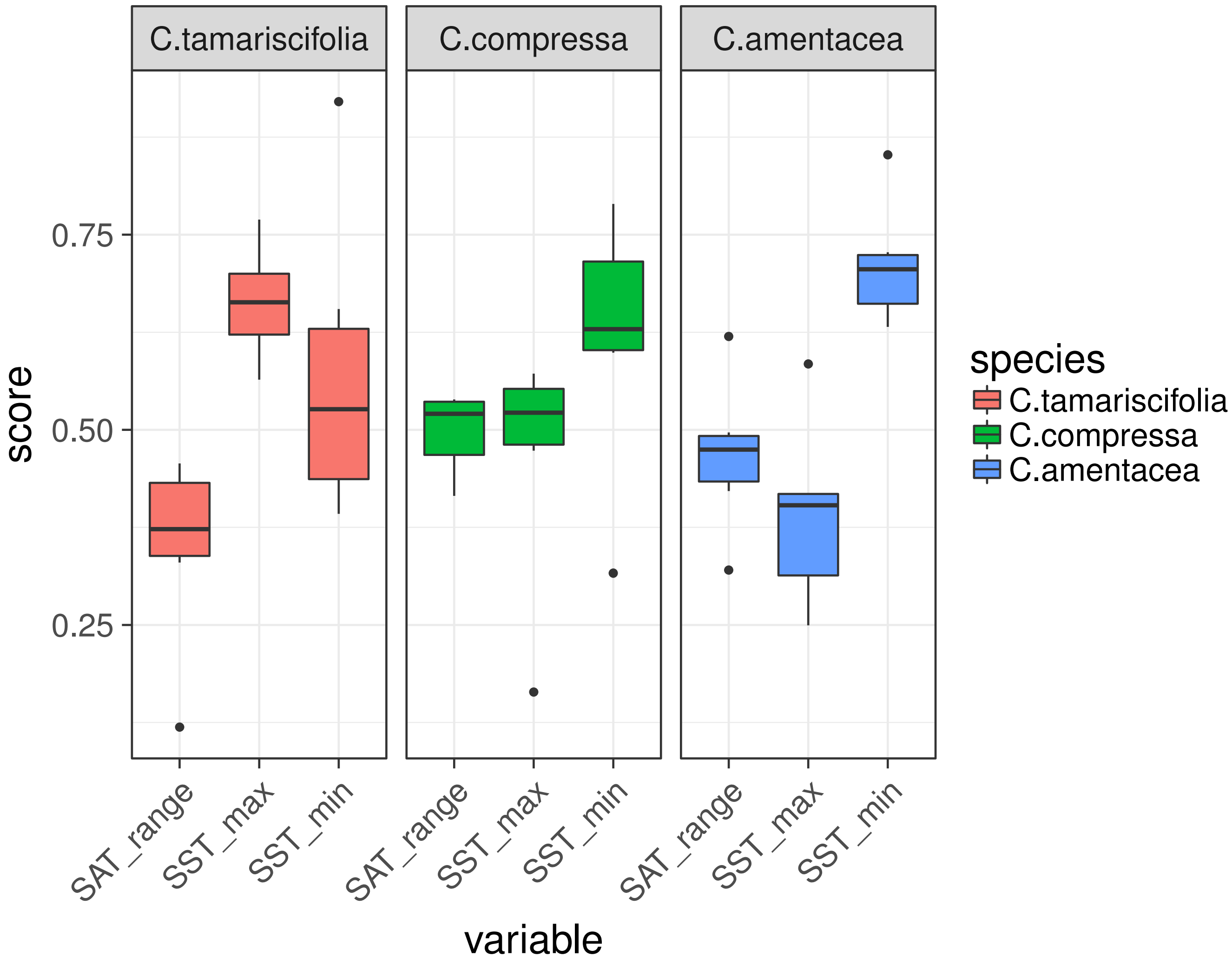

Figure 1 - Relative contribution of climatic variables to the overall models of the three *Cystoseira* species (*C. tamariscifolia, C. compressa* and *C. amentacea*) obtained with all the six algorithms. Boxplots limits show the 25th and 75th percentile and the horizontal bars show the median scores. Error bars indicates the 95 percent confidence interval around the medians while the dots are outlier models.

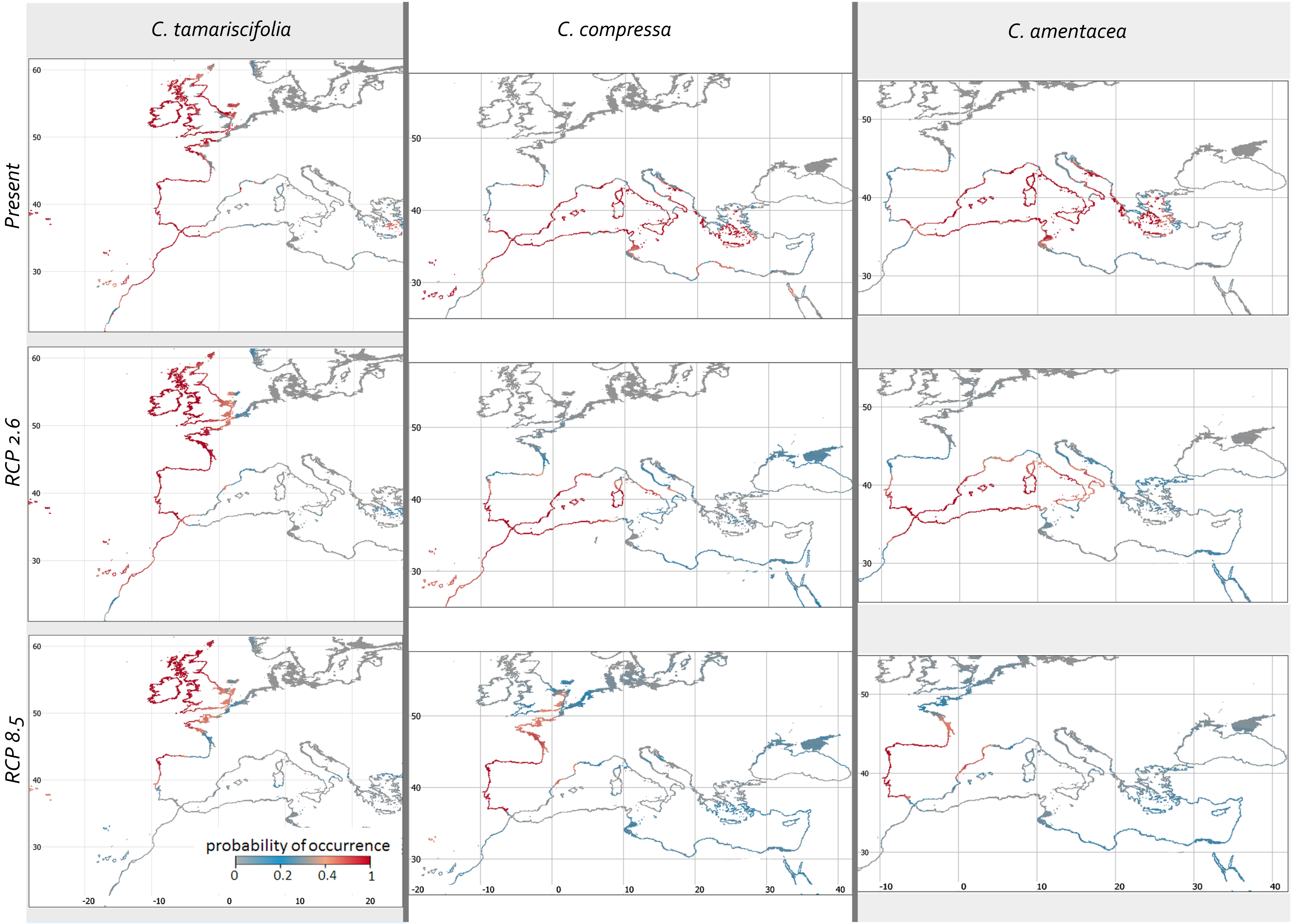

Figure 2 – Ensemble model maps showing the estimated probability of occurrence for C. tamariscifolia, C. compressa and C. amentacea for the present distribution and the two future scenarios.

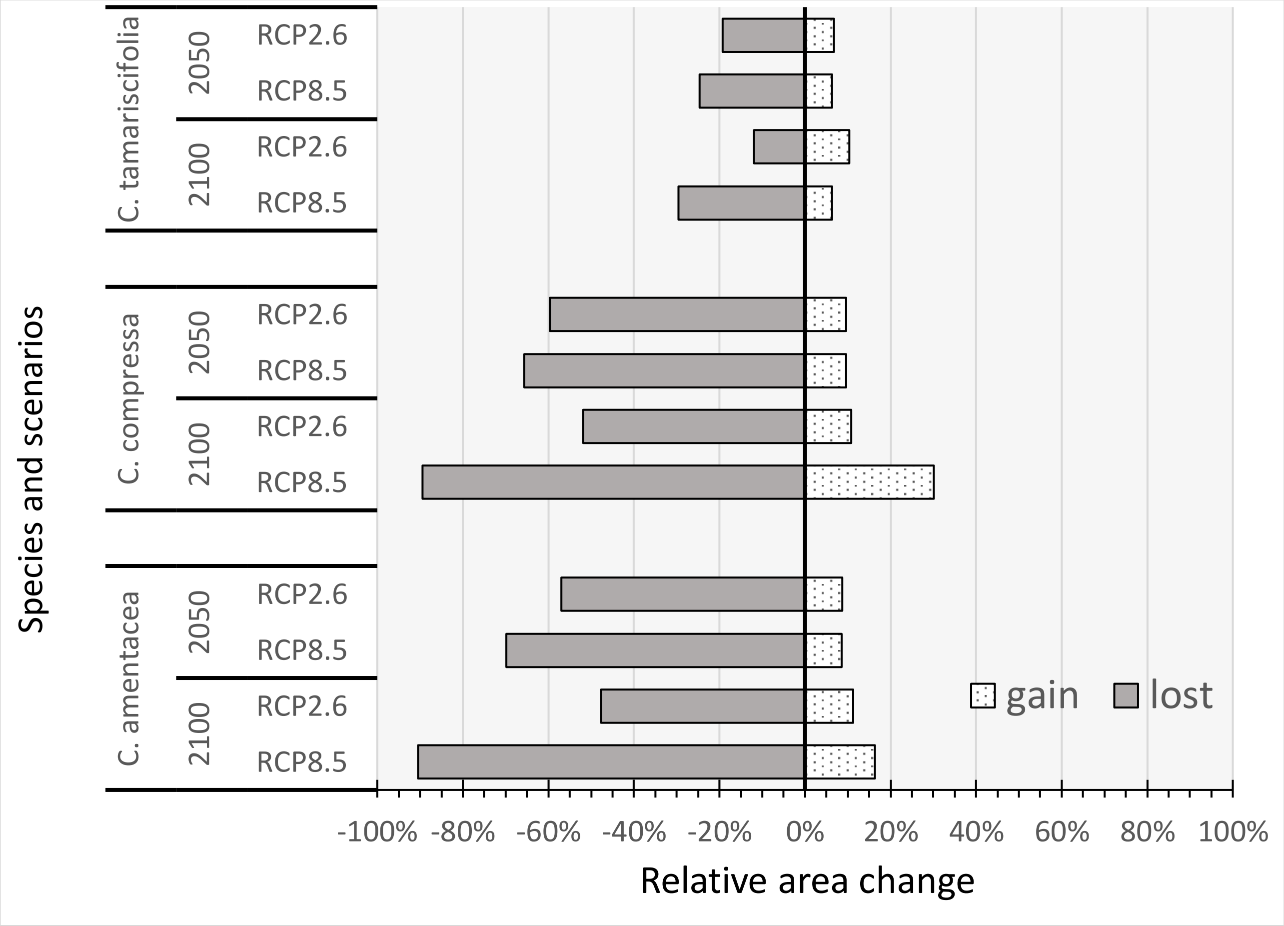

Figure 3 - relative area change between present and future predicted scenarios with at least a moderate probability of occurrence from the model (P> 0.3) according to each species, projection year and scenario.

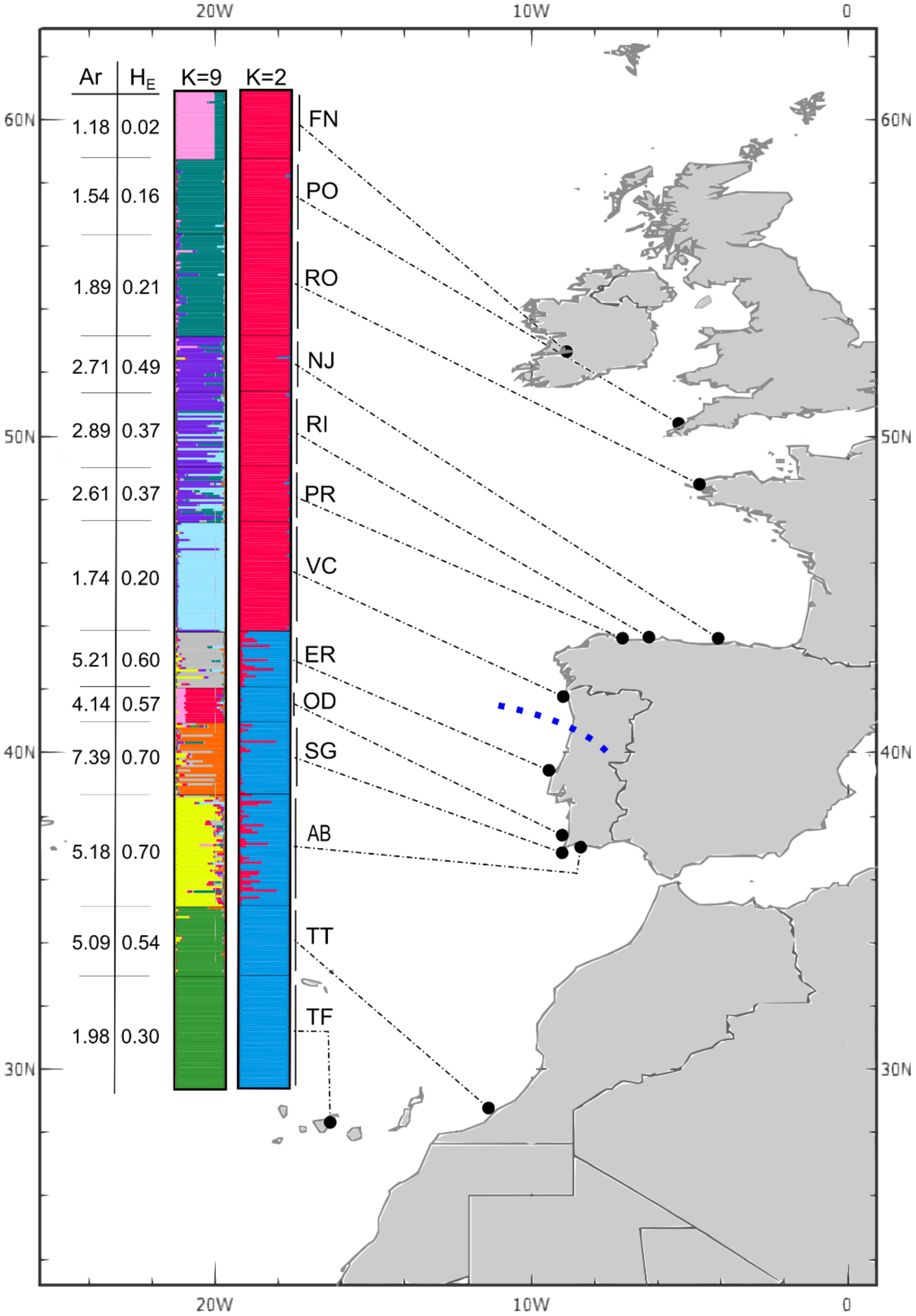

Figure 4 - *Cystoseira tamariscifolia* population genetic structure according to cluster analysis. Allelic richness (Ar) and expected heterozygosity (H_E) are indicated. Blue dotted line show the main separation individuated among north and southern linages. Locations: Finavarra (FN); Polzeath (PO); Roscoff (RO); Noja (NJ); Rieguabajo (RI); Porcia (PR); Viana Do Castelo (VC); Ericera (ER); Odeceixe (OD); Sagres (SG); Albufeira (AB); Tan-Tan (TT); Tenerife (TF)

Table 1 - Model performance statistics for the three *Cystoseira* species

Species	**Model**	**AUC (±SD)**	**TSS (±SD)**	**Sensitivity (±SD)**	**Specificity (±SD)**
C. amentacea	GLM	0.943(±0.006)	0.796(±0.013)	95.980(±2.421)	84.161(±2.339)
	GBM	0.944(±0.011)	0.796(±0.026)	93.431(±2.925)	86.781(±1.986)
	GAM	0.942(±0.005)	0.799(±0.014)	94.804(±2.813)	85.398(±1.643)
	FDA	0.937(±0.006)	0.756(±0.020)	95.490(±2.622)	81.384(±1.753)
	MARS	0.930(±0.010)	0.778(±0.014)	93.824(±2.657)	84.293(±1.939)
	RF	0.939(±0.009)	0.766(±0.026)	92.647(±1.863)	85.315(±2.914)
C. compressa	GLM	0.891(±0.011)	0.689(±0.022)	92.529(±3.305)	76.758(±3.703)
	GBM	0.928(±0.008)	0.732(±0.020)	90.471(±2.493)	83.609(±1.953)
	GAM	0.911(±0.005)	0.730(±0.018)	91.765(±2.496)	81.529(±1.368)
	FDA	0.909(±0.010)	0.709(±0.016)	92.706(±2.340)	78.858(±2.395)
	MARS	0.908(±0.009)	0.717(±0.018)	90.000(±1.754)	82.111(±1.562)
	RF	0.932(±0.009)	0.730(±0.022)	92.000(±1.885)	82.096(±2.373)
C. tamariscifolia	GLM	0.911(±0.005)	0.720(±0.016)	95.526(±2.109)	77.091(±2.151)
	GBM	0.919(±0.006)	0.717(±0.019)	94.368(±2.808)	78.890(±2.560)
	GAM	0.908(±0.006)	0.722(±0.021)	94.316(±2.351)	78.411(±1.613)
	FDA	0.904(±0.004)	0.676(±0.014)	92.684(±2.053)	76.363(±2.238)
	MARS	0.900(±0.009)	0.698(±0.020)	92.421(±3.475)	78.011(±2.333)
	RF	0.923(±0.008)	0.724(±0.025)	92.421(±3.386)	81.427(±3.095)

Average validation scores of the 20 models obtained for each algorithm are shown. GLM = generalized linear model, GBM = generalized boosting model, GAM = generalized additive model, FDA = flexible discriminant analysis, MARS = multiple adaptive regression splines, RF =RandomForest.

Table 2 – Genetic diversity within samping sites and geographical regions

	Population	*n*	*Ar*	*A'*	*He*	*Ho*	*F$_{IS}$*	
C. tamariscifolia	**North**		**6.66**	**1.50**				
	Finavarra	29	1.18	0.00	0.02	0.02	-0.03	
	Polzeath	32	1.54	0.00	0.16	0.16	0.00	
	Roscoff	44	1.89	0.50	0.21	0.19	0.17	
	Noja	24	2.70	0.33	0.49	0.58	-0.18	
	Rieguabajo	32	2.89	0.17	0.37	0.36	0.03	
	Porcia	24	2.61	0.00	0.37	0.37	-0.05	
	Viana Do Castelo	47	1.75	0.17	0.20	0.14	0.32	
	South		**22.33**	**17.17**				
	Ericera	24	5.22	1.67	0.60	0.57	0.03	
	Odeceixe	15	4.12	0.83	0.57	0.36	0.37	
	Sagres	31	7.44	4.00	0.70	0.73	-0.01	
	Albufeira	48	5.18	1.50	0.70	0.58	0.17	
	Tan-Tan	30	5.05	3.67	0.54	0.47	0.15	
	Tenerife	48	1.98	1.00	0.30	0.19	0.34	*
C. compressa	**West**		**8.30**	**4.10**				
	Albufeira	39	3.28	0.60	0.31	0.26	0.21	
	Murcia	38	3.93	0.80	0.42	0.33	0.41	*
	Mallorca	30	3.86	0.80	0.56	0.14	0.74	*
	Banyuls	35	2.18	0.40	0.32	0.00	0.99	*
	East		**6.90**	**2.70**				
	Sant'Elia	45	3.02	0.70	0.35	0.11	0.57	*
	Sciacca	26	2.99	0.90	0.41	0.10	0.68	*
	Porto Palo	28	2.73	0.30	0.39	0.32	0.37	*
	Montenegro	32	2.80	0.60	0.47	0.02	0.97	*
C. amentacea	**West**		**16.50**	**9.33**				
	Santa Pola	28	1.69	0.17	0.14	0.13	0.01	
	Tarragona	31	4.56	1.17	0.58	0.40	0.25	*
	Blanes	32	4.81	1.17	0.42	0.32	0.19	*
	Cap de Creus	28	4.93	2.00	0.57	0.34	0.43	*
	Mallorca	32	2.08	0.67	0.28	0.24	0.17	
	Marseille	30	3.87	2.17	0.42	0.38	0.06	
	East		**13.50**	**6.33**				
	Sant'Elia	30	4.57	1.67	0.52	0.45	0.14	
	Sciacca	25	3.12	0.50	0.31	0.24	0.33	
	Porto Palo	30	4.81	1.17	0.59	0.44	0.29	*
	Crotone	23	3.83	0.50	0.42	0.23	0.47	*
	Torre Uluzzo	25	1.98	0.33	0.17	0.15	0.05	
	Otranto	23	2.07	0.33	0.16	0.08	0.56	*

Ar: mean allelic richness per locus (999 resampling); A': mean number of private alleles per locus; He: Expected heterozygosity; Ho: observed heterozygosity; FIS: multi-locus inbreeding coefficient (*if significant).

Supplementary information

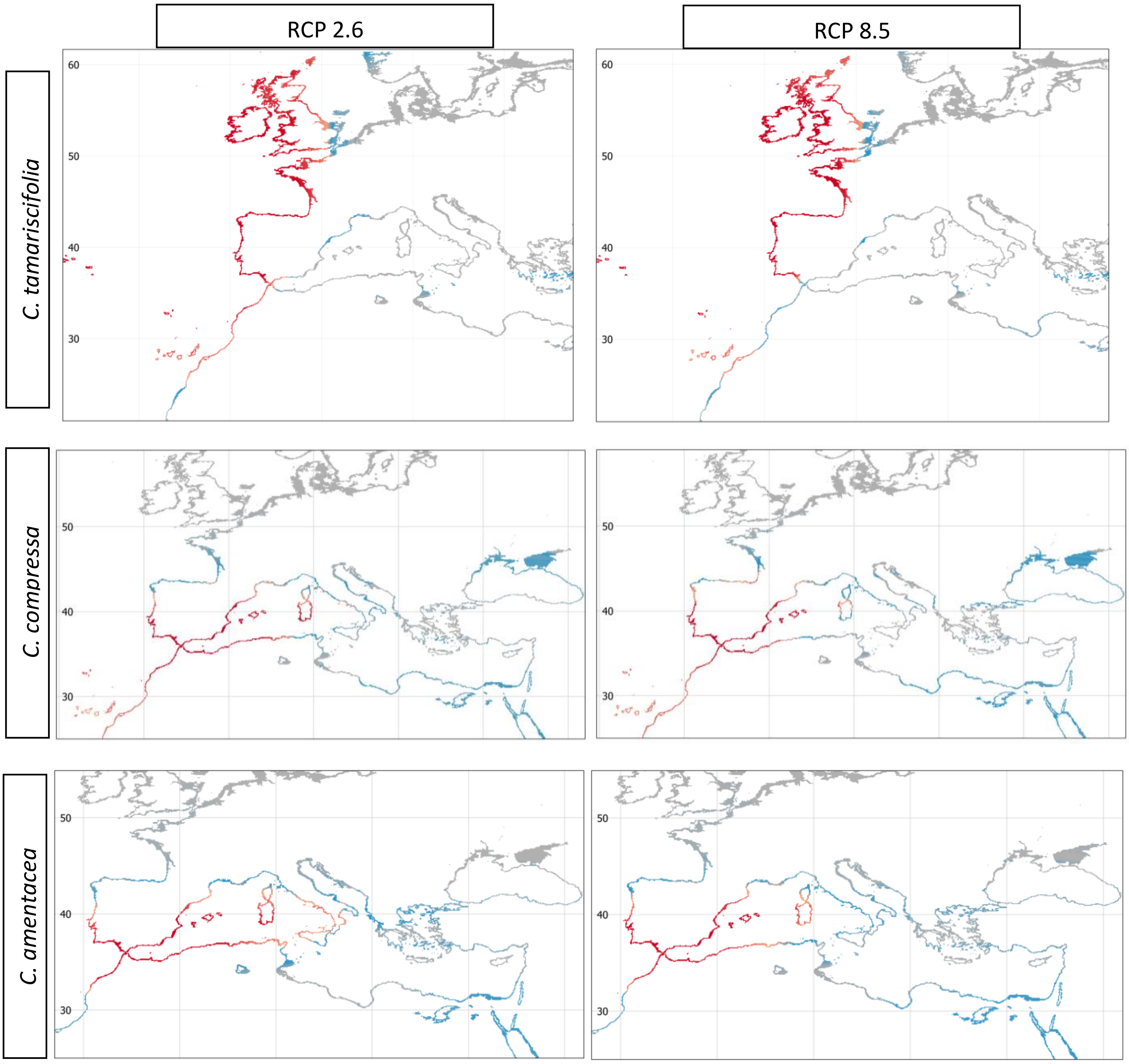

Figure S1 – Ensemble model maps showing the estimated probability of occurrence for *C. tamariscifolia, C. compressa* and *C. amentacea* by decade 2040-2050 for the two future scenarios.

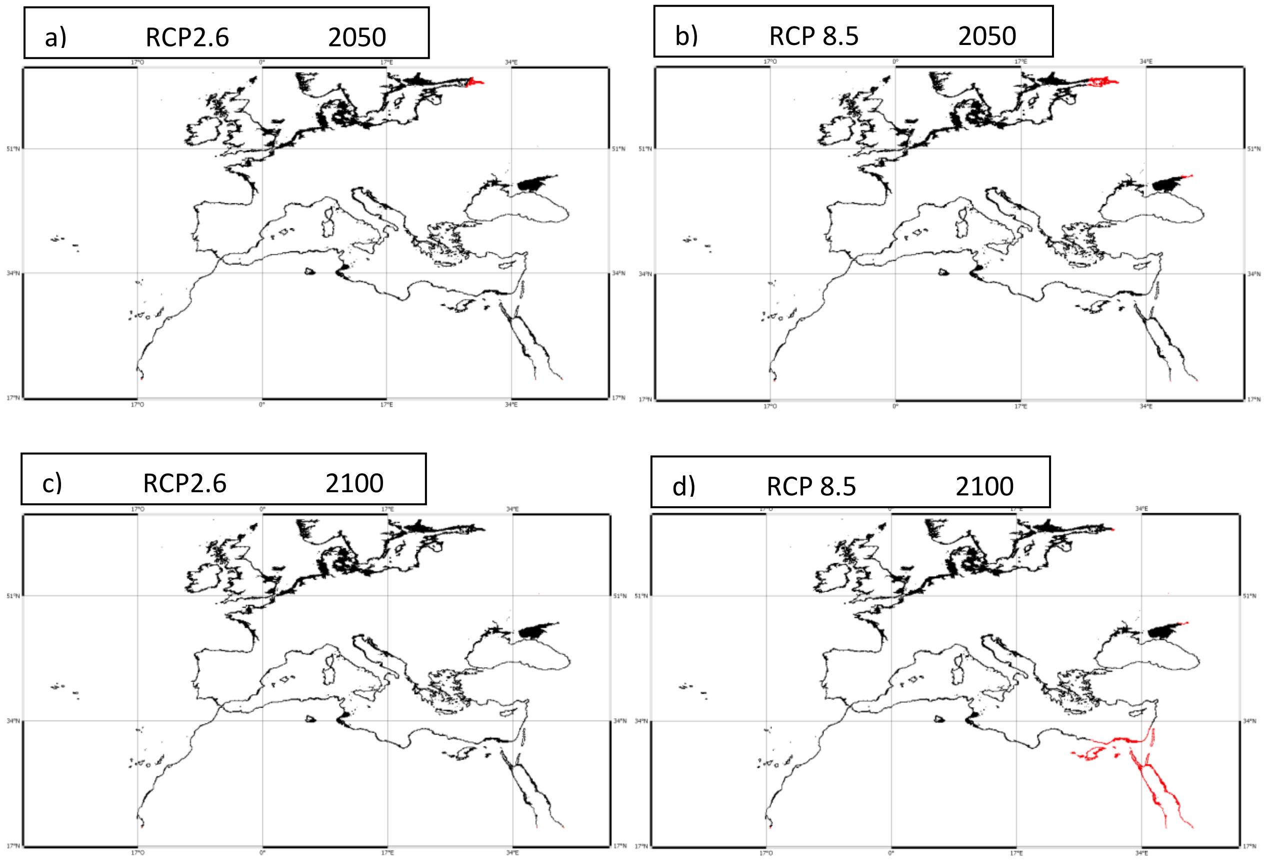

Figure S2 - Clamping mask for the scenarios RCP 2.6 (a) and RCP 8.5 (b) by 2040-2050 decade and RCP 2.6 (c) and RCP 8.5 (d) by 2090-2100 decade showing the locations where predictions are uncertain (in red) because the values of at least one of the variables are outside the range used for calibrating the models.

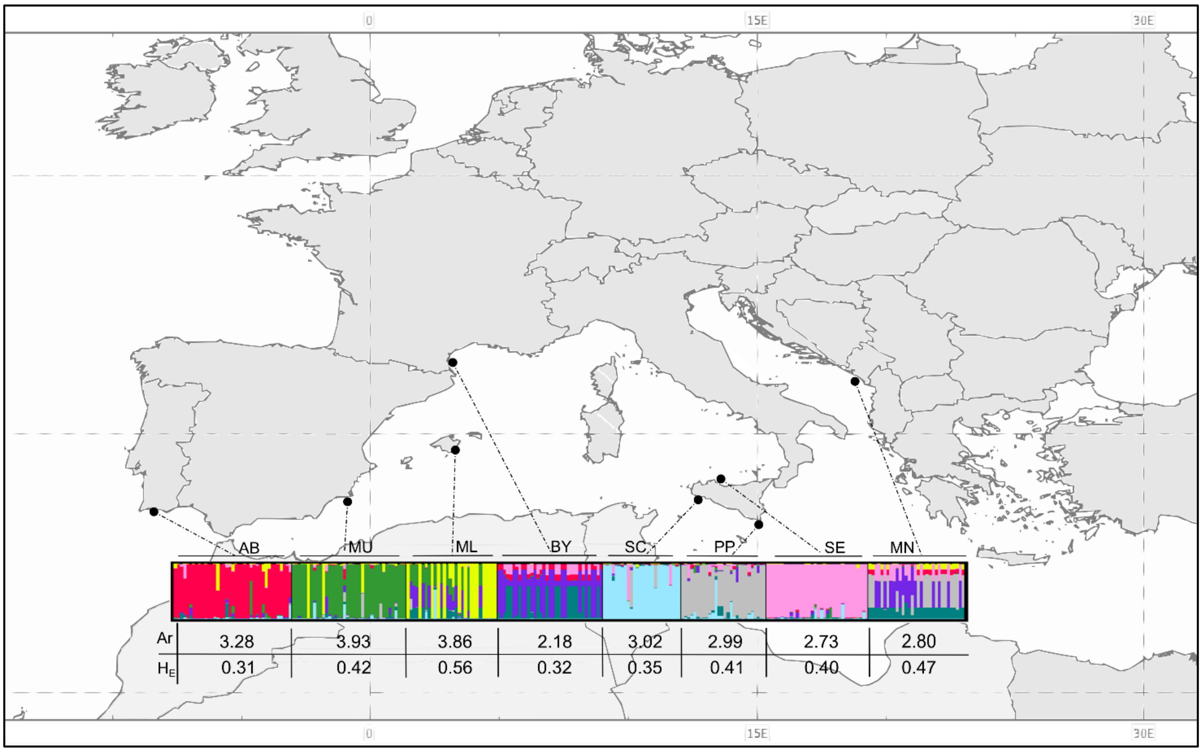

Figure S3 - *Cystoseira compressa* population genetic structure according to cluster analysis (K=8). Allelic richness (Ar) and expected heterozygosity (H_E) are indicated. Locations: Albufeira (AB); Murcia (MU); Mallorca (ML); Banyuls-sur-mer (BY); Sciacca (SC); Porto Palo di capo passero (PP); Sant'Elia (SE); Montenegro (MN).

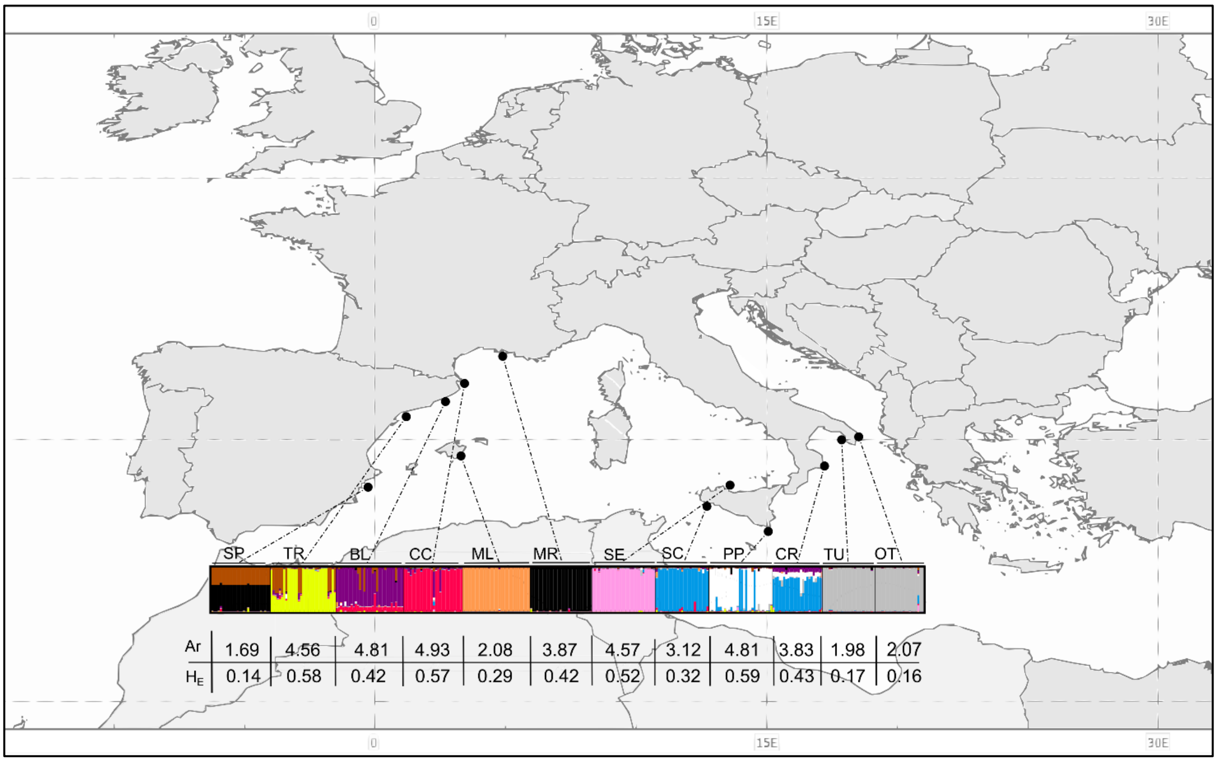

Figure S4 - *Cystoseira amentacea* population genetic structure according to cluster analysis (K=10). Allelic richness (Ar) and expected heterozygosity (HE) are indicated. Locations: Santa Pola (SP); Tarragona (TR); Blanes (BL); Cap de Creus (CC); Mallorca (ML); Marseille (MR); Sant'Elia (SE); Sciacca (SC); Porto Palo di capo passero (PP); Crotone (CR); Torre Uluzzo (TU); Otranto (OT).

Table S1 - References used to map the distribution of *C. compressa*, *C. tamariscifolia* and *C. amentacea*.

ID	Reference
1	Adrea, COSSU, Vittorio GAZALE, and Maura BAROLI. “La Flora Marina Della Sardegna: Inventario Delle Alghe Bentoniche.” 126 (1992): 651–707. Web.
2	Ainane, Tarik. *Valorisation de la biomasse algale du Maroc: Potentialités pharmacologiques et Applications environnementales, cas des algues brunes Cystoseira tamariscifolia et Bifurcaria bifurcata*. Diss. Faculté des Sciences Ben M'sik Université Hassan II Casablanca, 2011.
3	Antolić, Boris et al. “A Checklist of the Benthic Marine Macroalgae from the Eastern Adriatic Coast: II. Heterokontophyta: Phaeophyceae.” *Acta Adriatica* 51.1 (2010): 9–33. Web. 3 May 2014.
4	Aysel, V, and H Erduğan. “Marine Flora of Sinop (Black Sea, Turkey).” *EU Journal of …* (2004): n. pag. Web. 26 May 2015.
5	Aysel, V, H Erdugan, and EŞ Okudan. “Marine Algae and Seagrasses of Hatay (Mediterranean, Turkey).” *Journal of the Black Sea.* 12 (2006): 159–179. Web. 2 May 2014.
6	Bárbara, Ignacio, et al. "Nuevas citas y aportaciones corológicas para la flora bentónica marina del Atlántico de la Península Ibérica." (2012).
7	Barille, Anne-Laure, et al. "Mise en place de la DCE dans les masses d'eau côtières des Pays de la Loire-Prospection de la flore et de la faune benthiques et proposition d'un réseau de surveillance." (2007).
8	Benhissoune, S., C-F. Boudouresque, and M. Verlaque. "A checklist of the seaweeds of the Mediterranean and Atlantic coasts of Morocco. II. Phaeophyceae." *Botanica Marina* 45.3 (2002): 217-230.
9	Birje, Jamila, Marc Verlaque, and Frédéric Poydenot. "Macrophytobenthos des platiers rocheux intertidaux et semi-exposés da la région de Safi-Essaouira (Maroc occidental)." *Oceanologica acta* 19.5 (1996): 561-574.
10	Cabeçadas, G., et al. "Caracterização ambiental da zona costeira adjacente aos estuários do Tejo e Sado." *Relatórios Científicos e Técnicos IPIMAR, Série digital* 20 (2004): 40.
11	Catra, M, and S Giardina. “A Survey of the Marine Macroalgae of Karpathos Island (the Aegean Sea, Greece).” *Plant Biosystems* I.3 (2009): n. pag. Web. 8 May 2014.
12	Cecere, Ester, Mario Cormaci, and Giovanni Furnari. “Benthic Algal Flora of Cheradi Islands (Gulf of Taranto, Mediterranean Sea).” *Nova …* (1996): 191–214. Web. 8 May 2014.
13	Coppejans, Eric. "Végétation marine de la Corse (Méditerranée). III. Documents pour la flore des algues. Marine Vegetation of Corsica (Mediterranean Sea). III. Documents for the Algal Flora." *Botanica Marina* 22.4 (1979): 257-266.
14	Cormaci, M. et al. “Contribution to the Knowledge of Benthic Marine Algae on Rocky Substrata of the Maltese Islands (Mediterranean Sea).” *Botanica Marina* 40.1–6 (1997): 203–215. Web. 2 May 2014.
15	Cormaci, Mario et al. “The Benthic Algal Flora on Rocky Substrata of the Tremiti Islands (Adriatic Sea).” *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* 134.2 (2000): 133–152. Web. 8 May 2014.
16	Crisp, D. J., and Alan James Southward. "The distribution of intertidal organisms along the coasts of the English Channel." *Journal of the Marine Biological Association of the United Kingdom* 37.01 (1958): 157-203.
17	Diez, Isabel, et al. "Recovery stages over long-term monitoring of the intertidal vegetation in the ‘Abra de Bilbao’area and on the adjacent coast (N. Spain)." *European Journal of Phycology* 44.1 (2009): 1-14.
18	Einav, Rachel, and Alvaro Israel. "Checklist of seaweeds from the Israeli Mediterranean: Taxonomical and ecological approaches." *Israel Journal of Plant Sciences* 56.1-2 (2008): 127-191.
19	Falace, Annalisa, Elisa Zanelli, and Guido Bressan. “Morphological and Reproductive Phenology of Cystoseira Compressa (Esper) Gerloff & Nizamuddin (Fucales, Fucophyceae) in the Gulf of Trieste (North Adriatic.” *Annales. Series Historia Naturalis* 5.1 (2005): 5–12. Web. 21 Apr. 2014.
20	Feoli, Enrico, and Guido Bressan. "Affinità floristica dei tipi di vegetazione bentonica della Cala di Mitigliano (Massa Lubrense, Napoli)." *Plant Biosystem* 106.5 (1972): 245-256.

21	Fiorin, R et al. “Il Popolamento Ittico E Macroalgale Delle Scogliere Del Litorale Veneziano: Interazione Tra Le Alghe Brune Del Genere Cystoseira E Alcune Specie Di Pesci.” *Biol. Mar. Medit* 15 (2008): 304–305. Web. 25 May 2014.
22	Garreta, Amelia Gómez, et al. "Mapas de distribución de algas marinas de la Península Ibérica e Islas Baleares. VI. Cystoseira. C. Agardh: Grupos C. spinifero-opuntioides y C. discors-abrotanifolioides." *Botanica complutensis* 19 (1994): 119.
23	Giaccone, Giuseppe. “Raccolte Di Fitobenthos Sulla Banchina Continentale Italiana.” *Giornale botanico italiano* 103.6 (1969): 485–514. Web. 2 May 2014.
24	Godeh, M. M., M. Nizamuddin, and F. A. Elmenifi. "MARINE-ALGAE FROM EASTERN COAST OF LIBYA (CYRENAICA)." *Pakistan Journal of Botany* 24.1 (1992): 11-21.
25	Granja, A., J. Cremades, and I. Bárbara. "Catálogo de las algas bentónicas marinas de la Ría de Ferrol (Galicia, NO de la Península Ibérica) y consideraciones biogeográficas sobre su flora." (1992).
26	Guiry, Michael D. "The Marine Algal Flora of Bantry Bay, Co. Cork." (1973).
27	Güven, KC et al. “Über Die Marinen Algen an Den Küsten Der Türkei.” *Botanica marina* XIV.2 (1971): n. pag. Web. 8 May 2014.
28	Hily, C. "Lieux de surveillance du Benthos." *Région Bretagne-Suivi stationnel des sables intertidaux REBENT* (2005).
29	Jones, W. EIFION, and R. Williams. "The seaweeds of Dale." *Field Studies* 2.3 (1966): 303-330.
30	Lawson, G. W., and D. M. John. "The marine flora of the Cap Blanc peninsula: its distribution and affinities." *Botanical journal of the Linnean Society* 75.1 (1977): 99-118.
31	Levring, Tore. "The marine algae of the Archipelago of Madeira." (1974).
32	Lluch, J. Rull, et al. "Mapas de distribución de algas marinas de la Península Ibérica e Islas Baleares. VII. Cystoseira C. Agardh (Grupo C. baccata) y Sargassum C. Agardh (S. muticum y S. vulgare)." *Botanica Complutensis* 19 (1994): 131-138.
33	Maiz, NB, CF Boudouresque, and F Ouahchi. “Inventaire Des Algues et Phanérogames Marines Benthiques de La Tunisie.” *Plant Biosystem* June 2013 (1987): 37–41. Web. 2 May 2014.
34	Molinier, Roger. “Etude Des Biocénoses Marines Du Cap Corse.” *Vegetatio* (1960): n. pag. Web. 16 May 2014.
35	Neto, Ana I. "Checklist of the benthic marine macro algae of the Azores." (1994).
36	Nikolić, Vedran et al. “Cartography of Littoral Rocky-Shore Communities (CARLIT) as a Tool for Ecological Quality Assessment of Coastal Waters in the Eastern Adriatic Sea.” *Ecological Indicators* 34 (2013): 87–93. Web. 29 Apr. 2014.
37	Nizamuddin, M., JA West, and EG Menez. “A List of Marine Algae from Libya.” *Botanica marina* XXII (1979): 465–476. Web. 19 May 2014.
38	Ould-Ahmed, Nora, et al. "Checklist of the benthic marine macroalgae from Algeria. I. Phaeophyceae." *Anales del Jardín Botánico de Madrid*. Vol. 70. No. 2. Real Jardín Botánico, 2013.
39	Pereira, Sílvia G., et al. "Biogeographic patterns of intertidal macroinvertebrates and their association with macroalgae distribution along the Portuguese coast." *Marine Biodiversity*. Springer Netherlands, 2006. 185-192.
40	Perez, René, et al. "Répartition des grands champs d'algues brunes sur les côtes françaises de la Manche Occidentale entre l'Ile grande et l'Ile de Siec." *Science et Pêche* 226 (1973): 1-12.
41	Pérez-Cirera, J. L. "Catálogo florístico de las algas bentónicas de la ría de Cedeira, NO de España." *Anales del Instituto Botánico AJ Cavanilles*. Vol. 32. No. 2. 1975.
42	Polo Alberti, L., and Juan Antonio Seoane Camba. "Poblaciones y comunidades de algas bentónicas en la costa catalana." (1979).
43	Raphélis, A. "Algues du Maroc récoltées par MJ Gattefossé." *Bulletin de la Societe Botanique de France* 76.4 (1929): 719-730.

44	Rindi, Fabio, Gianfranco Sartoni, and Francesco Cinelli. “A Floristic Account of the Benthic Marine Algae of Tuscany (Western Mediterranean Sea).” *Nova Hedwigia* 74.1 (2002): 201–250. Web. 2 May 2014.
45	Robvieux, Pauline. “Conservation Des Populations de Cystoseira En Régions Provence-Alpes-Côte-d’Azur et Corse.” (2013): n. pag. Web. 2 May 2014.
46	Sartoni, Gianfranco, and AM De Biasi. “A Survey of the Marine Algae of Milos Island, Greece.” *Cryptogamie Algologie* 20.3 (1999): 271–283. Web. 8 May 2014.
47	Serio, Donatella et al. “Changes in the Benthic Algal Flora of Linosa Island (Straits of Sicily, Mediterranean Sea).” *Botanica Marina* 49.2 (2006): 135–144. Web. 2 May 2014.
48	Sibaja-Cordero, JEFFREY A., and Jesus S. Troncoso. "Upper and lower limits of rocky shore organisms at different spatial scales and wave exposure (Islas CÍES, NW Spain)." *Thalassas* 27.1 (2011): 81-100.
49	THIBAUT, T. et al. “Decline and Local Extinction of Fucales in French Riviera: The Harbinger of Future Extinctions?” *Mediterranean Marine Science* 16.1 (2014): 206–224. Web.
50	Tsekos, Ioannes, and Savas Haritonidis. "The marine algae of Rhodos island, Greece." *British Phycological Journal* 9.4 (1974): 399-406.
51	Tsirika, Anastasia, and Savvas Haritonidis. “A Survey of the Benthic Flora in the National Marine Park of Zakynthos (Greece).” *Botanica Marina* 48.1 (2005): 38–45. Web. 8 May 2014.
52	Vonthron-Sénécheau, Catherine, et al. "Antiprotozoal activities of organic extracts from French marine seaweeds." *Marine drugs* 9.6 (2011): 922-933.

CHAPTER 3

Published in *Molecular Ecology**

Habitat continuity and stepping-stone oceanographic distances explain population genetic connectivity of the brown alga Cystoseira amentacea

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Abstract

Effective predictive and management approaches for species occurring in a metapopulation structure require good understanding of inter-population connectivity. In this study we ask whether population genetic structure of marine species with fragmented distributions can be predicted by stepping-stone oceanographic transport and habitat continuity, using as model an ecosystem-structuring brown alga, *Cystoseira amentacea* var. *stricta*. To answer this question, we analyzed the genetic structure and estimated the connectivity of populations along discontinuous rocky habitat patches in southern Italy, using microsatellite markers at multiple scales. In addition, we modelled the effect of rocky habitat continuity and ocean circulation on gene flow by simulating Lagrangian particle dispersal based on ocean surface currents allowing multigenerational stepping-stone dynamics. Populations were highly differentiated, at scales from few meters up to 1000s of kilometers. The best possible model fit to explain the genetic results combined current direction, rocky habitat extension and distance along the coast among rocky sites. We conclude that a combination of variables suitable habitat and oceanographic transport is a useful predictor of genetic structure. This relationship provides insight into the mechanisms of dispersal and the role of life history traits. Our results highlight the importance of spatially explicit modeling of stepping stone dynamics and oceanographic directional transport coupled with habitat suitability, to better describe and predict marine population structure and differentiation. This study also suggests the appropriate spatial scales for the conservation, restoration and management of species that are increasingly affected by habitat modifications.

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Introduction

The application of landscape genetics in the marine environment, known as "seascape genetics", focuses mainly on the comparison of expected dispersal with observed pairwise genetic differentiation (Johansson *et al.* 2008; White *et al.* 2010; Coscia *et al.* 2012), detection of dispersal barriers to gene flow (Schultz & Feldheim 2008; Collins *et al.* 2010; Neiva *et al.* 2012) and testing specific ecological hypotheses (Collins *et al.* 2010; Crandall *et al.* 2012). As high-resolution data on ocean currents become available (Siegel & Kinlan 2003), connectivity and dispersal can be better described with improved oceanographic models simulating particle movement. However, in seascape genetics oceanographic data are often used only qualitatively to explain population genetic structure. Explicit tests of effects of dispersal probabilities derived from oceanographic models on estimated genetic data have been applied in the last decade (Galindo *et al.* 2006). This quantitative approach offers a framework to analyze the relevance and influence of different environmental features on gene flow and genetic structure (Storfer *et al.* 2007; Alberto *et al.* 2010, 2011; Sunday *et al.* 2014). Genetic differentiation and probability of migration from oceanographic models can also be combined with fine scale seascape features, such as canopy or temperature, to explain genetic structure for multiple species (Selkoe *et al.* 2010). The relative influence of seascape variables can be tested (e.g., Storfer *et al.* 2007; Alberto *et al.* 2010, 2011), to compare alternative ecological hypotheses on factors affecting the genetic structure of marine populations.

This study addresses two challenges in seascape genetics research, that are asymmetrical dispersal and multi-generation dispersal effects linking sites by stepping-stone movements. Although the most commonly tested seascape feature is Euclidian distance, predicted to be inversely related to gene flow (Rousset 1997), isolation-by-distance models can be insufficient if seascape features are unequally distributed between populations. For example, ocean currents are often predominantly unidirectional, therefore even close populations might be differentially isolated by ocean circulation (Collins *et al.* 2010). Asymmetrical dispersal can result in a source-sink population structure where the demographic context of each population influences its evolutionary potential (Kawecki & Holt 2002), but it is often an overlooked factor. Furthermore, population connectivity is increasingly affected by a variety of human modifications to the environment (Jones 2007), leading to habitat fragmentation and loss and genetic disjunction even at small spatial scales (Fraser et al. 2010; Alberto et al. 2011) or alternatively introducing novel artificial urban habitats that can act as a conduit for some species across the seascape (Airoldi et al 2015). Any of these effects would be particularly relevant for species with low dispersal ability, affecting their capability to recover from disturbances, colonize new habitats or migrate, as response to future climate changes (Opdam & Wascher 2004, Thrush et al 2013). The potential for local adaptation to environmental variations may also be modified by changing genetic variability and connectivity (Kawecki & Ebert 2004; Chevin *et al.* 2010). Potential limitations to dispersal in a marine metapopulation system can be caused by the species' dispersal biology, oceanographic transport and the spatial distribution of suitable habitat. These can therefore affect population genetic diversity and connectivity. The effects of these processes should be considered in coastal management and restoration (McKay *et al.* 2005). This goal can be achieved by modeling the stepping-stone dynamics of species among fragmented patches of suitable habitats.

Marine dispersal has rarely been evaluated in a stepping-stone mode, and the few cases that did so have generally focused on species with long pelagic larvae duration (Treml et al. 2007; Crandall *et al.* 2012). Here we focused on species with low dispersal capacity, expected to be highly differentiated and which might depend strongly on stepping stone dispersal to achieve genetic connectivity. This is expected to be the case of fucoid algae belonging to the genus *Cystoseira*, whose zygotes develop attached to the maternal alga and sink directly underneath, having no planktonic life stages. These are amongst the most relevant ecosystem engineering species in the Mediterranean Sea, and used to form extensive canopy “forests” on suitable rocky substrate (Airoldi & Beck 2007). Nowadays these biogenic habitats are threatened by increasing urbanization and habitat loss (Benedetti Cecchi *et al.* 2001, Mangialajo *et al.* 2008), sometimes to local extinction (Thibaut *et al.* 2015, Perkol-Finkel & Airoldi 2010, Airoldi *et al.* 2014). Recent work has tried to understand what factors can facilitate the persistence of populations of *Cystoseira* spp. across a range of increasing anthropogenic and climatic stressors and habitat modifications (Strain et al 2016), and what are the opportunities for restoration in natural and artificial habitats along increasingly urbanized coastlines (Perkol-Finkel et al 2012, Gianni et al 2013, Ferrario et al 2016). These programs should also incorporate knowledge of genetic structuring and connectivity to ensure that rehabilitation has the best possible chance of success by considering natural scales of dispersal and gene flow.

In this study we ask whether the genetic structure and genetic connectivity of populations of marine species that can only disperse by rafting can be better predicted by taking into account stepping stone migration between suitable habitat patches (i.e., rocky habitat continuity) and dispersal pathways inferred from oceanographic currents rather than distance alone. We use as model oceanographic region a complex coastline in southern Italy where geographical and oceanographic distances are uncoupled, therefore providing the opportunity to compare their effects. Our model species is *Cystoseira amentacea* var. *stricta* Montagne, chosen to represent low dispersal species whose propagules do not have a planktonic stage. This trait is expected to result in genetically isolated populations where suitable habitat is discontinuous, only counteracted by transport of reproductive drifting thalli in floating rafts. If connectivity is limited, the consequent smaller population gene pools and sizes render populations more vulnerable to threats that cause demographic instability (e.g., recruitment failure due to high sedimentation, Benedetti-Cecchi et al 2001, Soltan et al 2001, Ballesteros et al 2007). We compared genetic and oceanographic estimates of connectivity at different scales to assess the hypothesis that stepping stone transport by directional currents and habitat continuity could explain the main patterns of genetic differentiation. We chose as model region a complex oceanographic system where simple geographical distance can be distinguished from current transport and rocky habitat continuity.

Cystoseira amentacea life history

C. amentacea is an endemic Mediterranean species, restricted to the upper sublittoral zone and highly sensitive to environmental degradation, such as that occurring in proximity of sewage outfalls, ports and urban areas (Benedetti-Cecchi et al 2001; Soltan et al. 2001; Mangialajo *et al.* 2008; Falace et al 2010; Thibaut et al. 2014). It is used as an indicator of good environmental status within the EU Water-Framework Directive (2000/60/EC) (Ballesteros *et al.* 2007). The species has experienced local declines (Zavodnik *et al.* 2002; Mangialajo *et al.* 2008; Iveša *et al.* 2016), although in other regions it has been historically stable (Thibaut *et al.* 2016), and it is currently protected under the Bern Convention and Barcelona Convention. Little is known about the genetic diversity of this species or any other *Cystoseira* species (but see Susini *et al.* 2007; Robvieux *et al.* 2012; Robvieux 2013), limiting our understanding of populations and species resilience to local and global stressors.

Dispersal of *C. amentacea* is very restricted except for transport in floating rafts. Thalli are negatively buoyant, and propagules normally settle at less than 20-40 cm from the source (Mangialajo *et al.* 2012), strongly limiting the potential dispersal distance. Fragments of thalli can however also be transported entangled in floating rafts of other algae (Susini *et al.* 2007a), a relatively frequent and documented mechanism of marine dispersal (Thiel & Gutow 2005). Like most species in the genera *Cystoseira* and *Sargassum*, *C. amentacea* undergoes an annual thallus loss at the end of summer, where the entire frond breaks at the base. The holdfasts overwinter and regrow in the next year. Therefore, although individuals are perennial, the thalli are annual, forming large thallus loss events. To what extent these annual events release fragments that carry viable reproductive structures has not been studied in *C. amentacea*. However, massive drifting events of reproductive thalli are suggested to be a key factor in the invasion success of *Sargassum muticum* (e.g., Norton 1976).

Methods

Study area and sampling design

Samples were collected along the southern Italian coast at 8 different locations (Fig.1) encompassing the regions of Puglia, Calabria and the island of Sicily, to represent a complex coastline system regarding rocky shore continuity and current patterns. Shallow rocky habitats are naturally fragmented (Fig. 1), particularly due to separation of the mainland and the island of Sicily and because this area comprise the two regions (Sicily and Calabria) with the highest national extension of sandy coastlines (ISPRA 2015). Up to 75% of the local human populations live within 5 km from the coast, determining a variety of coastal pressures and transformations (Thompson *et al*. 2002) further modifying the suitable habitat available for species of *Cystoseira* (Perkol-Finkel & Airoldi 2010; Thibaut *et al.* 2014). In this region, the species presence is historically scattered (S1, supporting information) and limited by fragmentation of suitable substratum.

The eight locations sampled comprise three populations from the Italian mainland, and two and three populations from the southern and northern coast of Sicily, respectively. Population distances along the coast covered different scales, from hundreds of meters, the distance between three subpopulations at one location, Capo Gallo (Fig. 1), up to about 1000 km between the most distant population pair. The species was confidently identified in the field as it could be distinguished from other closely related taxa thanks to the expertise of the collectors and according to a local identification guide (Mannino & Mancuso 2009; identifications were later confirmed by the genetic results). Apical tissue, free from epiphytes, was collected from 30 to 35 individuals haphazardly selected and separated by at least 30 cm at each study site. Samples were preserved dry in silica gel.

At Capo Gallo an additional fine scale sampling scheme was applied along 230 m of coast. Three 10 m transects were sampled, located 100 m apart from each other, each consisting of 30 individuals collected every 30 cm. Coordinates were recorded for every individual to allow to estimate exact distances between pairs of individuals. Additionally, 5 individuals were collected every 25 m between the 3 transects, totaling 120 sampled specimens in Capo Gallo. We replicated the spatial autocorrelation analysis in Sant'Elia, but only up to a maximum distance of 34 m. It was not possible to replicate the entire sampling scheme as in Capo Gallo because due to the high degree of local population fragmentation no other site was found to hold a continuous population along 230 m of coastline.

Genetic analyses

Genomic DNA was extracted from algal tissue using a cetylatrimethyl ammonium bromide (CTAB) method optimized for brown algae (Hoarau *et al.* 2007). Ten microsatellites previously developed for *Cystoseira tamariscifolia* (Engelen *et al.* in press) were tested, eight of which successfully cross-amplified in *C. amentacea* after optimization. These were chosen instead of previously published microsatellites (Robvieux *et al.* 2012) for future data comparability between these two closely related species. The primer Ct 2.3, excluded for *C. tamariscifolia,* was included in our analysis because it showed polymorphism across populations (F: GTCGGATGCTCCTCCAGTC, R: AGTGATCTAGCCCAGCGAAG; $(AC)_{10}$). Fragment lengths were estimated on an ABI PRISM 3130 DNA analyzer (Applied Biosystems) with the LIZ 500 (GeneScan) standard. Alleles were scored using the free software STRand (Toonen & Hughes 2001) and further binned into size classes with the msatAllele R package (Alberto 2013).

The genotypes obtained per population were used to estimate population diversity (gene diversity and allelic richness) and to test for departures from the null hypothesis of Hardy-Weinberg equilibrium (H_0: F_{IS}=0) using permutations as implemented in GENETIX (Belkhir et al. 1996-2004). Presence of null alleles was estimated with Microchecker (Van Oosterhout *et al.* 2004) and corrected F_{ST} (θ, Weir & Cockerham 1984) accounting for null alleles were estimated with FreeNA (Chapuis & Estoup 2007). Results of corrected F_{ST} were compared with the original matrix of genetic distances between population pairs. Partition of variance was estimated with AMOVA (Excoffier *et al.* 1992) in GenALEx (Peakall & Smouse 2006). The null hypothesis of no pairwise differentiation (F_{ST}=0) was tested using 999 permutations of individuals across populations. Optimal population clustering and graphical visualization of population distance was obtained through the Discriminant Analysis of Principal Components (DAPC) approach implemented in the *adegenet* R package (Jombart 2008). DAPC minimized the within individuals genetic variance in the choice of discriminant functions to improve the between groups genetic variance description (Jombart *et al.* 2010).

Fine scale genetic differentiation was tested as variation of the probability of identity-by-descent between pairs of individuals over the total distance. We calculated the kinship autocorrelation coefficient as the r index (Smouse & Peakall 1999), a multiallelic codominant loci analysis closely related to Moran's-I index, and plotted the correlogram in GenALEx (Peakall & Smouse 2006). Statistical significance and confidence intervals were estimated by 999 random permutations.

Habitat continuity and particle dispersal

To infer the potential connectivity among *C. amentacea* populations, we developed individual-based Lagrangian Numerical Simulations (hereafter LNS) following the framework implemented by Assis et al. (2015) and Klein et al. (2016). This modeling approach mimics the species' capacity to disperse between favorable habitat patches via rafting of floating macroalgae (Susini *et al.* 2007a). The simulation tracked the geographical position of virtual particles advected by surface velocity fields (0 m depth layer) derived from the Hybrid Coordinate Ocean Model (HYCOM), a high-resolution product produced on a daily basis, forced by heat flux, precipitation, wind speed and wind stress (Chassignet *et al.* 2007). This oceanic model is already validated in the Mediterranean (Xu *et al.* 2007) and resolves oceanic fronts, filaments, meandering currents and eddies, essential processes to simulate the dispersion of floating rafts (Chassignet *et al.* 2007; Lett *et al.* 2008; Young *et al.* 2012).

Suitable near-shore rocky habitats in southern Italy were digitized (Quantum GIS Development Team 2014) and gridded to 0.01° x 0.01° cells (~0.9km), which is the spatial resolution of the simulation. Individual particles simulated floating rafts of C. *amentacea* by incorporating a set of state variables such as age (day), location (longitude and latitude) and status (drifting or settled). These were released from every cell with favorable habitat on a daily basis and allowed to drift for 30 days throughout summer (Jul-Sep; simulation period per year), the season when the individuals of C. *amentacea* are reproductive and may associate with other macroalgae, facilitating the drifting of the rafts, which otherwise would be limited due to the negative buoyance of the thalli.

The duration of rafts was established at 30 days according to viability tests on *Sargassum spp,* one of the plausible vector for the species (Thiel & Gutow 2005). The longevity of viable reproductive material in rafts is unknown so the best estimate is provided by laboratory studies in other fucoids that have kept unattached reproductive material releasing gametes for 24 days (Monteiro et al. 2016). Since unattached fucoid algae do not produce new reproductive structures (e.g., Norton & Mathieson 1983), the role of rafts on dispersal must be mediated by the longevity of the reproductive structures produced before detachment. The position of each particle (in geographical coordinates) was calculated every two hours (12 time steps per day) using bilinear interpolation of the daily velocity fields. A 4th-Order Runge-Kutta calculation scheme (sampling at midpoints along the distance) was integrated to determine the trajectory of particles throughout the simulated time steps (e.g., Lett et al. 2008).

Dispersal simulations were performed independently per year for an 11-year period (2002-2012). The individual trajectories were used to infer paired probability estimates by determining the steps in the path of each individual particle released from a given cell i, that achieved or crossed cell j, divided by the number of simulated steps (30 days PD * 12 time steps a day). Squared connectivity matrices between all pairs of sites were produced by averaging the probability outcomes from all particle release events performed in the simulation period of a given year (i.e., 92 particles per cell). Probabilities between samples in Capo Gallo were estimated as the retention probability inside the same cell, as the grid resolution of the oceanographic currents was not small enough to resolve connectivity at that scale. An averaged connectivity matrix between every pair of cells was determined for the 11-year period.

We implemented a graph-theoretical framework to account for the hypothesis of genotypes migrating between suitable habitats patches on a year to year stepping-stone scenario. The Floyd-Warshall's algorithm (Floyd 1962) was used to find the shortest directed path between cells (i.e., the graph nodes), using the paired probabilities from the averaged connectivity matrix as the direct weight between edges (i.e., the graph links). The stepping-stone probabilities were determined by multiplying the probabilities found in the shortest path between all pairs of cells. The averaged and stepping-stone connectivity matrices were used to extract the connectivity estimates between the sites sampled for genetics. LNS were developed in R (R Core Team 2014) with packages: calibrate, e1071, parallel and raster.

Directionality of oceanographic transport

An index of directionality was designed to be proportional to the directionality of currents driving particles between sites, being 0 where completely bidirectional and 1 when exclusively unidirectional and calculated as $I_{(ij)} = \frac{(P_{min} - P_{max})}{P_{min}}$ where P_{min} is the natural logarithm of the minimum probability between sites i and j from the LNS model and P_{max} the logarithm of the maximum probability between the same sites. The index was developed to test the hypothesis that sites connected with bidirectional gene exchange show lower genetic differentiation compared to sites with unidirectional gene flow.

Modeling

Linear regressions were used to explain pairwise genetic distances between populations estimated as F_{ST} / (1- F_{ST}) (Rousset & Raymond 1997). In order to test the regression models, all the pairwise probabilities were transformed with the natural logarithm to account for the wide variability and the skewness toward zero values. Correlation was tested using the shortest marine distance, distance along the shore, habitat continuity (km of rocky shore/km of shore), average probability obtained from the LNS model and the index of directionality as predictors. The slope of linear regression between genetic differentiation and logarithm of geographic distance was used to derive the distance at which a limit of $F_{ST} = 0.2$ was reached. We assessed the potential of multiple regressions models from a combination of predictors to improve the data fitting when these met assumptions for multiple regression. Akaike information criteria (AIC) and Bayesian information criterion (BIC) were adopted to assess the model efficiency in predicting the data in the model selection phase. We generated a confidence set of models to help formal inference using model likelihood and Akaike weight (Johnson & Omland 2004).

Results

No significant linkage between loci was detected after Bonferroni correction. Most loci showed high polymorphism, ranging from 12 (Ct2.10 and Ct4.4.) to 36 (Ct4.2) total alleles, with the exception of locus Ct2.3 with only three alleles. No multilocus genotype clones were found.
Significant deviation from HWE was observed at most of the loci and populations (Table 1). Null alleles were detected at three loci (Ct2.10, Ct4.2 and Ct4.3), but these were not consistent across populations. Even after repeated extractions some populations showed lower amplifications rates at these loci (especially Capo Gallo and Altavilla), possibly inflating null allele detection. Global F_{ST} estimated with the ENA method for null allele correction (0.292) showed little difference from uncorrected F_{ST} (0.308). The highest allelic richness, standardized by sampling to the maximum common population size (N=25) was observed in Capo Gallo (4.76) and the lowest in the eastern populations of Otranto and Torre Uluzzo with 2.75 and 2.29 respectively (Table 1).

Genetic differentiation

Genetic divergence was significant between all populations (P = 0.001), including at 100 m distances (P = 0.012). The smallest but significant F_{ST} value was found between Capo Gallo sites A and B ($F_{ST} = 0.017$) while the highest genetic distance was detected between Mazara and Torre Uluzzo populations, the western most and one of the eastern most populations, respectively, with an F_{ST} of 0.510 (S2, supporting information).
Only few individuals could be attributed to another population based on their genotype as first generation migrants based on an assignment test with a frequencies-based method (Paetkau *et al.* 1995) implemented by GENECLASS2 (Piry *et al.* 2004) (S3, supporting information). These were mainly between sites A, B and C inside Capo Gallo population, with an evident predominant direction from the exposed side (A) towards the inside of the bay (C).

Cluster analysis showed that the optimal number of clusters identified by lower BIC is 10, which corresponds to the actual number of populations (including Capo Gallo as subpopulations). The strongest decrease in BIC was found at the first three clusters. Two of them correspond, as expected, to the cluster of populations around Palermo and the cluster of eastern populations (Otranto and Torre Uluzzo), as locations inside these clusters were geographically closer. However, the third cluster included the populations of southern Sicily and the mainland population of Crotone, which are hundreds of kilometers apart and separated by the Strait of Sicily (S4, supporting information). Yet, these locations were predicted to be connected by oceanographic currents, and particle dispersal simulation confirmed this observation (Fig. 2).

The analysis of molecular variance attributed 64% of the genetic variance to the intra-population level. The remaining 36% was attributed to inter-populations variance: 20% was assigned to the distance among clusters and 16% among populations. F-statistic among clusters resulted to be similar to the F-statistics among population within clusters (0.201 and 0.195 respectively).

Fine scale autocorrelation

We discarded one of the molecular markers (Ct2.10) for this analysis because the locus showed a low rate of amplification in some transects. Spatial autocorrelation analysis across sites showed an overall decline in r with distance. At small spatial scales (within the 10 meters distance) significant positive r values were observed in the first meter distance and an x-intercept at about 3.3 meters (Fig. 3a). Patterns of decline in r values were consistent across transects A, B and C, and we found no significant differences associated to distance classes (T2 and omega heterogeneity tests: P<0.01). Higher scales analysis, considering pairwise distances between elements of transects showed that kinship decreased faster within the smaller classes up to 10 meters while slowly decreasing and oscillating inside the null hypothesis CI for higher distances, and declining again reaching significantly negative values at about 200 meters (Fig. 3). We replicated the spatial autocorrelation analysis in Sant'Elia, where the coordinates of each of the 35 specimens were recorded. The maximum distance among sample pairs was only 34 m, however similar patterns of decrease in kinship coefficients could be found (S5, supporting information).

Modeling

A total of 125 favorable habitat patches were found based on satellite image of rocky shores. After gridding, this resulted in a habitat continuity index expressed as rate of rocky shore by coastline distance, varying from 0.38 (discontinuous patches, separated by unsuitable habitats) to 1 for the contiguous suitable rocky coastlines. The lowest values were recorded between the eastern populations of Crotone and Torre Uluzzo, as most of the coast of the Gulf of Taranto and the Calabria Ionian shore is sandy and unsuitable for the species.

The LNS using HYCOM data tracked the geographic position of 134,640 particles released from 1,496 cells with favorable habitats (1,481,040 particles for the 11-year period) (Figure 2). Most connectivity events occurred in the first days of simulation, as the average time of effective connectivity events was ca. 2 days (1.97 day ± 1.77). Despite the maximum allowed pelagic duration of 30 days, used in this study, the longer connectivity event was accomplished in only 17.83 days. This period of ca. 18 days is well within the time when fucoid reproductive material are viable while unattached (at least for 24 days, Monteiro *et al.* 2016). After this maximal connectivity event observed, particles were pushed offshore or transported to regions outside the scope of our study.

The resulting trajectories yielded two asymmetric matrices: a direct connectivity matrix averaged for the 11-year period and a stepping-stone matrix. Direct connectivity resulted in few valid pairwise probabilities, as most of the populations were not oceanographically connected by our simulated floating rafts. In contrast, stepping stone probabilities were always resolved in at least one direction between paired sites. Probabilities ranged widely, from maximum values (averaged between sites) of 0.997 for the very close populations of Sant'Elia and Altavilla to a minimum of 5.46E-15 between Altavilla and Torre Uluzzo.

All the predictors tested were significant, but there were important differences in the ability to explain the data (Table 2). Linearized F_{ST} levels were highly correlated to geographical distance, both expressed as shortest marine distance (Adjusted $R^2 = 0.59$) and as coastline distance (Adjusted $R^2 = 0.68$). Including only the 12 site pairs which presented direct connectivity, without taking into account the stepping stone option, still lead to a positive correlation between genetic distance and coastal distance ($R^2 = 0.55$, $p<0.01$) as well as between genetic distance and the combination of coastal distance and average direct probability ($R^2 = 0.70$, $p<0.01$). The single term model of coastline distance was not significantly different (delta AIC = 0.99) from the stepping stone model of average probability of connectivity. Both were highly correlated to estimated genetic distance (Adjusted $R^2 = 0.68$ and 0.67 respectively), and both performed better than the shortest marine distance to explain our data (model likelihood $L_{marine} = 0.02$). However, the model based on average probability from stepping-stone oceanographic transport did not appear to produce a better fit than a combination of habitat continuity and coastal distance. These two variables explained 73% of the variation in genetic differentiation, and were a better predictor model according to Akaike criteria, but the difference to the stepping stone model was not significant according to model likelihood (AIC = -21.57; of $L_{two\text{-}term} = 0.88$) and was just as good as best single terms models with a more conservative diagnostic (BIC = -14.40 and -14.33 respectively).

The directionality index was not a very good predictor of genetic differentiation ($R^2 = 0.27$; $P_{(F\ test)} = 0.0015$), but significantly increased the fitting of the observations when added to other models. Our best-fit model (Fig. 4) was obtained adding the information of directionality to the two term model ($R^2=0.78$; AIC = -28.80), significantly interacting with coastline distance ($P_{(t\ test)} < 0.05$) and rocky shore percentage ($P_{(t\ test)} = 0.001$).

Discussion

Populations of *C. amentacea* were strongly differentiated and connected by very low migration rates, leading to significant genetic distance even at small spatial scales. The capacity to predict genetic differentiation was improved when dispersal probability across available rocky habitat was estimated by simulating stepping stone directional transport mediated by ocean currents.

Genetic differentiation between populations

Populations of *C. amentacea* had strong differentiation at all geographical scales, from a few meters to hundreds of kilometers. Genetic differentiation was comparable to populations of other brown algae (Durrant *et al*. 2014), such as the fucoids *Fucus vesiculosus* (Tatarenkov *et al.* 2007; Assis et al. 2014), *Pelvetia canaliculata* (Neiva et al. 2014) or *Bifurcaria bifurcata* (Neiva et al. 2015). Similar differentiation had been estimated for the same species using random amplified polymorphic DNA (RAPD) markers at similar spatial scales (Susini et al. 2007a). As expected from intertidal algae with such a low-dispersal life history, the sampled populations were almost completely genetically isolated.

Geographically closer populations were not always the most connected. The sites along the southernmost coastlines of the mainland (Crotone) and Sicily island (Porto Palo and Mazara) were more connected than expected, while sites south and north of Sicily were highly differentiated from each other. This supports the hypothesis that the Ionian Stream in the Sicily channel represents a dispersal barrier between these populations, limiting rafting drift around the Sicily Island. This hydrographic feature and the difference in water density between the water masses of the Western and Eastern Mediterranean across the Sicily Strait had been already reported as a main driver of genetic differentiation in various species (Zardoya *et al.* 2004; Debes *et al.* 2008; Serra et al. 2010; Villamor *et al.* 2014), which could contribute to maintain the divergence established by past vicariant events (Arnaud-Haond *et al.* 2007).

The eastern populations of mainland southern Italy, Otranto and Torre Uluzzo, were very isolated. LNS simulation showed that the marine currents in this area tend to push onshore the drifting particles and allow only occasional rafts to move offshore towards other suitable sites.

Genetic structure within populations

The genetic variance attributed to intra-population differences was high and consistent with a previous study using RAPD markers (Susini *et al.* 2007a). Our spatial autocorrelation analyses showed that individuals start to diverge within distances of a few meters, and significant genetic differences can be found among sites only 100 m apart along a continuous rocky fringe. This is consistent with the observation that recruitment of *C. amentacea* from settling embryos occurs predominantly within 20-40 cm from a natural population (Mangialajo *et al.* 2012), while large distance dispersal may only take place by rafting (Thibaut et al. 2014), and with the high intra-population genetic variance here found. We hypothesize that the observed kinship similarity pattern may be explained by different dispersal processes acting on the species at different geographical scales. On one side, fucoid algae release their gametes under calm water conditions (Pearson & Serrão 2006) that minimize gamete dilution, suggesting that mating occurs by selfing or between neighboring individuals that are expected to be more related on average. Our results support this hypothesis because nearly all populations had significant heterozygote deficits. Plus the negatively buoyant propagules tend to fall close to the parent algae, leading to clustering of related individuals and strong correlation of kinship with distance. On the other side, in the genus *Cystoseira* the female gametes remain attached to the parental algae even after fertilization and germination, for a few days (e.g., Engelen et al. 2008), therefore the water motion conditions may change to higher exposure between the timing of gamete release/mating and the timing of settlement a few days later. Since *C. amentacea* is most common along exposed coastlines, it is conceivable that occasionally such propagules can be transported further apart and mixed in the water column. Furthermore, when water movement increases enough, dislocated fertile algae, although not buoyant, could be transported hundreds of meters away or even further if associated to other floating rafts (Thiel & Gutow 2005), in agreement with the observed colonization of artificial structures at over 3 km from natural populations (Thibaut et al. 2014). The latter process would act along the whole geographical scale while the former would cover distances up to a few tens of meters. Our autocorrelation analysis could only be partially replicated and caution should be taken before generalizing the results to other geographical areas with different oceanographic conditions. Replicating this analysis across sites would allow to understand if similar general processes and patterns can be found, and support that is to be linked to the reproductive life history of the species.

Stepping-stone directional oceanographic transport

Gene flow was strongly related to marine currents even though connectivity between sites was often only possible by means of stepping stone dispersal because direct oceanographic transport was never achieved. The oceanographic transport model revealed that, where direct connectivity of particles was possible, transport by currents explained the observed genetic differentiation better than coastal distance alone even in species with propagules that sink and attach immediately below the parents. Furthermore, the inclusion of a directionality index based on the stepping stone simulation produced the best-fit model explaining genetic differentiation of our populations. This provides strong support for a key role of rafting in population connectivity of low dispersive marine taxa, allowing them to be strongly influenced by oceanographic transport as observed in species with planktonic life stages that persist in the water column for long (e.g., Klein et al. 2016), or short (e.g., Alberto et al. 2011) periods.

Population genetic structure results from past historical events and present reproductive and migratory patterns. Our results suggest that at the multiple spatial scales analysed in this study, the present population pattern is determined and maintained by present low gamete dispersal within population and by raft drifting between populations rather than colonization history. Although all populations were highly differentiated, rare successful migration events might be sufficient to preserve the natural levels of connectivity, as reported for giant kelp based on present oceanographic patterns simulated over 11 years (Alberto *et al.* 2011).

Stepping-stone dispersal explained observed connectivity better than habitat continuity. However, habitat continuity can well describe observed genetic differentiation over an extended spatial scale, comprising discontinuities (Alberto *et al.* 2011), even better when combined with other predictors. The addition of a directionality index predictor is an innovative approach that produced the best-fit model, accounting for the evident asymmetry in currents flow, and therefore indicating a coupled asymmetric genetic flow among populations.

Future directions and implications

Our results showed a dependency of dispersal on ocean currents, supporting the raft association hypothesis, but some genetic patterns still remain unexplained by the simulation. The drifting of reproductively mature algae has been suggested as one of the main mechanisms for dispersal over long distances, for species sharing similar life history traits and reproductive strategies as *C. amentacea* (Coleman & Brawley 2005). However, *C. amentacea* lacks flotation vesicles, and rafts tend to sink rapidly. Our simulation model was based on the hypothesis that reproductive fragments can float for longer distances when associated to other species rafting with floating vesicles, namely *Sargassum spp.* and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, often closely coexisting in the same areas (direct observation). However, rafts might not be able to cover the distance between populations separated by long continuous sandy shores (e.g. between Otranto and Crotone) where observed levels of genetic differentiation were explained by a combination of habitat discontinuity and geographic distance. This is an aspect where more work is clearly needed. Experimental approaches to test raft duration and viability in different associations could help clarifying the issue and possibly increase model accuracy. Even more important, the model was based on inferred habitat presence, but mapping of the actual distribution of *C.amentacea* would be essential for better extrapolation as well as a priority for conservation.

Our results have important implication for two of the main conservation and management strategies suggested for canopy-forming algae: the use of marine protected areas (MPAs) and restoration practices. The establishment of MPAs potentially favors the recovery of macroalgae forests by increasing predation pressure on grazers (Babcock *et al.* 2010) or by eliminating sources of anthropogenic disturbance (Thibaut *et al.* 2016). Networks of MPAs should also allow maintaining connectivity among fragmented populations and increase resilience to future environmental changes (Frankham *et al.* 2002; Coleman *et al.* 2011). However, little is known about the real connectivity of Mediterranean MPAs (Marti-Puig *et al.* 2013). Urbanization and other anthropic pressures on the seaside are locally depleting many populations of canopy forming seaweeds (Benedetti-Cecchi et al 2001, Mangialajo et al 2008, Perkol-Finkel & Airoldi 2010; Thibaut et al. 2015, Strain et al 2015), while currently offering limited possibilities for expansion (Ferrario et al 2016). Lack of connectivity among MPAs and habitat degradation along the coast may decrease the genetic variability and hamper the resilience and adaptation of these endangered habitat-forming species, possibly limiting the expected ecological and socio-economic benefits of protection. Restoration of *Cystoseira* forests on both natural and artificial substrata is an alternative management strategy to counteract the local decline of these species (Falace *et al.* 2006; Susini *et al.* 2007b; Perkol-Finkel et al 2012; Gianni *et al.* 2013). However, precaution should be taken when designing restoration interventions for *C. amentacea* as the connectivity between populations on short temporal scales can only be guaranteed at very small distances. The loss of the species from large, fragmented areas would be hardly reversible even by restoration, as limited dispersal would not allow recolonization in short time scales. This implies that restoration can still be effective to foster recovery if some natural populations are present in the area, and if adequate habitat patches can serve as stepping stones for migrants. This knowledge can help select priority areas for conservation and restoration, as well as incentive a better planning, design and management of marine urbanized areas to mitigate habitat degradation and provide potentially suitable stepping stones (e.g. green-blue infrastructure) for threatened native populations (Perkol-Finkel et al 2012).

In conclusion, this study is pioneer in combining local scale genetic analysis and oceanographic modeling to understand mechanisms of dispersal and to give insight into species life history traits for Mediterranean canopies of brown algae. Explicit modeling of stepping stone dynamics and inclusion of habitat continuity allowed explaining most of the genetic variability in the region, suggesting that integrating the life history processes and directional information can help to better describe and predict population patterns and evolution. This is crucial to understand the potential for adaptation and restoration under scenarios of increasing anthropogenic and climatic stressors and habitat modifications.

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References

Airoldi L, Beck MW (2007) Loss , status and trends for coastal marine habitats of europe. *Oceanography and Marine Biology: An Annual Review*, **45**, 345–405.

Airoldi L, Ballesteros E, Buonomo R *et al.* (2014) Marine forests at risk: solutions to halt the loss and promote the recovery of Mediterranean canopy-forming seaweeds. *Proceedings of the 5th Mediterranean Symposium on Marine vegetation. Portoroz, Slovenia. 2014.*

Airoldi L, Turon X, Perkol-Finkel S, Rius M (2015) - Corridors for aliens but not for natives: effects of marine urban sprawl at a regional scale. *Diversity and Distributions*, **21**, 755-768

Alberto F, Raimondi P, Reed D (2010) Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. *Ecology*, **91**, 49–56.

Alberto F, Raimondi PT, Reed DC *et al.* (2011) Isolation by oceanographic distance explains genetic structure for *Macrocystis pyrifera* in the Santa Barbara Channel. *Molecular Ecology*, **20**, 2543–54.

Alberto F (2013) MsatAllele: Visualizes the scoring and binning of microsatellite fragment sizes. R package version 1.05. http://CRAN.R-project.org/package=MsatAllele

Arnaud-Haond S, Migliaccio M, Diaz-Almela E *et al.* (2007) Vicariance patterns in the Mediterranean Sea: east?west cleavage and low dispersal in the endemic seagrass Posidonia oceanica. *Journal of Biogeography*, **34**, 963–976.

Assis J, Serrão EA, Claro B, Perrin C, Pearson GA (2014) Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. *Molecular Ecology*, **23**, 2797-2810.

Assis J, Zupan M, Nicastro KR, Zardi GI, McQuaid CD, Serrão EA (2015) Oceanographic conditions limit the spread of a marine invader along southern Africa shores. *PLoS ONE*, **10**, e0128124.

Babcock RC, Shears NT, Alcala AC *et al.* (2010) Decadal trends in marine reserves reveal differential rates of change in direct and indirect effects. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 18256–61.

Ballesteros E, Torras X, Pinedo S *et al.* (2007) A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. *Marine Pollution Bulletin*, **55**, 172–80.

Benedetti-Cecchi L, Pannacciulli F, Bulleri F *et al.* (2001) Predicting the consequences of anthropogenic disturbance: large-scale effects of loss of canopy algae on rocky shores. *Marine Ecology Progress Series*, **214**, 137–150.

Belkhir K., Borsa P., Chikhi L., Raufaste N. & Bonhomme F. 1996-2004 GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (Fr)

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, **24**, 621–31.

Chassignet EP, Hurlburt HE, Smedstad OM *et al.* (2007) The HYCOM (HYbrid Coordinate Ocean Model) data assimilative system. *Journal of Marine Systems,* **65**, 60–83.

Chevin L-M, Lande R, Mace GM (2010) Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biol*, **8**, e1000357.

Coleman MA, Brawley SH (2005) Are life history characteristics good predictors of genetic diversity and structure? A case study of the intertidal alga *Fucus spiralis* (heterokontophyta; phaeophyceae). *Journal of Phycology*, **41**, 753–762.

Coleman MA, Chambers J, Knott NA et al. (2011) Connectivity within and among a network of temperate marine reserves. *PloS one,* **6**, e20168.

Collins CJ, Fraser CI, Ashcroft A, Waters JM (2010) Asymmetric dispersal of southern bull-kelp (*Durvillaea antarctica*) adults in coastal New Zealand: testing an oceanographic hypothesis. *Molecular Ecology,* **19**, 4572–80.

Crandall ED, Treml EA, Barber PH (2012) Coalescent and biophysical models of stepping-stone gene flow in neritid snails. *Molecular Ecology,* **21**, 5579–98.

Coscia I, Robins P, Porter J (2012) Modelled larval dispersal and measured gene flow: seascape genetics of the common cockle Cerastoderma edule in the southern Irish Sea. *Conservation Genetics,* **14**, 451-466

Debes P V, Zachos FE, Hanel R (2008) Mitochondrial phylogeography of the European sprat (*Sprattus sprattus* L., Clupeidae) reveals isolated climatically vulnerable populations in the Mediterranean Sea and range expansion in the northeast Atlantic. *Molecular Ecology*, **17**, 3873–88

Durrant HMS, Burridge CP, Kelaher BP *et al.* (2014) Implications of macroalgal isolation by distance for networks of marine protected areas. *Conservation biology : the Journal of the Society for Conservation Biology*, **28**, 438–45.

Engelen A, Espirito-Santo C, Simões T, Monteiro C, Serrão EA, Pearson GA, Santos R. (2008) Periodicity of propagule expulsion and settlement in the competing native and invasive brown seaweeds, *Cystoseira humilis* and *Sargassum muticum* (Phaeophyta). *Eur J Phycol*, **43**, 275-282

Engelen AH, Costa J, Bermejo R, Marba N, Duarte CM, Serrão EA (2016) A population genetics toolbox for the threatened canopy-forming brown seaweeds *Cystoseira tamariscifolia* and *C. amentacea* (Fucales, Sargassaceae). *Journal of Applied Phycology*, 1-3, doi: 10.1007/s10811-016-0964-7, in press.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics,* **131**, 479–491.

Falace A, Zanelli E, Bressan G (2006) Algal transplantation as a potential tool for artificial reef management and environmental mitigation. *Bulletin of Marine Science*, **78**, 161–166.

Falace A, Alongi G, Cormaci M *et al.* (2010) Changes in the benthic algae along the Adriatic Sea in the last three decades. *Chemistry and Ecology*, **26**, 77–90.

Ferrario F, Iveša L, Jaklin A, et al. (2016) The overlooked role of biotic factors in controlling the ecological performance of artificial marine habitats. *Journal of Applied Ecology*, **53**, 16-24.

Floyd RW (1962) Algorithm 97: Shortest path. *Communications of the ACM*, **5**, 344-348.

Frankham R, Briscoe DA, Ballou JD (2002) *Introduction to Conservation Genetics*. Cambrige University Press. Cambridge.

Fraser CI, Thiel M, Spencer HG, Waters JM (2010) Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp. *BMC Evolutionary Biology*, **10**, 203.

Galindo HM, Olson DB, Palumbi SR (2006) Seascape genetics: a coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology : CB*, **16**, 1622–6.

Gianni F, Bartolini F, Airoldi L *et al.* (2013) Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. *Advances in Oceanography and Limnology*, **4**, 83–101.

Hoarau G, Coyer JA, Stam WT, Olsen JL (2007) A fast and inexpensive DNA extraction/purification protocol for brown macroalgae. *Molecular Ecology Notes*, **7**, 191–193.

ISPRA (2015) *Annuario dei dati ambientali*. Istituto Superiore per la Protezione e la Ricerca Ambientale – Settore Editoria. Roma.

Iveša L, Djakovac T, Devescovi M (2016) Long-term fluctuations in Cystoseira populations along the west Istrian Coast (Croatia) related to eutrophication patterns in the northern Adriatic Sea. *Marine pollution bulletin*, **106**, 162–73.

Johansson ML, Banks MA, Glunt KD, Hassel-Finnegan HM, Buonaccorsi VP (2008) Influence of habitat discontinuity, geographical distance, and oceanography on fine-scale population genetic structure of copper rockfish (*Sebastes caurinus*). *Molecular Ecology*, **17**, 3051–61.

Johnson JB, Omland KS (2004) Model selection in ecology and evolution. *Trends in Ecology & Evolution*, **19**, 101–8.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics (Oxford, England)*, **24**, 1403–5.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC genetics*, **11**, 94.

Jones P (2007) Point-of-View: Arguments for conventional fisheries management and against no-take marine protected areas: only half of the story? *Reviews in Fish Biology and Fisheries*, **17**, 31–43.

Kawecki T, Holt R (2002) Evolutionary consequences of asymmetric dispersal rates. *The American Naturalist*. **160**, 333-347

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.

Klein, M., Teixeira, S., Assis, J., Serrão, E.A., Gonçalves, E.J., Borges, R. (2016) High interannual variability in connectivity and genetic pool of a temperate clingfish, matches oceanographic transport predictions. *Plos One*, **11**, e0165881.

Lett C, Verley P, Mullon C *et al.* (2008) A Lagrangian tool for modelling ichthyoplankton dynamics. *Environmental Modelling & Software*, **23**, 1210–1214.

Mangialajo L, Chiantore M, Cattaneo-Vietti R (2008) Loss of fucoid algae along a gradient of urbanisation, and structure of benthic assemblages. *Marine Ecology Progress Series*, **358**, 63–74.

Mangialajo L, Chiantore M, Susini M-L *et al.* (2012) Zonation patterns and interspecific relationships of fucoids in microtidal environments. *Journal of Experimental Marine Biology and Ecology*, **412**, 72–80.

Mannino AM, Mancuso FP (2009) Guida all'identificazione delle Cistoseire (Area Marina Protetta "Capo Gallo-Isola delle Femmine"). Progetto AMPOCYS. Tipografia Alba, Palermo.

Marti-Puig P, Costantini F, Rugiu L, Ponti M, Abbiati M (2013) Patterns of genetic connectivity in invertebrates of temperate MPA networks. *Advances in Oceanography and Limnology*, **4**, 138–149.

McKay JK, Christian CE, Harrison S, Rice KJ (2005) "How Local Is Local?"-A Review of Practical and Conceptual Issues in the Genetics of Restoration. *Restoration Ecology*, **13**, 432–440.

Monteiro CA, Paulino C, Jacinto R, Serrão EA, Pearson GA (2016) Temporal windows of reproductive opportunity reinforce species barriers in a marine broadcast spawning assemblage. *Scientific Reports*, **6**, 29198.

Neiva J, Pearson GA, Valero M, Serrão EA (2012) Fine-scale genetic breaks driven by historical range dynamics and ongoing density-barrier effects in the estuarine seaweed *Fucus ceranoides* L. *BMC Evolutionary Biology*, **12**, 78.

Neiva, J, Assis, J, Fernandes, F, Pearson, GA, Serrão, EA. (2014) Species distribution models and mitochondrial DNA phylogeography suggest an extensive biogeographical shift in the high-intertidal seaweed *Pelvetia canaliculata*. *Journal of Biogeography,* **41**, 1137-1148.

Neiva J, Assis J, Coelho NV, Fernandes F, Pearson GA, Serrão EA (2015) Genes left behind: climate change threatens cryptic genetic diversity in the canopy-forming seaweed *Bifurcaria bifurcata*. *PLoS ONE*, **10**, e0131530.

Norton, T. A. (1976) Why is Sargassum muticum so invasive? *British Phycological Journal*, **11**, 197-198.

Norton, T.A. & Mathieson, A.C. (1983) The biology of unattached seaweeds. *Progress in Phycological Research*, **Vol. 2**, 333–386.Opdam P, Wascher D (2004) Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation*, **117**, 285–297.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pearson G a, Serrão E a (2006) Revisiting synchronous gamete release by fucoid algae in the intertidal zone: fertilization success and beyond? *Integrative and Comparative Biology*, **46**, 587–97.

Perkol-Finkel S, Ferrario F, Nicotera V, Airoldi L (2012) Conservation challenges in urban seascapes: promoting the growth of threatened species on coastal infrastructures. *J Appl Ecol*, **49**, 1457–1466

Perkol-Finkel S, Airoldi L (2010) Loss and recovery potential of marine habitats: an experimental study of factors maintaining resilience in subtidal algal forests at the Adriatic Sea. *PLoS ONE*, **5**, e10791.

Piry S, Alapetite A, Cornuet J-M *et al.* (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. *The Journal of Heredity*, **95**, 536–9.

Quantum GIS Development Team (2014). Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Robvieux P (2013) Conservation des populations de Cystoseira en régions Provence-Alpes-Côte-d’Azur et Corse.

Robvieux P, Videment J, Ribout C *et al.* (2012) First characterization of eight polymorphic microsatellites for Cystoseira amentacea var. stricta (Fucales, Sargassaceae). *Conservation Genetics Resources*, **4**, 923–925.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **1228**, 1219–1228.

Rousset F, Raymond M (1997) Statistical analyses of population genetic data: new tools, old concepts. *Trends in Ecology & Evolution*, **12**, 313–7.

Schultz J, Feldheim K (2008) Global phylogeography and seascape genetics of the lemon sharks (genus Negaprion). *Molecular Ecology*, **17,** 5336–48.

Selkoe K a, Watson JR, White C *et al.* (2010) Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology*, **19**, 3708–26.

Serra IA, Innocenti AM, Di Maida G, Calvo S, Migliaccio M, Zambianchi E, Pizzigalli C, Arnaud-Haond S, Duarte CM, Serrão EA, Procaccini G (2010) Genetic structure in the Mediterranean seagrass Posidonia oceanica. Disentangling past vicariance events from contemporary patterns of gene flow. *Molecular Ecology*, **19**, 557-568

Siegel D, Kinlan B (2003) Lagrangian descriptions of marine larval dispersion. *Marine Ecology Progress series,* **260**, 83–96.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity,* **82**, 561–573.

Soltan D, Verlaque M, Boudouresque CF, Francour P (2001) Changes in macroalgal communities in the vicinity of a Mediterranean sewage outfall after the setting up of a treatment plant. *Mar. Pollut. Bull.*, **42**, 59–70.

Storfer A, Murphy MA, Evans JS *et al.* (2007) Putting the “landscape” in landscape genetics. *Heredity,* **98**, 128–42.

Strain EMA, van Belzen J, van Dalen J, *et al* (2015) Management of local stressors can improve the resilience of marine canopy algae to global stressors. PLoS One **10**, e0120837.

Strain EMA, Thomson RJ, Micheli F, et al (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. Glob *Chang Biol,* **20**, 3300–3312.

Sunday JM, Popovic I, Palen WJ, Foreman MGG, Hart MW (2014) Ocean circulation model predicts high genetic structure observed in a long-lived pelagic developer. *Molecular Ecology*, **23**, 5036–47.

Susini ML, Thibaut T, Meinesz A, Forcioli D (2007a) A preliminary study of genetic diversity in *Cystoseira amentacea* (C. Agardh) Bory var. stricta Montagne (Fucales, Phaeophyceae) using random amplified polymorphic DNA. *Phycologia*, **46**, 605–611.

Susini ML, Mangialajo L, Thibaut T, Meinesz A (2007b) Development of a transplantation technique of *Cystoseira amentacea* var. *stricta* and *Cystoseira compressa*. *Hydrobiologia*, **580**, 241–244.

Tatarenkov A, Jönsson RB, Kautsky L, Johannesson K (2007) genetic structure in populations of fucus vesiculosus (phaeophyceae) over spatial scales from 10m to 800km. *Journal of Phycology*, **43**, 675–685.

Thibaut T, Blanfuné A, Markovic L *et al.* (2014) Unexpected abundance and long-term relative stability of the brown alga Cystoseira amentacea, hitherto regarded as a threatened species, in the north-western Mediterranean Sea. *Marine Pollution Bulletin*, **89**, 305–323.

Thibaut T, Blanfuné A, Boudouresque CF, Verlaque M (2015) Decline and local extinction of Fucales in the French Riviera: The harbinger of future extinctions? *Mediterranean Marine Science*, **16**, 206–224.

Thibaut T, Blanfuné A, Boudouresque C-F *et al.* (2016) Unexpected Temporal Stability of Cystoseira and Sargassum Forests in Port-Cros, one of the Oldest Mediterranean Marine

National Parks. *Cryptogamie, Algologie*, **37**, 61–90.

Thiel M, Gutow L (2005) The ecology of rafting in the marine environment. I. The floating substrata. *Oceanography and Marine Biology: an Annual Review*, **42**, 181–264.

Thompson RC, Crowe TP, Hawkins SJ (2002) Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. *Environmental Conservation*, **29**. 168-191.

Thrush SF, Hewitt JE, Lohrer AM, Chiaroni LD (2013) When small changes matter: the role of cross-scale interactions between habitat and ecological connectivity in recovery. *Ecological Applications*, **23**, 226–238.

Toonen R, Hughes S (2001) Increased throughput for fragment analysis on an ABI Prism® 377 automated sequencer using a membrane comb and STRand software. *Biotechniques*, **31**, 1320–1325.

Treml E a., Halpin PN, Urban DL, Pratson LF (2007) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. *Landscape Ecology*, **23**, 19–36.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: Software for Identifying and Correcting Genotyping Errors in Microsatellite Data. *Molecular Ecology Notes*, **4**, 535–538.

Villamor A, Costantini F, Abbiati M (2014) Genetic Structuring across Marine Biogeographic Boundaries in Rocky Shore Invertebrates (M Hart, Ed,). *PLoS ONE*, **9**, e101135.

Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

White C, Selkoe KA, Watson J *et al.* (2010) Ocean currents help explain population genetic structure. *Proceedings. Biological sciences / The Royal Society*, **277**, 1685–94.

Xu X, Chassignet EP, Price JF, Özgökmen TM, Peters H (2007) A regional modeling study of the entraining Mediterranean outflow. *Journal of Geophysical Research: Oceans*, **112**, 2156-2202.

Young CM, He R, Emlet RB *et al.* (2012) Dispersal of deep-sea larvae from the intra-American seas: simulations of trajectories using ocean models. *Integrative and Comparative Biology*, **52**, 483–96.

Zardoya R, Castilho R, Grande C *et al.* (2004) Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. *Molecular Ecology*, **13**, 1785–98.

Zavodnik N, Ivesa L and Travizi A (2002) Note on recolonisation by fucoid algae *Cystoseira spp*. and *Fucus virsoides* in the North Adriatic Sea. *Acta Adriat*., **43**, 25–32.

Data Accessibility:

Microsatellite scorings, information on populations' sites and all the data used in the analysis can be found in the Dryad repository with entry doi:10.5061/dryad.dk563.

Figures and Tables

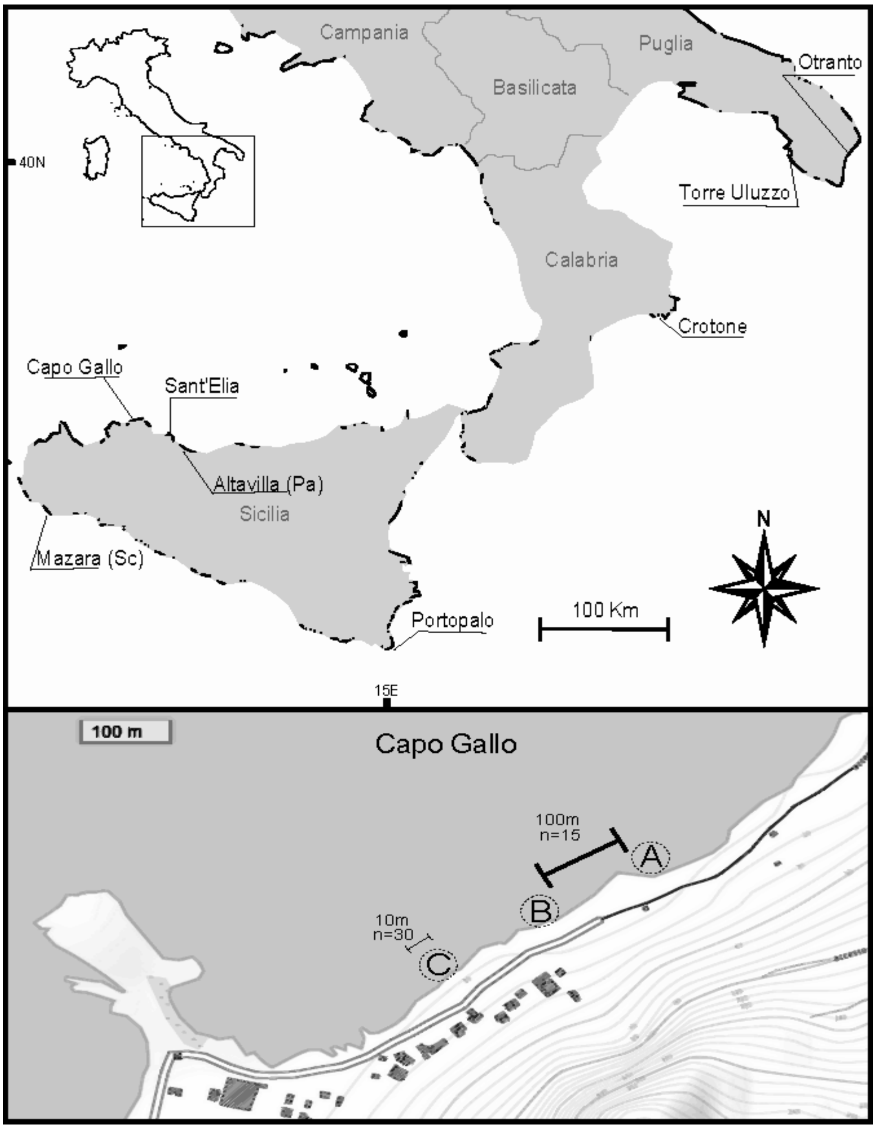


Figure. 1 - Map of the study area with the location of the eight sampled *Cystoseira amentacea* populations. Darker coastline represents areas with rocky shore. Transects A, B and C within Capo Gallo are also shown with respective distances and samples sizes.

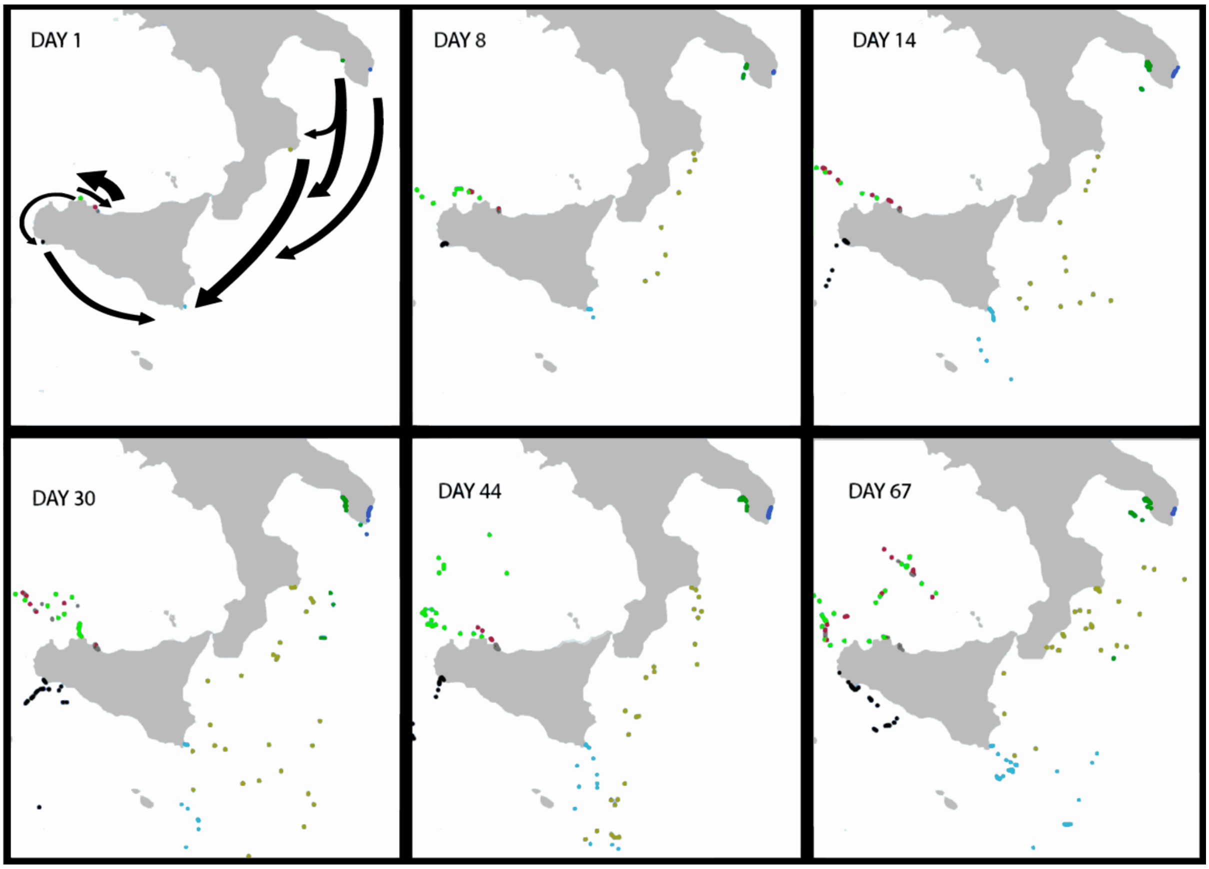


Figure. 2 - Example snapshots of particle dispersal simulations. Shown after 1, 8, 14, 30, 44 and 67 days of simulation of particles originating from eight populations over one season. Each colour represents a particle released from one sampling site floating for 30 days and advected by oceanic velocity fields derived from HYCOM. Arrows at day 1 are proportional to the estimated probabilities of direct connectivity among populations based on the particle simulation

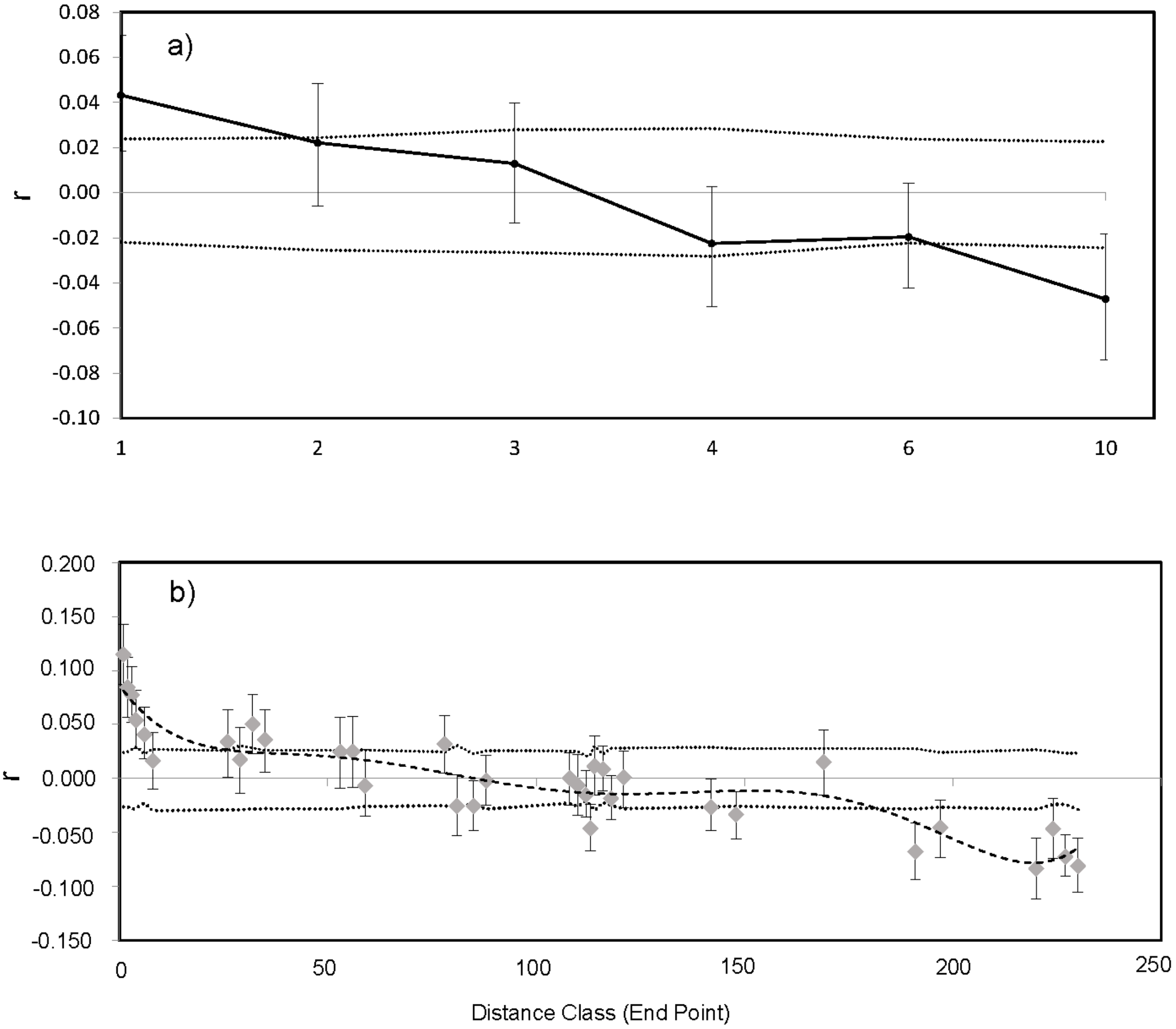


Figure 3 - Correlograms showing mean kinship coefficient (diamonds) as a function of geographical distance classes within transect (combined correlogram of the three transects) (a) and across all transects along the whole area (b) at Capo Gallo. Classes were chosen in order to keep similar number of observations/class. Dotted lines represent 95% confidence interval around the null hypothesis of relatedness by chance. In (b) the dotted line shows a fitted 6th order polynomial line

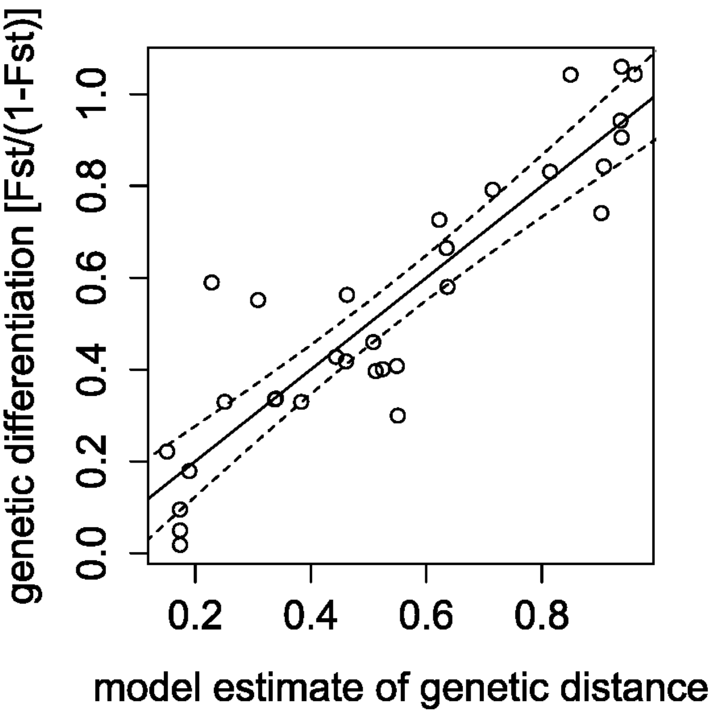


Figure 4 - Best fit regression model (using coastal distance, habitat continuity and directionality index) to predict genetic differentiation among 8 *C. amentacea* populations in southern Italy (see Table 2).

Table 1 Summary population genetics statistics for each sampling site.

POPULATION	*N*	*H_E*	*H_O*	*ALLELIC RICHNESS*	*F_{IS}*	
CAPOGALLO A	30	0.60	0.53	4.29	0.121	
CAPOGALLO B	30	0.65	0.53	4.76	0.190	*
CAPOGALLO C	30	0.56	0.50	4.51	0.109	*
CROTONE	31	0.54	0.37	3.92	0.317	*
OTRANTO	31	0.36	0.28	2.75	0.230	*
ALTAVILLA	33	0.52	0.37	2.97	0.296	*
PORTOPALO	34	0.62	0.53	4.26	0.148	*
MAZARA	29	0.47	0.36	3.42	0.219	*
SANTELIA	35	0.55	0.48	3.8	0.134	*
TORRE ULUZZO	25	0.26	0.26	2.29	0.011	

N, number of sampled individuals; H_e, expected heterozygosity; Ho, observed heterozygosity; Allelic richness after 999 bootstrapping; F_{IS}, inbreeding coefficient. Asterisks indicate significant probability for $F_{IS}>0$.

Table 2 Summary of model testing

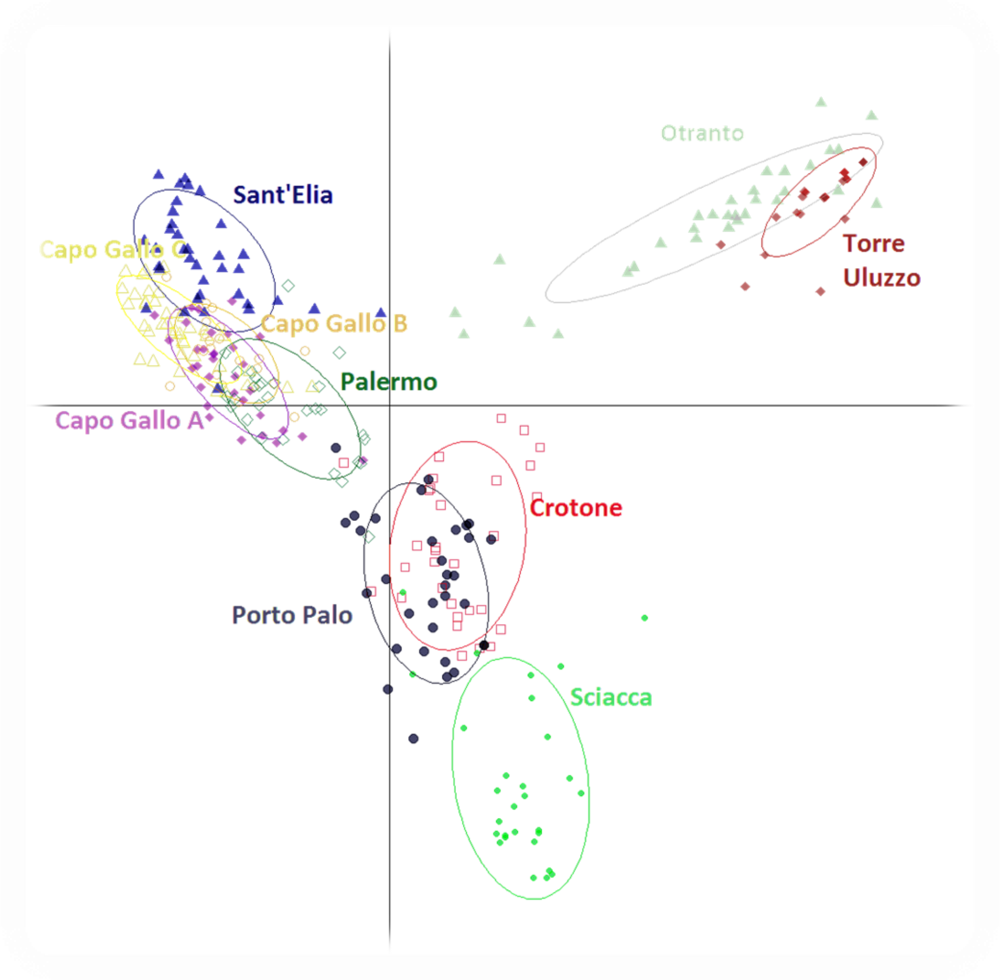
Predictor variable	*Adjusted r^2/ Adjusted r^2 after ENA correction*	*P values*	*AIC/BIC*	*Model likelihood*
Coastline distance	0.68/0.60	***	-18.63/ -14.33	0.017
Marine distance	0.59/0.54	***	-11.22/-6.92	0.0004
Habitat continuity	0.57/0.54	***	-9.57/ -5.27	0.0002
Directionality index	0.27/0.24	**	6.98/ 11.28	4.616e-08
Average probability	0.67/0.58	***	-17.65/ -13.35	0.010
Coastline distance + habitat continuity	0.73/0.67	** Coast:rock*	-21.57/-14.40	0.073
Coastline distance + habitat continuity + Directionality index	0.78/0.73	*** Coast:rock** Coast:index *	-26.80/ -16.76	W_{best} = 0.91

The adjusted coefficients of determination (r2) before and after ENA correction for null alleles are reported. Significance of regression is also indicated (*=0.05; **=0.01; ***= 0.001). AIC and BIC= Akaike and Bayesian model selection criteria Model likelihood (L) and Akaike weight (Wbest) are also shown (following Johnson and Omland 2004).

Supporting information

	CGA	CGB	CGC	AL	SE	CR	PP	MA	TU	OT
CGA	0.000	0.012	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CGB	0.017	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CGC	0.095	0.054	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
AL	0.196	0.186	0.250	0.000	0.001	0.001	0.001	0.001	0.001	0.001
SE	0.249	0.200	0.240	0.235	0.000	0.001	0.001	0.001	0.001	0.001
CR	0.289	0.262	0.269	0.310	0.303	0.000	0.001	0.001	0.001	0.001
PP	0.245	0.199	0.204	0.292	0.271	0.228	0.000	0.001	0.001	0.001
MA	0.352	0.301	0.313	0.351	0.353	0.289	0.252	0.000	0.001	0.001
TU	0.467	0.438	0.493	0.487	0.492	0.416	0.437	0.510	0.000	0.001
OT	0.444	0.408	0.447	0.462	0.416	0.364	0.395	0.449	0.142	0.000

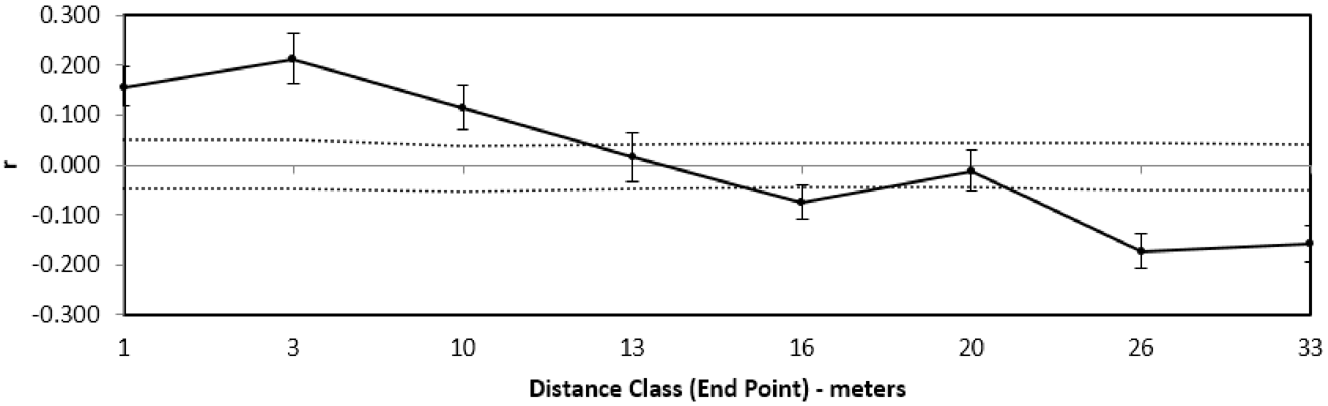
S1 - Triangular matrix of genetic differentiation. Fst Values below diagonal. Probability, P(rand >= data) based on 999 permutations is shown above diagonal. CGA: Capo Gallo A; CGB: Capo Gallo B; CGC: Capo Gallo C; AL: Altavilla (Palermo); SE: Sant'Elia; CR: Crotone; PP: Portopalo; MA: Mazara (Sciacca); TU: Torre Uluzzo (Nardò); OT: Otranto.



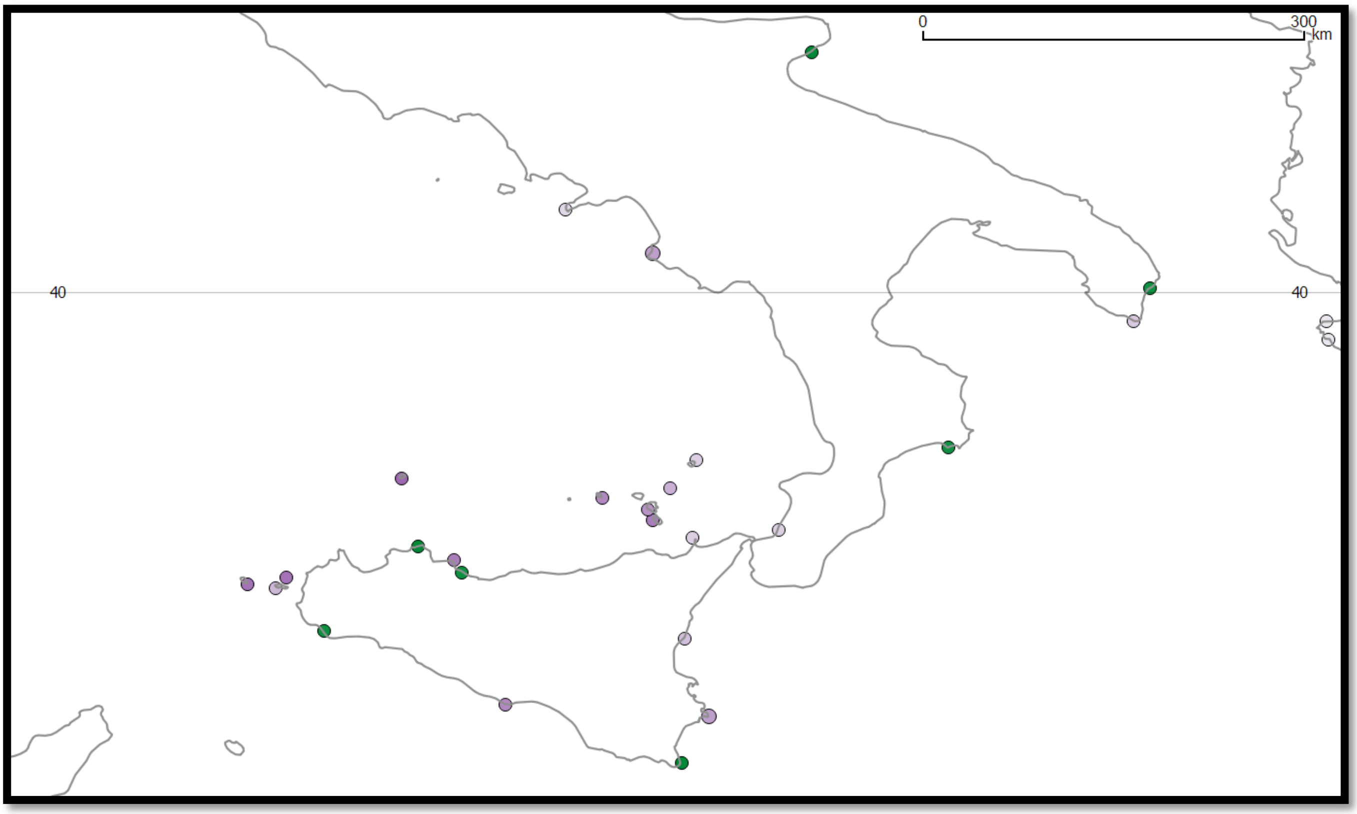
S2 - DAPC plot. Color and cluster are relative to each population

From/To	**CGA**	**CGAB**	**CGB**	**CGBC**	**CGC**	**CR**	**OT**	**AL**	**PP**	**MA**	**SE**	**TU**
CGA	0	1	8	1	1	0	0	0	0	0	0	0
CGAB	5	0	7	2	3	0	0	0	0	0	1	0
CGB	1	2	0	4	3	0	0	0	0	0	1	0
CGBC	1	0	2	0	5	0	0	0	0	0	0	0
CGC	0	0	0	6	0	0	0	0	0	0	0	0
CR	0	0	0	0	0	0	0	0	0	0	0	0
OT	0	0	0	0	0	0	0	0	0	0	0	6
AL	0	0	0	0	0	0	1	0	0	0	0	0
PP	0	0	0	0	0	0	0	0	0	0	0	0
MA	0	0	0	0	0	0	0	0	4	0	0	0
SE	0	0	0	0	0	0	0	1	0	0	0	0
TU	0	0	0	0	0	0	7	0	0	0	0	0

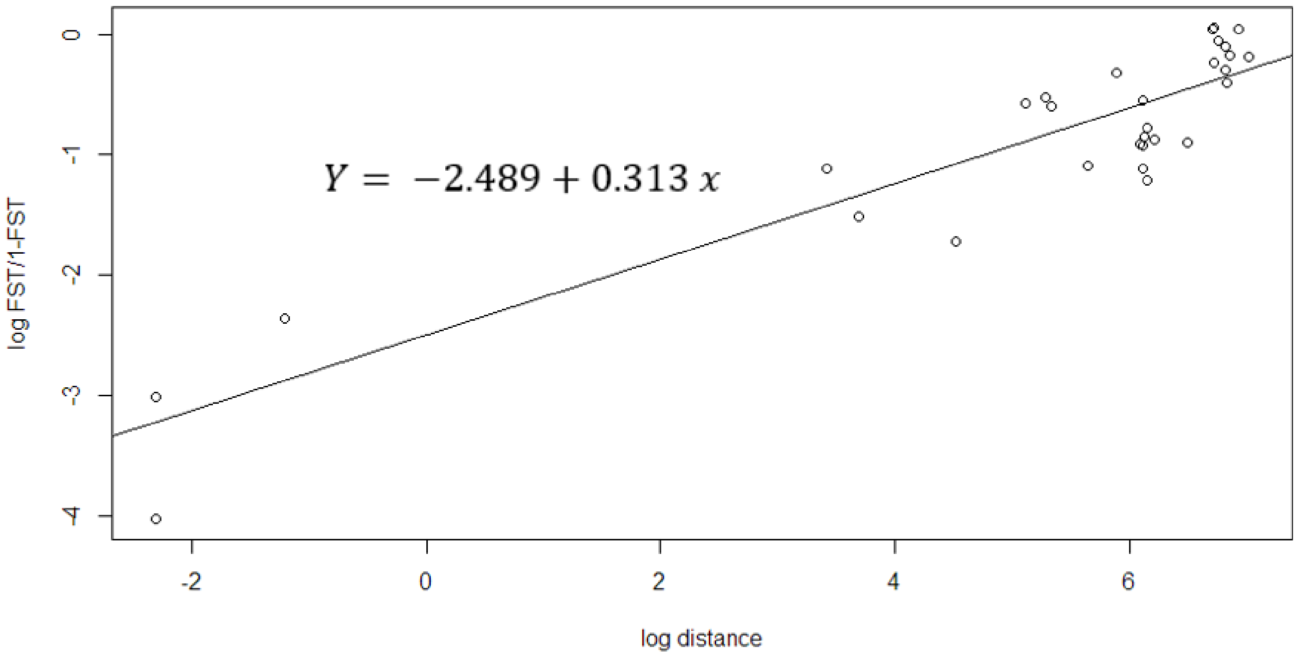
S3 - Number of first generation migrant for each population pairs estimated according to Paetkau at al. (1995) criterion in Geneclass2. CGA: Capo Gallo A; CGB: Capo Gallo B; CGC: Capo Gallo C; AL: Altavilla (Palermo); SE: Sant'Elia; CR: Crotone; PP: Portopalo; MA: Mazara (Sciacca); TU: Torre Uluzzo (Nardò); OT: Otranto.



S4 - Autocorrelation analysis with samples from Sant'Elia population



S5 - Historical records of C. amentacea from the literature. Green dots represents recent records while violet were documented between the 1962 and the 1975. Shades is proportional to the age of the record with darker violet showing older records.



S6 Linear regression between genetic differentiation and logarithm of geographic distance.

CHAPTER 4

Geography and host influence phylogenetic and functional variability of microbial communities associated to littoral fucoid seaweeds

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Abstract

The complexity of bacterial communities associated with seaweeds contributes to the development and functioning of healthy and resilient bacteria-seaweed holobiont. Alteration of the bacterial community can concur with other detrimental effects of natural or anthropogenic stressors on canopy algae, facilitating the colonization by pathogens and epiphytes or causing the loss of some key function for the holobiont. We analysed the specificity and geographical variability of the microbial community associated with vulnerable populations of habitat-forming littoral species of fucoid algae belonging to the genus *Cystoseira* and Sargassum., We also explored the presence of a possible core of shared bacteria for the most widespread species *C. compressa*, and its potential functional role by predicting metagenome functional content from marker gene using a bioinformatics approach. Results showed large spatial differences in the composition and structure of the microbial communities, but with consistent differences among species at regional scales. The community structures of these species-specific bacterial communities are locally shaped by geographical and environmental local conditions. Additionally, the bacterial community of *C. compressa* followed a geographical isolation-by-distance pattern, clearly distinguishable among basins and possibly driven by the population genetic structure of the host. However, across its distribution *C. compressa* has a small set of shared bacteria forming the core community of its holobiont system. This core comprised bacteria with different functional roles, including important defenses from xenobiotics pollutants, probably contributing to the capability of the holobiont system to withstand increasing environmental stressors.

Introduction

Canopy forming seaweeds are a key habitat forming group, providing three-dimensional complexity and increasing productivity and biodiversity of coastal ecosystems (Bulleri *et al.* 2002; Steneck *et al.* 2002). However, many of these seaweed species experience (strong) local decline and contraction of distribution ranges (Airoldi & Beck 2007; Smale & Wernberg 2013; Assis *et al.* 2014) often associated with an increase of low-complexity species (Airoldi & Beck 2007; Strain *et al.* 2014). Local stressors, such as eutrophication, might play an important role in driving this shift (Benedetti-Cecchi *et al.* 2001; Bulleri *et al.* 2016). Furthermore, the synergistic interaction of local and global drivers have the potential to greatly increase the pressure on these important habitat formers with cascading effects on coastal ecosystems (Strain *et al.* 2015).

A consistent body of literature recognizes that seaweed-associated bacteria mediate the complex interaction with the surrounding environment (Goecke *et al.* 2010; Wahl *et al.* 2012). That bacteriome can contribute to different important functions of the algal host, such as morphological development, growth and nutrient acquisition, spore release and settlement, and protection from fouling (Aires *et al.* 2013; Egan *et al.* 2012). Furthermore, the bacterial communities associated to macroalgae are often highly species-specific (Lachnit *et al.* 2009; Hollants *et al.* 2013, Aires et al., 2015), strongly differing from communities in the surrounding seawater and substrate (Tujula *et al.* 2010; Burke *et al.* 2011b; Mancuso *et al.* 2016). For this reason, it has been suggested that the complex algae-microbiome can be considered a holobiont, where the hosts and all their specific associated microbiota behave as a singular organism (Barott *et al.* 2011; Egan *et al.* 2012, Wahl *et al.* 2012, Zilber-Rosenberg & Rosenberg 2008). Alteration of the associated microbial community may come along with the detrimental effects of natural and anthropogenic stressors observed in canopy algae, facilitating the colonization by pathogens and epiphytes (Mancuso *et al* 2016) or causing the loss of some key function for the holobiont (Wahl *et al.* 2012). The study of associated bacterial communities can have a central role in understanding the future of canopy algae, and their capability to cope with increasing environmental stressors. However, its exploration with molecular techniques is just beginning to be applied to the understanding of diversity and specificity of macroalgal-bacterial relationships (Hollants *et al.* 2013).

The composition and abundance of operational taxonomic units (OTUs) in the bacteriome can vary across algal species and populations as well as across different algal tissues, time and space (Tujula *et al.* 2010). Environmental conditions could also play an important role in rearranging the structure for specifically adapted microbial communities (Busch *et al.* 2015; Aires *et al.* 2016). Due to this high level of variability and the numerous contributing factors, it has been challenging to understand if bacterial community associations are specific of individuals, species or higher taxonomic levels (Hester *et al.* 2015). An additional degree of complexity is given by the functional redundancy of bacterial species. As different bacteria can provide identical functions in the holobiont, changes in functional identity rather than taxonomic identity could be most relevant in affecting the responses of the holobiont system (Burke *et al.* 2011a). According to the niche hypothesis, the ecological role of each element of the associated microbiome allow to occupy a specific niche in the bacteriome defined by a set of micro-environmental condition and resources on the algal surface (Burke *et al.* 2011a; Eigemann *et al.* 2013). Better adapted bacteria species will eventually outcompete less adapted ones and tend towards a dynamic equilibrium of the ecological relationship which persists until environmental conditions do not change. The dynamic of the microbial community should therefore follow a universal pattern as, under the same conditions, the relationship among bacterial species will tend to develop and maintain similar bacterial composition (Bashan *et al.* 2016).

In this study we explore the specificity and spatial variability of the bacterial community associated with canopy forming seaweeds from the genus *Cystoseira*, also comparing it with the microbial composition of other co-occurring macroalgal species forming a phylogenetic and distributional gradient. Although the temporal variability of the bacterial community associated to *Cystoseira compressa* has been studied recently (Mancuso *et al.* 2016), nothing is known about its spatial variability nor the degree of similarity with other related species. Here we ask whether bacterial community composition diverges at different locations and if it can be related to the underlining genetic differentiation of the host macroalgal species. Additionally, we describe the core community of shared bacterial species that characterize the *Cystoseira* holobiont and try to identify the possible associated functions. Understanding if and how these communities vary along the spatial scale and which are the drivers of these variations is critical for such key habitat-forming species as could help in the future to forecast and manage impacts and loss of these canopy forming algae.

Materials and Methods

Study area and sampling design

Samples were collected in six locations, two locations in southern Portugal (Olhos de Água and Praia do Castelo), two in the Adriatic Sea (Mattinata and Vasto) and two along the coast of Sicily in the central Mediterranean Sea (Portopalo and Sant'Elia) (Fig. 1). Biotic and abiotic characteristics vary greatly among the locations. The Mediterranean sites have a smaller tidal range (average tidal amplitude ≈ 30 cm) but a wider temperature range (8°C – 30°C) compared to the Atlantic sites (average tidal amplitude ≈ 210 cm; temperature range 16°C – 22°C) (data from "Istituto Superiore per la Protezione e Ricerca Ambientale," ISPRA, www.mareografico.it and “Instituto Hidrográfico”, http://www.hidrografico.pt, 2000– 2013 period). Patches of mussels (*Mytilus galloprovincialis*) and ephemeral algae (*Ulva spp.*) dominate rocky shores at the Adriatic locations and the presence of *C. compressa* is limited to isolated individuals or small patches, while in Sicily and Algarve sublittoral fringes are characterized mainly by dense stands of brown seaweeds.

Bacterial communities were sampled from whole thalli of *C. compressa, C. tamariscifolia, C. amentacea* and *Sargassum vulgare* between August and early September. Three replicate samples of *C. compressa* were collected at every location from epiphyte-free thalli. Three replicates of *C. amentacea* and *C. tamariscifolia* were sampled only at one location (Sant’Elia and Praia do Castelo respectively) while *Sargassum vulgare* was sampled in both Portuguese locations where it was found. In samples from Italy, sterile cotton swabs were used to rub the surface and immediately placed in sterile 1.5 ml tubes and frozen. Whole tissue was collected in Atlantic samples and preserved in a stabilization buffer (Xpedition Lysis/Stabilization solution, Zymo Research) in the field. In Olhos de Água we found sediment at the bottom of the same rockpool of the macroalgae and sampled three replicates (preserved in buffer). Three water samples were collected at each location with the exception of Praia do Castelo.. Water was collected in 500 ml bottles and first filtered with 3.0 µm pore size cellulose acetate filters (Millipore), to remove suspended particles and eukaryotes, and then with 0.2 µm pore size cellulose nitrate filters (Sartorius) to retain the bacteria. Tubes were transported cool and preserved at -80°C until extraction.

Bacterial DNA extraction was performed using PowerSoil® DNA Isolation Kit (MoBio Laboratories). The total 16S rRNA region was amplified using the universal primers 27F and 1492r (Lane, 1991) with the following changes to the original protocol: after an initial denaturation at 95°C for 2 min, conditions were as follows: 35 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 20 s, and extension at 72°C for 90 s. The final extension was at 72°C for 3 min. The 25 µl reaction mixture contained 250 µM dNTPs, 0.6 µM of each primer, 1 × 2 PCR buffer mix, 2 µl of template DNA (with a final concentration of about 10 ng μl^{-1}) and 0.3 µl of Taq polymerase. Extracted DNA was outsourced to MrDNA (Molecular Research LP - Texas) for Illumina Miseq sequencing after nested amplification of the total 16S rRNA PCR products with modified primers 799F (5’-AACMGGATTAGATACCCKG-3’) and 1193r (5’-ACGTCATCCCCACCTTCC- 3’) for region V5, V6 and V7 of the 16S rRNA gene, avoiding most plastid contamination (Bodenhausen *et al.* 2013). A proof-reading *Taq* polymerase and reduced nested PCR cycles (10) were used to avoid introduction of amplification errors.

Bacterial community analysis

16S amplicon sequences were processed through the recently developed MICCA pipeline version 1.6.0 (Albanese *et al.* 2015) which rely on OTUCLUST, a de novo OTUs clustering algorithm providing a more realistic estimate of the sample complexity. Sequences were quality filtered allowing a maximum expected error rate of 0.5% and truncated at a length of 300 bp. Operational taxonomic units (OTUs) were assigned with a greedy clustering method using a sequence identity threshold of 97%. Chimeric sequences were filtered out with the embedded UCHIME algorithm. Taxonomy was assigned in QIIME v.1.8.0 (Caporaso *et al.* 2010) to representative sequences up to genus level using RDP classifier version 2.11 (Wang *et al.* 2007) against the Greengenes 16S rRNA gene dataset (release 13_8). Sequences identified as mitochondria or chloroplasts were removed for subsequent analysis. In order to display the bar plot of relative abundance by taxonomic classification, only OTUs comprising the 90% of the community were retained. Analyses were performed in R software 3.2.4 (R Core Team, 2016) using the “phyloseq” R-package (McMurdie & Holmes 2013).

Alpha diversity was estimated as OTU richness after rarefaction at lowest sequencing depth while Chao1 and Shannon index were estimated before rarefaction. Differences were statistically tested with permutational ANOVA (PERMANOVA+, Anderson et al., 2008) for PRIMER v.6 (Clarke and Gorley, 2006). Differences were tested between habitat type (2 levels: Seawater and Algae, fixed) across the regions (Re: 3 levels, fixed) and controlling for the location factor nested within regions (Si (Re): 6 levels, random). Differences among alpha diversity values of sediment and other habitat types (seawater and macroalgae) were tested with Student’s t test within Olhos de Água. Differences in alpha diversity measures among species were tested by PERMANOVA based on Euclidean distance matrix at each site. Modified Levene’s test for homogeneity of variance was calculated (Anderson 2006), as PERMANOVA is not reliable in unbalanced design with heterogeneous dispersions (Anderson & Walsh 2013). Where unique permutation number were low (<100), significance values (alpha = 0.05) were estimated with Monte Carlo permutations.

For Beta diversity analysis, a Bray-Curtis distance matrix was computed from square-root transformed OTU abundances and visualized with principal coordinate analysis (PCoA). The significance of observed distances was tested with PERMANOVA based on Bray-Curtis dissimilarity matrix and 9999 permutations. OTUs contributing the most to the differentiation among samples were obtained by SIMPER analysis in PRIMER v.6 (Clarke and Gorley, 2006) using a cut off of 50%. To test for specificity of bacterial associations, we used linear regression with geographical distance to explain pairwise Bray-Curtis dissimilarity between *C. compressa* associated bacterial communities at different regions. Additionally, we tested whether the genetic structure of the sampled populations increased the amount of variability explained. Populations were genotyped using 10 specific microsatellite markers previously developed (Chapter 1). Spatial/genetic correlation was compared with the correlation of pair-wise distances among communities associated with different macroalgal species and the host phylogenetic distance. Shortest distance among populations was estimated in R (R Core Team 2014) with packages raster, gdistance and rgdal. Microsatellite amplified fragment were measured on an ABI PRISM 3130 DNA analyzer (Applied Biosystems) with the LIZ 500 (GeneScan) standard. Alleles were scored using the free software STRand (Toonen & Hughes 2001) and further binned into size classes with the msatAllele R package (Alberto 2013). Pairwise population distances were estimanted in GENALEX (Peakall & Smouse 2006). Phylogenetic distance was based on NCBI deposited partial sequences of *psbA* gene and estimated in R with “phangorn” and “ape” packages under a Jukes-Cantor evolution model.

The core OTUs of *Cystoseira compressa* were defined as those present in at least two replicates at each location and shared across all sampled sites. In this way we tried to account for individual variability of replicates to obtain a OTU selection that represents the macroalgal population at each location. Bacterial OTUs were obtained in QIIME with the *compute_core_microbiome.py* command. Microbial core was than obtained and graphically displayed by merging those in JVENN online software (Berdou et al. 2014).

Prediction of the potential function of associated bacterial communities was performed with PICRUSt (Langille *et al.* 2013). OTU selection was computed using the closed-reference OTU picking strategy, against Greengenes (version 2013/05) at 97% identity. The number of sequences was normalized by the 16S copy number of each OTU. Resulting functional predictions were analyzed with STAMP (Parks *et al.* 2014), using up to KO (KEGG orthology) tier 3 for all genes. Only tier 1 functions of “organismal systems” and “human disease” were filtered out as less relevant to environmental samples while “metabolism,” “genetic information processing,” “environmental information processing,” and “cellular processes” were analyzed further.

Results

A total of 3,888,223 paired-end raw reads were obtained from the Miseq sequencing. After trimming, quality filtering and singletons and chimera removal 62% of the sequences remained

(2,407,178) with an average of 53,492 reads per sample (min = 32,802; max = 85,525; SD = 11,957). OTUs classification identified 4064 unique taxonomic units with an average of 1117 OTUs per sample. After rarefaction at the lowest sampling depth total richness decreased to 4020 unique OTUs (Table 1). About 10% of the sequences matched identified OTUs in the Greengenes database at the genus level. Classification success increased sharply at higher taxonomic levels, with 70% and 87% already at the Order and Class levels respectively (Fig. S1).

Alpha diversity measures

Diversity of associated bacterial communities differed among habitats and between the region of origin (p = 0.05 and p(MC)=0.008 respectively, Table S1). Seawater accounted for the lowest OTUs richness (mean number of OTUs = 819) and Shannon diversity index but showed variability across location (min =447, max =1189) (Fig. 2, Fig. S2), with the minimum richness found in the Adriatic Sea. The highest OTU richness was found in the few sediment samples available (mean= 1471): within Olhos de Água higher than seawater (t = 8.2279, p (MC) = 0.0008), and macroalgae (*C. compressa:* t = 6.4432, p (MC) = 0.003; *S. vulgare:* t = 2.7053, p (MC) = 0.05). *Cystoseira compressa* showed 1.4 times higher Shannon indexes compared to seawater (p = 0.03), while it did not differ in OTU richness or Chao1 index. The Shannon diversity of *C. compressa* was lower in the Atlantic region (3.89) compared to both Adriatic (4.69) and central Mediterranean (4.37) ones (p(MC) = 0.009). Bacteriomes did not differ among species in terms of alpha diversity within locations where comparison was possible.

Habitat related diversity

Principal coordinate analysis showed highly similar seawater samples across the locations compared to a wider variability of epiphytic microbial assemblages (Fig. 3). Habitat type factor was substantial in determining the OTU assemblages. Seawater bacterial community structure (Pseudo $F_{2,5}$ = 14.661, p = 0.0003) and composition (Pseudo $F_{2,5}$ = 4.0412, p = 0.0001) differed strongly from macroalgae in every location and pairwise tests at Olhos de Água showed significant divergence also among sediment, water and algae (Table S2). Those differences were reflected also at higher taxonomical classification (Fig. 4, Table S5-S6). At Phylum level seawater showed higher abundance of *Proteobacteria* while *Planctomycetes* representatives were almost absent. The former comprised on average 53% of the relative abundance in seawater samples compared to 26% in macroalgal samples. *Proteobacteria* and *Bacterioidetes*, accounted together for 85% of the total bacterial abundance in seawater. Other Phyla less represented in those samples were *Actinobacteria* (3.5%), *SAR406* (0.6%) and *Fusobacteria* (0.2%). In both macroalgae and sediment samples, the three most abundant Phyla were

Proteobacteria, *Bacterioidetes* and *Planctomycetes* in similar proportions (26.2%, 27.8 and 28.3% respectively in algae samples). Sediment samples showed higher abundances of *Firmicutes* (0.4%), *Acidobacteria* (0.15%) and *Fusobacteria* (1%) while *Chloroflexi* (0.14%) and *Thermi* (1.8%) were unique for seaweed samples. The latter, however, was present in much higher proportions in *Cystoseira amentacea* samples (17% on average).

At family level, phylogenetic characterization identified 69 families which, together with the OTUs that could not be classified at this level, comprised 90 % of the diversity in the dataset (Fig. 4B). *Pirellulaceae* (27.9%), *Saprospiraceae* (11.7%), *Flavobacteriaceae* (11.6%), *Rhodobacteraceae* (5.5%), *Hyphomonadaceae* (3.9%) were the most abundant taxa, with an average of 18.7% of unclassified families. The main representatives taxa in sediment were *Pirellulaceae* (27.6%), *Flavobacteriaceae*(17.1%) and *Hyphomonadaceae* (4.4%), while seawater bacterial communities were mainly characterized by members of the families *Flavobacteriaceae* (26.3%) with a larger component of unclassified groups (44.1%), mostly represented by a unique OTU of *Alphaproteobacteria*.

Macroalgal bacterial community structure

Structure of macroalgal associated bacterial communities differed among species. The bacterial community associated to *C. compressa* differed from all the other species at each respective region (*C. amentacea*: Pseudo F = 3.8144, p (MC) = 0.01; *C. tamariscifolia*: Pseudo F = 2.6502, p (MC) = 0.05; *S. vulgare*: Pseudo F = 2.6674, p = 0.01), however *S. vulgare* community structure was not different from *C. tamariscifolia* (p (MC) = 0.12). SIMPER analysis underlined main OTUs contribution to dissimilarity (Table S3). *Cystoseira amentacea* was characterized by much higher abundance of a single member of *Trueperaceae* family (order: *Deinococcales*) compared to the other species and contributing at 2.7% of dissimilarity with *C. compressa* at the same location. It showed also the highest relative abundances of genus *Leucothrix* (*Thiotrichaceae*, *Proteobacteria*) and lower occurrence of *Actinobacteria* compared to local *Cystoseira compressa*. *Sargassum vulgare* showed the highest abundances of *Hyphomonadaceae* (9.7% of relative abundance) and of *Flavobacteriaceae* family representatives (19.2%) compared the others species and with levels more similar to what observed in sediment, particularly of the genus *Olleya*, *Winogradskyella*, *Aquimarina* and *Nonlabens* (found only associated to *S. vulgare*). *Cystoseira tamariscifolia* was characterized, among others, by highest abundance of *Planctomycetaceae*, *Lewinella* (*Saprospiraceae*), *Maribacter* (*Flavobacteriaceae*) and lowest *Phyllobacteriaceae* compared to the other species at Praia do Castelo.

Diversity along geographic distribution

Within *Cystoseira compressa,* we found significant differentiation among locations within regions (pseudo F_5 = 3.9288, p =0,0001). Locations within region level were not significantly different with the exception of Adriatic sites (p(MC) = 0.0182). Bacterial community associated to *Cystoseira* samples collected at the location Mattinata showed, in fact, some peculiarities which contributed to differentiate it from the others samples (Fig. S3). Specifically, OTUs belonging to the family of *Rhodobacteraceae* were overly represented at Mattinata compared to all the other *C. compressa* bacterial communities (13.7% against 2.8% of relative abundance, p =0.033). *Loktanella* and *Ruegeria* were some of the most represented genera of the family (2.5% and 0.5% respectively). Various sulfate-reducing bacteria groups (*Desulfovibrio, Desulfobacter, Desulfococcus, Desulfonema*) were also exclusively or more represented in Mattinata samples compared to any other location.

The bacterial community associated with *C. compressa* showed some differences in structure also among the Atlantic, central Mediterranean and Adriatic regions (Table S2). Atlantic samples showed higher relative abundance of *Planctomycetes*, accounting for the 43% of all the Phlya, while in both Adriatic and central Mediterranean samples *Bacteroidetes* was the most represented Phyla (36% and 43% respectively). Bacteria belonging to the Phylum *Actinobacteria* were more abundant in samples of *C. compressa* from the central Mediterranean region (14%) compared to both Adriatic (7%) and Atlantic (1%) ones.

We found a clear linear pattern of isolation-by-distance, with pairwise Bray-Curtis distance variation along the geographical distance (Fig 4, Table S4) (adjusted $R^2 = 0.45$, $p < 0.001$). Closer populations showed higher degree of similarity among the associated bacterial structures (Fig. 5c). The genetic distance among populations also resulted to be positively correlated with the distance among bacteriome structures (adjusted $R^2 = 0.17$, $p < 0.001$). However, the addition of genetic distance did not improve the model of isolation-by-distance (Table S4).

The correlation with phylogenetic distance among species did not show any significant relation. Even though bacterial communities were significantly different among the macroalgal species, we did not find a clear pattern of co-evolutionary variation in bacterial structure with the underling host phylogeny (Fig. 5b). More related species did not show significantly lower Bray-Curtis distance in their bacterial community structure. A subtler pattern could be however masked by the high degree of inter-sample variability found.

C. compressa core bacterial community

Across the distribution of *C. compressa*, in total 413 OTUs were identified as part of the core microbiome (Fig.6) from a set of 952 different OTUs on average, shared by at least two replicates per location and representing 14% of the OTUs found in all *C. compressa* samples. The core community included members of the phyla *Bacteroidetes*¸ *Proteobacteria*, *Planctomycetes*, *Actinobacteria*, *Thermi*, *Chloroflexi*, *Fusobacteria*, *Verrucomicrobia* and *WPS-2*. The most represented taxa of the microbial core represented the *Bacteroidetes* phylum (33.8%), with representatives mainly of the classes *Flavobacteriia* (11.6%) and *Saprospirae* (22.2%) (Table S7). The single most represented class were the *Planctomycetia*, with a relative abundance of 32.5%. The most represented OTUs originated from the *Pirellulaceae*, accounting alone for 13.9% of the total abundance and a *Bacteoriodetes* of the order *Saprospirales* (6.8%). OTUs exclusive for the Adriatic region belonged mainly to the orders *Pirellulales*, *Flavobacteriales* and *Saprospirales*, with the most represented unique OTU belonging to order *Marinicellales.* The Central Mediterranean region showed unique OTUs particularly abundant for the candidate Order *Saprospirales* and *CV90* (28% and 27% respectively). The Atlantic region was instead characterized mainly by OTUs belonging to the order *Alteromonadales* (Table S8).

Predicted functions

PICRUSt functional assignments allocated most of the genes to metabolic pathways (55%) among tier 1 of the KO categories (Table S9). Other highly represented categories were “environmental information processing” and “genetic information processing” with 15% and 19% of the assigned genes respectively. The mean nearest sequence taxon index (NSTI) of the samples was 0.15 ± 0.05, a value lower than observed in reference sediment (Langille *et al.* 2013) and indicating PICRUSt inferences were likely to be accurate. At the lowest KO category level, PCA among samples showed again a clear and significant ($p = 0.0001$) differentiation of seawater from other samples based on predicted functional groups (Fig. S4). Algae and sediment compared to seawater had higher tier 1 “environmental information processing” but lower frequency of “metabolism” and “genetic information processing” related functions ($p < 0.0001$). More in detail, at level 2 of KO, macroalgae showed higher proportion of sequences allocated to cell motility, signaling related genes, transcription, transport and xenobiotics biodegradation when compared to seawater ($p<0.0001$). Sediment bacterial community showed to be functionally more similar to macroalgae community than seawater. Sediment showed however lower level of xenobiotic biodegradation (p=0.04) but higher proportion of translation (p <0.001).

Predicted functionality of macroalgal associated bacterial communities showed host species-specific differences, however the overall functional profile among species based on Bray-Curtis dissimilarity was not significantly different (table S10). Only *S. vulgare* showed a significantly different functional profile from *C. compressa* (p = 0.02) and *C. tamariscifolia* (p = 0.05). *C. amentacea* showed lower proportion of sequences related to carbohydrate metabolism (p <0.001), membrane transport and metabolism of secondary products as polyketides and xenobiotics (p<0.05). It also showed higher proportion of predicted functions associated to motility (p=0.01) compared to *C. compressa* at the same location. Degradation of xenobiotic functions was more expressed in *Sargassum vulgare* compared to both *C. tamariscifolia* and *C. compressa*, especially those related to P450 protein and biodegradation of benzoates (p<0.001). Only small functional differences were found between *C. tamariscifolia* and *C. compressa*; mainly in the oxidative phosphorylation and methane metabolism pathways, which were more present in the former species (p<0.001).

The functions inferred to be associated to the bacterial communitiy derived from *C. compressa* samples were different among the geographical regions (Pseudo-F = 9.691, p(MC) = 0.02). Atlantic region showed higher proportion of genes related to bacterial movement and chemotaxis (p < 0.001) while carbohydrates metabolism functions were lower compared to the other regions. Adriatic and central Mediterranean region where functionally more closely related, showing only the group of “unknown function” significantly higher in Adriatic region (p < 0.001).

Functional assignment of the core bacterial community associated with *Cystoseira compressa* revealed consistent abundance of functions related to bacterial motility and chemotaxis, as the methyl-accepting chemotaxis protein (K03406). The PICRUSt analysis found, among others, high proportion of transcription factors and transcription related proteins, such as the ECF sigma factor (K03088), and signal transducers as the membrane histidine kinase sensor (K00936). Finally, to be noted, also the presence of functions related to xenobiotics biodegradation, to degradation of aminobenzoate/benzoate, chloroalkane or polycyclic aromatic hydrocarbon, as well as to sulfur metabolism, as the arylsulfatase (K01130) or the iduronate 2-sulfatase (K01136).

Discussion

This is the first study to address the specificity and spatial variability of bacterial community associated with the ecologically important ecosystem-structuring macroalgae of the genus *Cystoseira*. We evaluated the differences among bacterial community structure living in association with *Cystoseira compressa* and cohabiting intertidal macroalgae. Differences were linked to the spatial distribution, with the dissimilarity of bacterial associated community correlated with geographical distance among host specimens. We also found that species identity represents an important factor in shaping bacterial associations: species-specific bacterial community was consistent at regional scale but community structure was modulated by geographical and environmental local conditions. However, no co-evolutionary pattern between the bacterial community and the macroalgal hosts was detected. Finally, in this study, a set of shared bacteria forming the core community of *C. compressa* was identified, characterized by key functions for the macroalgal holobiont.

Specificity of bacterial association

We observed a clear differentiation of bacterial assemblages on macroalgae compared to those in seawater. The community structure on each of the tested substrates was considerably different, suggesting that selective processes from the pool of available bacteria in the water column is indeed taking place. This selection process in macroalgae has been previously identified for several species, including brown seaweed, where biofilm communities always resulted markedly distinct from surrounding seawater (Staufenberger *et al.* 2008; Lachnit *et al.* 2009; Burke *et al.* 2011b). Differences in terms of richness were not so strong, in contrast to what is observed in a previous study on *Cystoseira* (Mancuso *et al.* 2016), where seawater community richness was consistently lower. However, similarly, the richness and diversity in *Cystoseira* was significantly larger than that of seawater at the same location (Vasto) where a comparable study took place (Mancuso *et al.* 2016). That underlines the comparability between the two studies and the importance of including a spatial component to enable broader generalizations of results. All the habitats tested in the present study were able to modulate the recruitment of the bacterial assemblage from the surrounding environment. A first selection of bacterial OTUs could be simply due to the presence of a solid substrate, facilitating the taxa better adapted to a biofilm environment rather than planktonic life. However, sediments hosted a richer and distinct community from the algal biofilms, suggesting that other levels of selective pressures are acting on the microbial community of seaweeds. A conspicuous amount of literature depicts the complex chemical bidirectional interaction among the two components of the holobiont as possibly able to specifically shape the microbial layer composition and abundances (Goecke *et al.* 2010 for a review). This relation could be so specific to identify unique communities for each macroalgal species (Staufenberger *et al.* 2008), however the degree of stability of this association is not clear (Hester *et al.* 2015) as some species have been found to harbor different communities across space and time (Tujula *et al.* 2010; Aires *et al.* 2016; Mancuso *et al.* 2016). In this study, host species identity and geographical distribution both contributed to shaping diversity and structure of the associated microbial community. Despite the strong inter and intra-species structuring of bacterial diversity, a high degree of spatial variability was also evident. Nevertheless, the microbiome composition of *Cystoseira compressa* from different locations inside the same geographical region was more related than interspecific associated communities inhabiting the same area. It suggests that host identity has a primary role in selecting the associated community as suggested by Hollants et al. 2013 for bacterial endosymbionts. Sporadic symbionts could guide this latter differentiation and be a product of availability of bacteria within the guild and responses to habitat pressures (algal and environmental) (Hester *et al.* 2015; Aires *et al.* 2016). Even if our results underline the specificity of the holobiontic association, we did not find evidence that this association is following a common evolutionary path between the host and its bacteriomes. It has been previously demonstrated in mammals that closely related species tend to share a more similar microbial community compared to less related ones (Ley *et al.* 2008). However, this co-evolution was limited to gut-community in mammal species with diverging diets and gut morphologies, which create specific differences among the enclosed habitats. Macroalgae, instead, share a common open habitat, where free exchange with the mutual environmental guild is allowed. This means a high variability in community structure, which could mask that effect, if there is any.

Our results showed a clear correlation of bacteriome structure and spatial distance. Neighbor individuals were hosting more similar bacterial communities. This result could be linked to the influence of different environmental guilds of the holobiont. However, environmental samples did not follow the same pattern of isolation by distance nor the abundances of common OTUs between seawater and macroalgae relatedness (Figure S6). The alternative explanation would be that genotypic divergence of the host is driving the differentiation of associated bacteriomes. Genetic differentiation of algae populations was indeed correlated with the microbial communities. However, we could not analyze the actual single genotypes corresponding to the *Cystoseira* specimens hosting the microbes. Therefore, generalizing the population genetic results to the single individual differences is not the best analysis to test this hypothesis.

Functional profiling and core community

Despite variability across the samples, the epiphytic community included a sub-population of bacteria that were consistently associated with *Cystoseira compressa.* Only a relatively small fraction of the original 2950 unique OTUs were present across the entire distribution of *C. compressa* from Atlantic southern Portugal to the Adriatic. The resulting core community showed a high proportion of *Planctomycetes,* consistent with microbial communities associated to *Laminaria* (Bengtsson *et al.* 2012). Conversely, *Plancotmycetes* were much less represented in the single previous study on *C. compressa* at Vasto (Mancuso *et al.* 2016). Some bacterial genera commonly found across brown, green and red macroalgae (Hollants *et al.* 2013) were present, such as *Alteromonas* and *Pseudoalteromonas.* However, we also found *Tenacibaculum* which is reported here for the first time to be in stable association with a brown seaweed. Interestingly, we found just one unique representative for *Thermi*, *Fusobacteria* and *WPS-2* phyla in the core community. The member of *Fusobacter* was identified as belonging to genus *Propionigenium,* a bacteria group that are able to grow by decarboxylation of succinate to propionate and have been attribute the ability to debrominate di- and tribromophenols in marine infauna (Watson *et al.* 2000). Bromophenols have been found in brown algae and associated mainly to chemical defense mechanism (Liu *et al.* 2011).

In the context of functional redundancy, functional profiles of the microbial communities could be a better descriptor of species-specific relation among algal and microbial components (Burke *et al.* 2011a). Some of the KEGG pathways that were consistently more represented in the macroalgae compared to environmental samples also characterized the core community of *C. compressa.* The core microbial community could be related to various potential functions that can be important in driving the composition and structure of the microbial layer and the chemical interactions with the host. For instance, the genes associated to motility and chemotaxis are essential for the colonization and aggregation of the symbiotic bacteria, guiding the detection and movement toward the host surface and the formation of the microbial biofilm (Burke *et al.* 2011a). The high fraction of environmental signal transducers and transcriptional regulators, instead, could be indicative of the need to respond to modifications of the host environment (Burke *et al.* 2011a). Sigma factor has been also associated to changes in the transcriptional responses induced by bacterial wall interface interactions (Bashyam & Hasnain 2004). The analysis results also underlined the potential role that microbial core community can have in degradation of pollutants. The presence of sulfatases play important roles in the cycling of sulfur in the environment and their increase can be also related to increase in the supply of organic nutrients (Olapade *et al.* 2006) or the degradation of sulfated polysaccharides of algal exudates (Goecke *et al.* 2010). In addition, functions related to degradation of hydrocarbons and other xenobiotic substances have been observed. Many of these pollutants remain floating at the surface and therefore intertidal macroalgae such as *Cystoseira compressa* are particularly exposed to those substances. The ectomicrobiome is exposed to the pollutants which get trapped in the polysaccharide mucus at the algal surface and selection for bacteria that are resistant or use these compounds takes place (Busch *et al.* 2015). Mutualistic association with bacteria able to degrade it might help to reduce the impact of this substances on one side (Semenova et al. 2009) and provide energy source for the bacteria on the other (Leahy & Colwell 1990).

All replicates from Mattinata showed increased level of *Rhodobacteraceae*, which was previously associated with aging of *Cystoseira* canopy (Mancuso *et al.* 2016). Furthermore, the augmented presence of sulfate-reducing bacteria that we observed can be related to increase in the supply of organic nutrients (Olapade *et al.* 2006). Considering that samples were all collected in approximately the same period, it is unlikely that quiescence *per se* is the main cause of the observed shift. Instead, we hypothesize that premature decaying of the health status of the fronds, induced by environmental pressures as nutrient enrichment, reflects in an alteration of the community structure. The holobiont under stress condition might reduce the active selection of mutualistic bacteria, by for example reducing the amount of energy dedicated to secondary metabolites production in exudates (e.g. furanones), and be more subject to colonization by pathogens. At the same time, bacteria better adapted to altered environmental conditions could find a competitive advantage over the “healthy” community. Testing this hypothesis was outside the scope of this work and could not be adequately verified. Nonetheless understanding this aspect further should be of main concern as this microbial community shift can be an early warning sign of longer term degradation of the algal community. On the other side, functional characterization of the microbial community based on the PICRUSt annotations did not find differences based on location, including the location Mattinata, which did not diverge significantly from the Adriatic cluster. Additionally, our results did not show significant differences among the functional profiles of the host species of the genus *Cystoseira*, but only among species of different genera (Table S10). This could either suggest that a certain amount of functional redundancy is maintained across the locations and among the host species (Burke *et al.* 2011a; Staley *et al.* 2014), or a limitation of the inferences obtained by the procedure for populations clustering (Nagpal *et al.* 2016). With the values obtained of NSTI scores and considering that derivation of true functional diversity is limited by the genomes available in sequence databases, we cannot exclude a priori this latter hypothesis (Langille *et al.* 2013;

Sharpton 2014). However, potential functional profiles inferred by PICRUSt still allowed to obtain a significant differentiation among habitat and regions, suggesting a likely overall good performance of the functional reconstruction. Moreover, functional description of core community associated to *C. compressa* revealed the presence of sound functions for the microbial biofilm formation and activity.

Conclusions

In conclusion, our results confirmed that species-specific seaweed associated bacterial communities can be identified and are intra-regionally consistent, yet they present inter-regional differentiation. The structure of bacterial communities associated with *Cystoseira* followed a geographical isolation-by-distance pattern, clearly distinguishable among basins (Atlantic, Mediterranean and Adriatic) and possibly driven by population genetic structure of the host. Nevertheless, we could not identify a correlation of phylogeny with bacteriome similarities, suggesting that microbial components and their host do not follow a shared evolutionary pathway.

Differences in bacterial community structures are reflected in the variation of functional assignments across microhabitats. However, our study suggested that functional redundancy is mostly conserved when comparing community functions among host species and, with some regional adjustments, spatial distance. This might indicate functional redundancy and convergence toward a shared core of bacteria genes associated to the *Cystoseira* group.

The identified *C. compressa* core microbial community could be described across all regions and its bacterial identity and functions can clearly contribute to the structure and stability of the holobiont, which is particularly important in a system that is constantly exposed to a changing environment and potentially invasive organisms. Future studies should focus on experimental manipulation of the environmental conditions to test the stability of the microbial-algal association and, associated to metatranscriptomics studies, the eventual shifts in the functioning and fitness of the *Cystoseira* holobiont.

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References

Aires T, Moalic Y, Serrao EA, Arnaud-Haond S (2015) Hologenome theory supported by cooccurrence networks of Species-specific bacterial communities in siphonous algae (Caulerpa). *FEMS Microbiology Ecology*, **91**, fiv067.

Aires T, Serrão EA, Engelen AH (2016) Host and Environmental Specificity in Bacterial Communities Associated to Two Highly Invasive Marine Species (Genus Asparagopsis). *Frontiers in Microbiology*, **7**, 1–14.

Airoldi L, Beck MW (2007) Loss, Status and Trends for Coastal Marine Habitats of Europe. *Oceanography and Marine Biology: An Annual Review*, 345–405.

Albanese D, Fontana P, De Filippo C *et al.* (2015) MICCA: a complete and accurate software for taxonomic profiling of metagenomic data. *Scientific Reports*, **5**, 9743.

Anderson MJ (2006) Distance-Based Tests for Homogeneity of Multivariate Dispersions. *Biometrics*, **62**, 245–253.

Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, **83**, 557–574.

Assis J, Serrão EA, Claro B, Perrin C, Pearson GA (2014) Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. *Molecular Ecology*, **23**, 2797–2810.

Bashan A, Gibson TE, Friedman J *et al.* (2016) Universality of human microbial dynamics. *Nature*, **534**, 259–262.

Bashyam MD, Hasnain SE (2004) The extracytoplasmic function sigma factors: role in bacterial pathogenesis. *Infection, Genetics and Evolution*, **4**, 301–308.

Benedetti-Cecchi L, Pannacciulli F, Bulleri F *et al.* (2001) Predicting the consequences of anthropogenic disturbance: large-scale effects of loss of canopy algae on rocky shores. *Marine ecology. Progress series*, **214**, 137–150.

Bengtsson MM, Sjøtun K, Lanzén A, Øvreås L (2012) Bacterial diversity in relation to secondary production and succession on surfaces of the kelp Laminaria hyperborea. *Isme J*, **6**, 2188–2198.

Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. *PLoS ONE*, **8**.

Bardou P,Mariette J, Escudié F,Djemiel C and Klopp C (2014) Jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics*,2014, **15**,293Sharpton TJ (2014) An introduction to the analysis of shotgun metagenomic data. *Frontiers in plant science*, **5**, 209.

Bulleri F, Benedetti-cecchi L, Acunto S, Cinelli F, Hawkins SJ (2002) The influence of canopy algae on vertical patterns of distribution of low-shore assemblages on rocky coasts in the northwest Mediterranean. *Journal of Experimental Marine Biology and Ecology*, **267**, 89–106.

Bulleri F, Cucco A, Dal Bello M *et al.* (2016) The role of wave-exposure and human impacts in regulating the distribution of alternative habitats on NW Mediterranean rocky reefs. *Estuarine, Coastal and Shelf Science*.

Burke C, Steinberg P, Rusch D, Kjelleberg S, Thomas T (2011a) Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 14288–93.

Burke C, Thomas T, Lewis M, Steinberg P, Kjelleberg S (2011b) Composition, uniqueness and variability of the epiphytic bacterial community of the green alga Ulva australis. *The ISME journal*, **5**, 590–600.

Busch J, Nascimento JR, Magalhães ACR, Dutilh BE, Dinsdale E (2015) Copper tolerance and distribution of epibiotic bacteria associated with giant kelp Macrocystis pyrifera in southern California. *Ecotoxicology*, **24**, 1131–1140.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**, 335–336.

Eigemann F, Hilt S, Salka I, Grossart H-P (2013) Bacterial community composition associated with freshwater algae: species specificity vs. dependency on environmental conditions and source community. *FEMS Microbiology Ecology*, **83**, 650–663.

Goecke F, Labes a, Wiese J, Imhoff J (2010) Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, **409**, 267–299.

Hester ER, Barott KL, Nulton J, Vermeij MJ, Rohwer FL (2015) Stable and sporadic symbiotic communities of coral and algal holobionts. *The ISME Journal*, 1–13.

Hollants J, Leliaert F, De Clerck O, Willems A (2013) What we can learn from sushi: a review on seaweed-bacterial associations. *FEMS microbiology ecology*, **83**, 1–16.

Lachnit T, Blümel M, Imhoff JJF, Wahl M (2009) Specific epibacterial communities on macroalgae: Phylogeny matters more than habitat. *Aquatic Biology*, **5**, 181–186.

Langille MGI, Zaneveld J, Caporaso JG *et al.* (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, **31**, 814–821.

Leahy JG, Colwell RR (1990) Microbial degradation of hydrocarbons in the environment. *Microbiological reviews*, **54**, 305–15.

Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI (2008) Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology*, **6**, 776–788.

Liu M, Hansen PE, Lin X (2011) Bromophenols in marine algae and their bioactivities. *Marine drugs*, **9**, 1273–92.

Mancuso FP, D'Hondt S, Willems A, Airoldi L, De Clerck O (2016) Diversity and Temporal Dynamics of the Epiphytic Bacterial Communities Associated with the Canopy-Forming Seaweed Cystoseira compressa (Esper) Gerloff and Nizamuddin. *Frontiers in microbiology*, **7**, 476.

McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, **8**.

Nagpal S, Haque MM, Mande SS *et al.* (2016) Vikodak - A Modular Framework for Inferring Functional Potential of Microbial Communities from 16S Metagenomic Datasets (N Ahmed, Ed,). *PLOS ONE*, **11**, e0148347.

Olapade OA, Depas MM, Jensen ET, McLellan SL (2006) Microbial communities and fecal indicator bacteria associated with Cladophora mats on beach sites along Lake Michigan shores. *Applied and environmental microbiology*, **72**, 1932–8.

Parks DH, Tyson GW, Hugenholtz P, Beiko RG (2014) STAMP: Statistical analysis of taxonomic and functional profiles. *Bioinformatics*, **30**, 3123–3124.

Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Semenova EV, Shlykova DS, Semenov AM, Ivanov MN, Shelyakov OV, Netrusov AI (2009) Bacteria epiphytes of brown macroalga in oil utilization in North Sea ecosystems. *Moscow Univ Biol Sci Bull* **64**, 107 – 110

Smale DA, Wernberg T (2013) Extreme climatic event drives range contraction of a habitat-forming species. *Proceedings. Biological sciences / The Royal Society*, **280**, 20122829.

Staley C, Gould TJ, Wang P *et al.* (2014) Core functional traits of bacterial communities in the Upper Mississippi River show limited variation in response to land cover. *Frontiers in Microbiology*, **5**.

Staufenberger T, Thiel V, Wiese J *et al.* (2008) Phylogenetic analysis of bacteria associated with Laminaria saccharina. *FEMS microbiology ecology*, **64**, 65–77.

Steneck RS, Graham MH, Bourque BJ *et al.* (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, **29**, 436–459.

Strain EMA, van Belzen J, van Dalen J, Bouma TJ, Airoldi L (2015) Management of local stressors can improve the resilience of marine canopy algae to global stressors. *PloS one*, **10**, e0120837.

Strain EMA, Thomson RJ, Micheli F, Mancuso FP, Airoldi L (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology*, **20**, 3300–3312.

Tujula N a, Crocetti GR, Burke C *et al.* (2010) Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. *The ISME journal*, **4**, 301–11.

Wahl M, Goecke F, Labes A, Dobretsov S, Weinberger F (2012) The second skin: ecological role of epibiotic biofilms on marine organisms. *Frontiers in microbiology*, **3**, 292.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and environmental microbiology*, **73**, 5261–7.

Watson J, Matsui GY, Leaphart A *et al.* (2000) Reductively debrominating strains of Propionigenium maris from burrows of bromophenol-producing marine infauna. *International journal of systematic and evolutionary microbiology*, **50**, 1035–1042.

Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews*, **32**, 723–735.

Figures and Tables

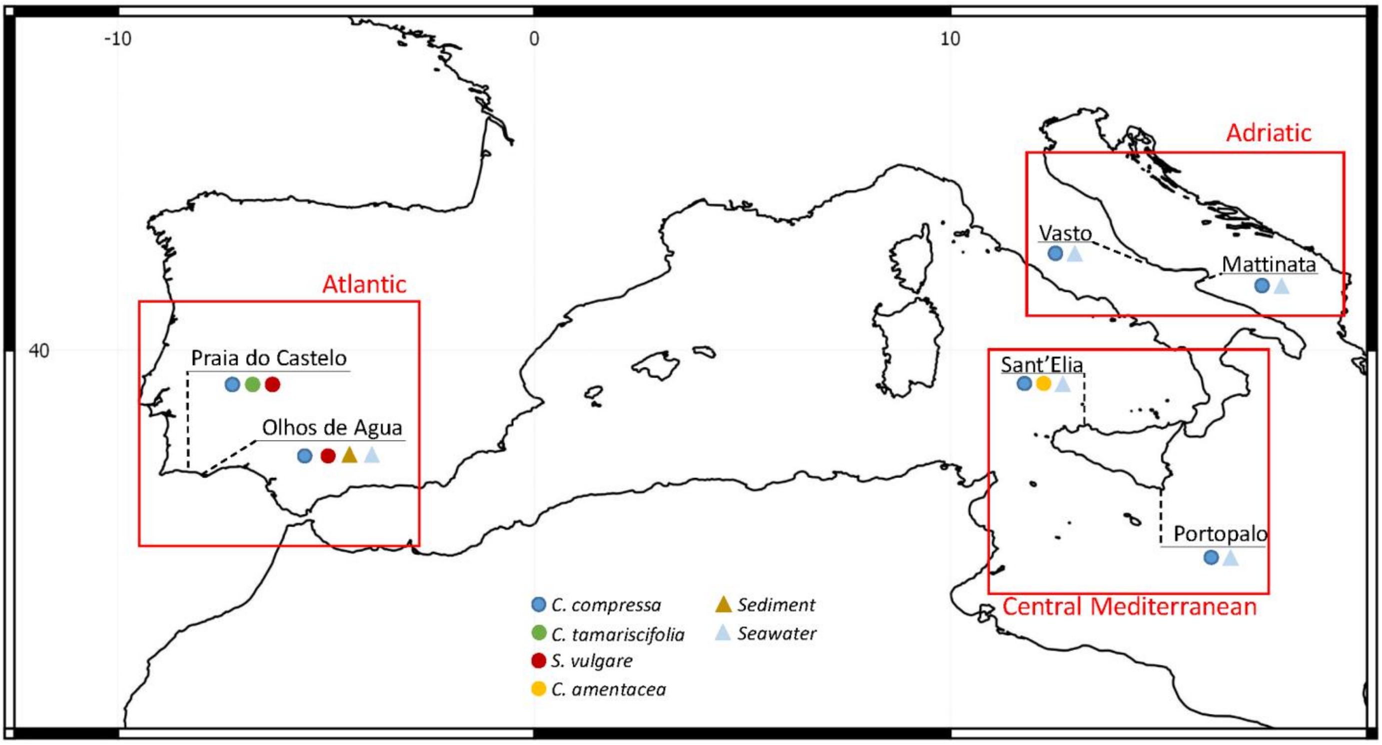


Figure 1 - Map showing the sampling locations and geographical regions (red boxes). Habitat where the microbial community was sampled at each location is also indicated (circles = macroalgae and triangles = environmental samples).

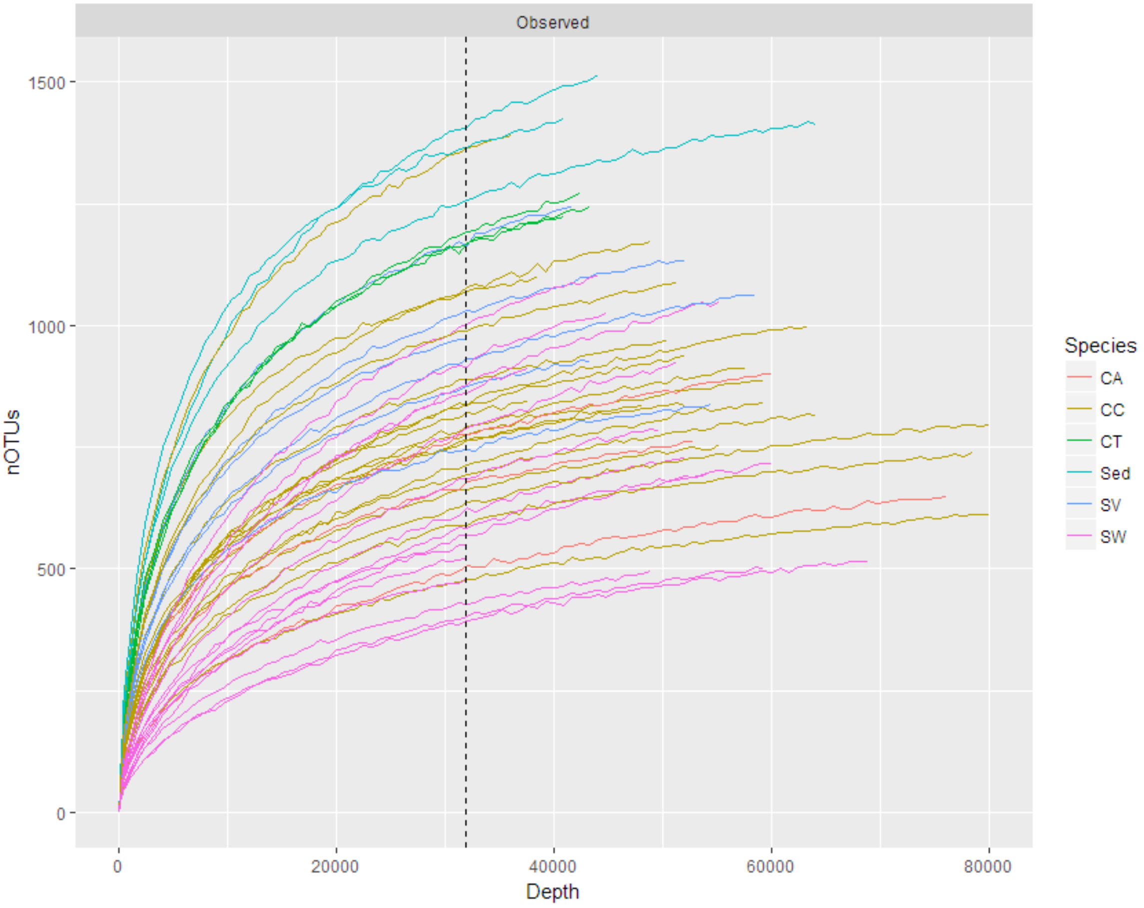


Figure 2 - Rarefaction curves showing the number of OTUs as a function of sequencing depth for each sample. Colours are according to media with: Ca = *Cystoseira amentacea*; Cc = *Cystoseira compressa*; Ct = *Cystoseira tamariscifolia*; Sed = sediment; Sv = *Sargassum vulgare*; SW = seawater. Dotted line represent the rarefaction depth used.

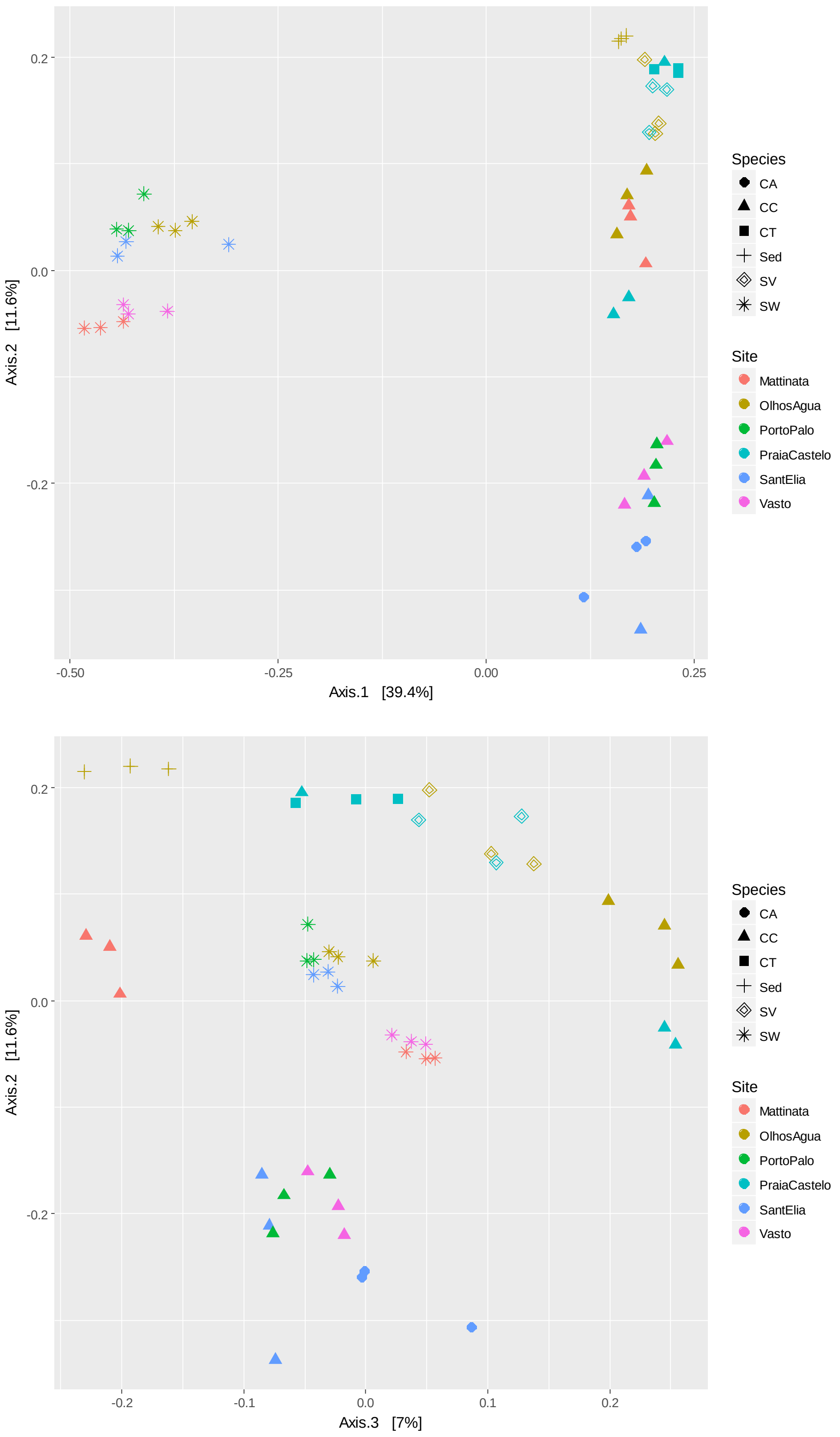


Figure 3 - Principle component analysis (PCOA) of square root transformed OTUs abundances data of bacterial communities associated to seaweeds, seawater and sediments (shape) and locations (colors) distributed from the Atlantic coast of Portugal (Olhos de Água and Praia do Castelo), central Mediterranean (Portopalo and Sant'Elia) and Adriatic sea (Mattinata and Vasto). CA= *Cystoseira amentacea*, CC = *Cystoseira compressa*, CT = *Cystoseira tamariscifolia*, Sed = sediment, SV= *Sargassum vulgare*, SW = seawater

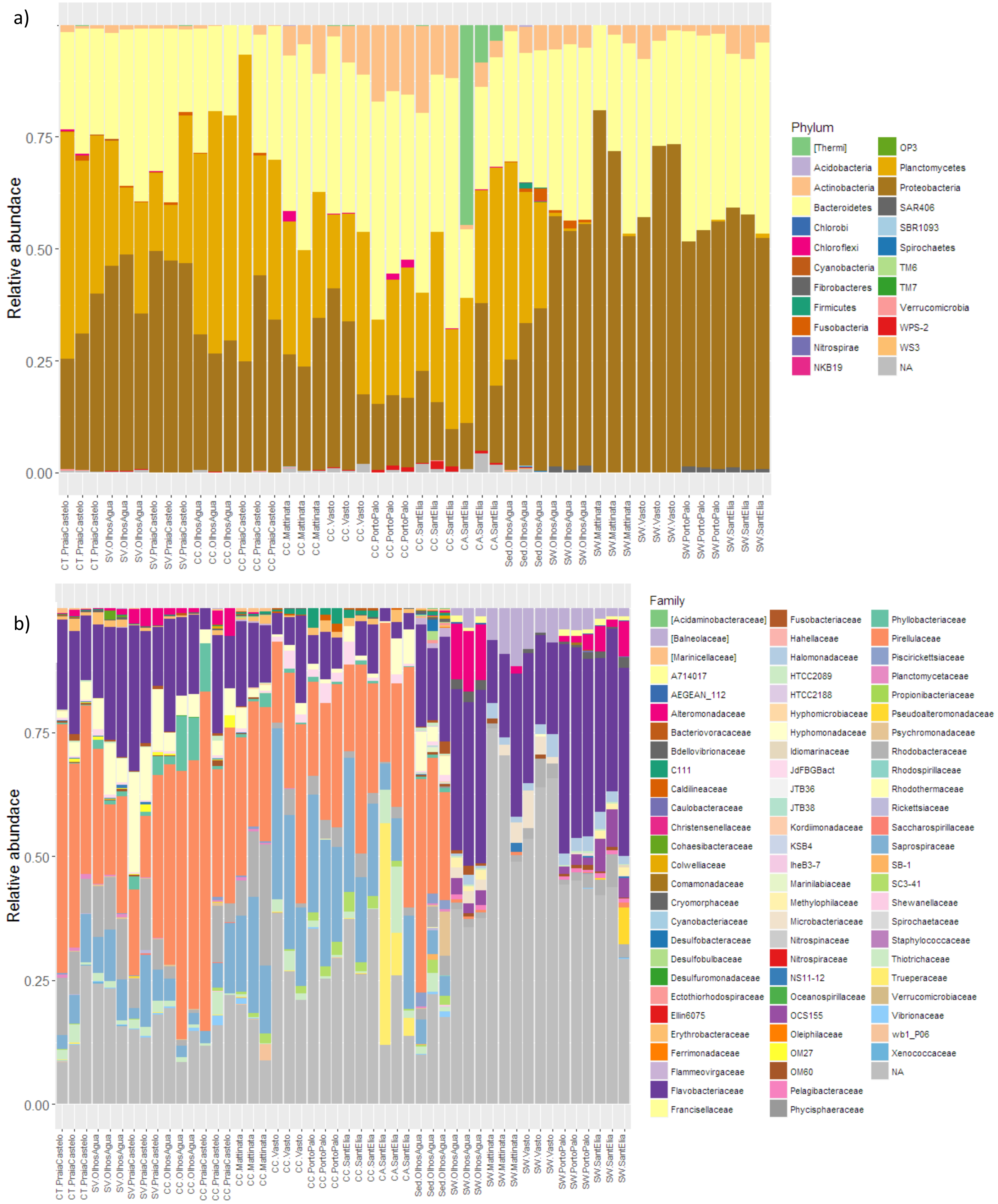


Figure 4 - Relative abundances of taxa at Phylum level (**a**) and Family (**b**) for each replicate of species and location combination. CA= *Cystoseira amentacea*, CC = *Cystoseira compressa*, CT = *Cystoseira tamariscifolia*, Sed = sediment, SV= *Sargassum vulgare*, SW = seawater

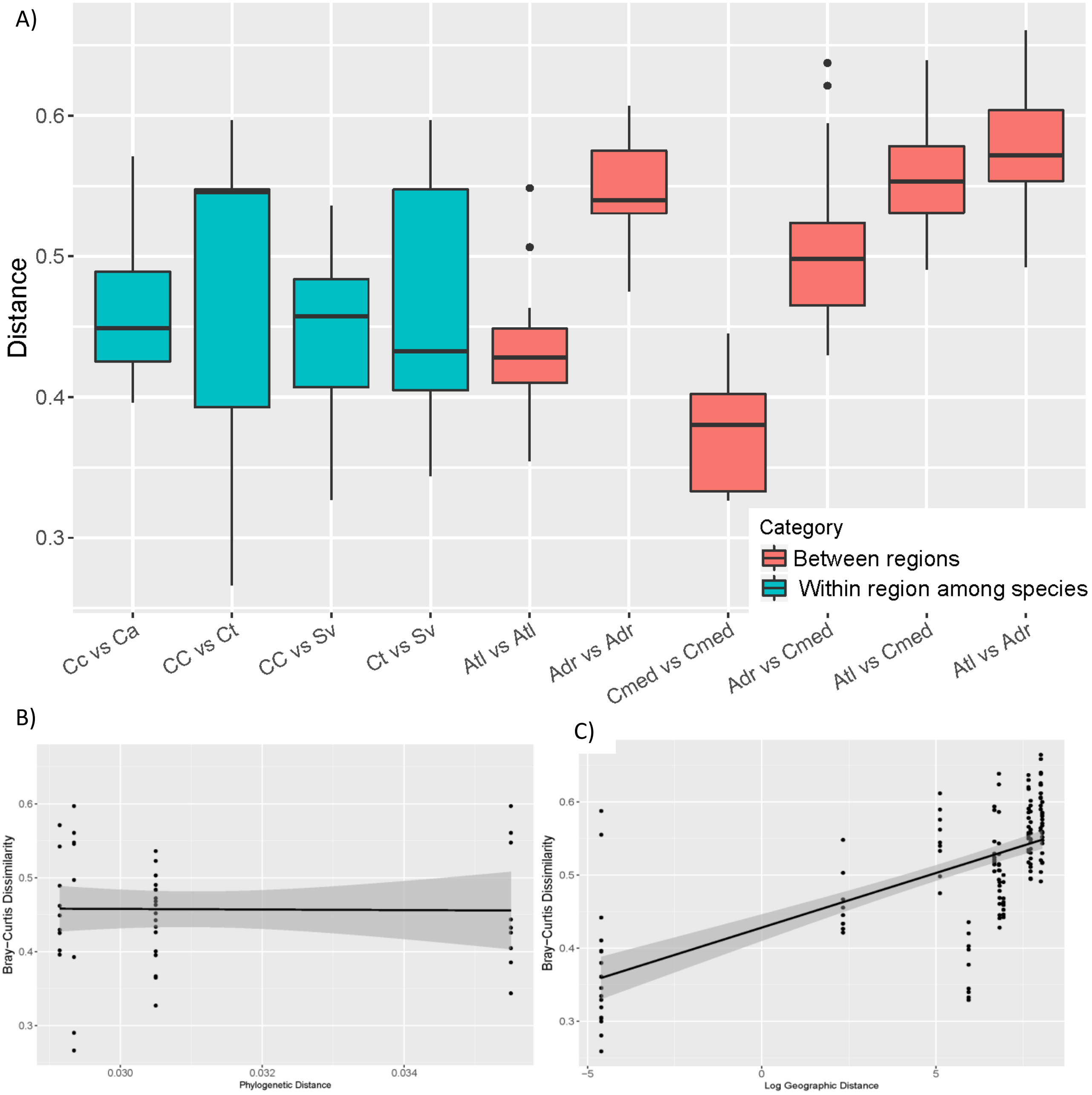


Figure 5 - **A**) Boxplots showing Bray-Curtis pairwise distances between different locatios grouped by region for the same species (in red) and whithin region among different species (in blue). Species paris are from left to right in increasing order of phylogenetic distance while locations comparison are in increasing order of geographic distance. Between location comparision labels indicate the region pairs. Within locations labels indicate the two species contrasted. Boxplots show median (horizontal line), first and third quartile (box frame) and 95% confidence interval of median (vertical line). **B**) linear regressions of pair-wise Bray-Curtis dissimilarity with species pairs phylogenetic distance and **C**) with the natural logarithm of shortest geographical distance among samples pairs. Shaded area around the regression line show 95% confidence intervals.

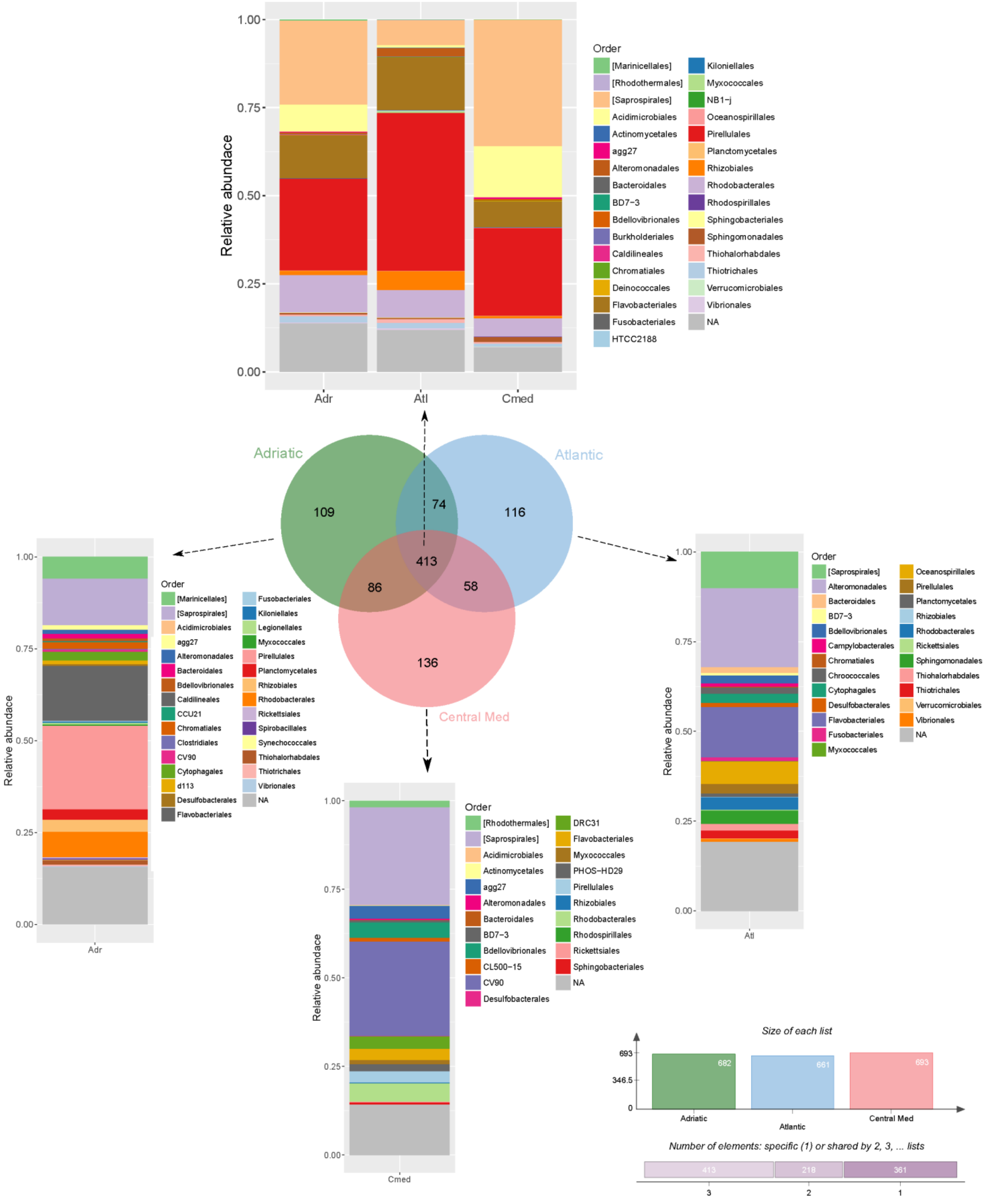


Figure 6 - Bar charts representation of the core microbial community of *Cystoseira compressa* at Order level (common OTUs shared by 2/3 of the replicates within each location). Bar charts showing the relative abundance of different Order of the exclusve OTUs of each geographic region are also presented. At the bottom right cornern is shown the number of OTUs at each region and the number of OTUs shared by 1, 2 or 3 regions.

Table 1 - Number of OTUs and sequences of each replicate after quality control and removal of chimeras, chloroplast and singletons.

Species	Location	Region	Number of OTUs	Number of Sequences	Number of OTUs after rarefaction	% of unique OTUs
C.compressa	Mattinata	Adriatic	1234	38956	1184	2.70%
C.compressa	Mattinata	Adriatic	1339	48809	1179	1.95%
C.compressa	Mattinata	Adriatic	1221	51563	1086	1.75%
C.compressa	Vasto	Adriatic	1019	59943	828	0.48%
C.compressa	Vasto	Adriatic	1106	50804	995	0.30%
C.compressa	Vasto	Adriatic	986	48678	849	0.24%
Seawater	Mattinata	Adriatic	648	59802	477	0.00%
Seawater	Mattinata	Adriatic	660	69580	438	0.68%
Seawater	Mattinata	Adriatic	615	49044	494	0.40%
Seawater	Vasto	Adriatic	879	54716	688	1.02%
Seawater	Vasto	Adriatic	920	52283	716	0.56%
Seawater	Vasto	Adriatic	917	60069	669	0.60%
C.compressa	OlhosAgua	Atlantic	1094	52339	934	0.32%
C.compressa	OlhosAgua	Atlantic	948	81174	671	0.60%
C.compressa	OlhosAgua	Atlantic	1006	59375	792	0.25%
C.compressa	PraiaCastelo	Atlantic	738	85524	518	0.00%
C.compressa	PraiaCastelo	Atlantic	1619	36164	1561	0.45%
C.compressa	PraiaCastelo	Atlantic	851	78625	632	0.16%
C.tamariscifolia	PraiaCastelo	Atlantic	1425	41295	1309	0.46%
C.tamariscifolia	PraiaCastelo	Atlantic	1434	43695	1295	0.23%
C.tamariscifolia	PraiaCastelo	Atlantic	1480	42970	1351	1.04%
S.vulgare	OlhosAgua	Atlantic	1077	43523	973	0.00%
S.vulgare	OlhosAgua	Atlantic	1148	32790	1143	0.00%
S.vulgare	OlhosAgua	Atlantic	1450	42322	1341	0.52%
S.vulgare	PraiaCastelo	Atlantic	1303	52287	1117	0.36%
S.vulgare	PraiaCastelo	Atlantic	1231	59098	1036	0.29%
S.vulgare	PraiaCastelo	Atlantic	968	54691	821	0.12%
Seawater	OlhosAgua	Atlantic	789	32955	687	0.00%
Seawater	OlhosAgua	Atlantic	776	32880	674	0.00%
Seawater	OlhosAgua	Atlantic	810	32897	607	0.00%
Sediment	OlhosAgua	Atlantic	1754	44640	1596	1.44%
Sediment	OlhosAgua	Atlantic	1588	41352	1501	5.60%
Sediment	OlhosAgua	Atlantic	1576	64258	1338	3.36%
C.amentacea	SantElia	C.Mediterranean	781	76197	562	0.71%
C.amentacea	SantElia	C.Mediterranean	896	53159	762	0.39%
C.amentacea	SantElia	C.Mediterranean	1061	60590	859	0.35%
C.compressa	PortoPalo	C.Mediterranean	952	64582	753	0.13%
C.compressa	PortoPalo	C.Mediterranean	963	52433	832	0.60%
C.compressa	PortoPalo	C.Mediterranean	1073	57672	884	0.23%
C.compressa	SantElia	C.Mediterranean	885	55504	748	0.53%
C.compressa	SantElia	C.Mediterranean	1002	37937	950	0.32%
C.compressa	SantElia	C.Mediterranean	1172	63789	929	0.11%
Seawater	PortoPalo	C.Mediterranean	1289	55815	996	1.41%
Seawater	PortoPalo	C.Mediterranean	1271	45038	1085	1.38%
Seawater	PortoPalo	C.Mediterranean	1353	44466	1161	1.46%
Seawater	SantElia	C.Mediterranean	1165	51561	921	0.65%
Seawater	SantElia	C.Mediterranean	988	49622	791	0.51%
Seawater	SantElia	C.Mediterranean	1150	38176	1060	0.75%

OTUs number before and after rarefaction at minimum sample size is shown as well as the % of exclusive OTUs of each replicate.

Supplementary material

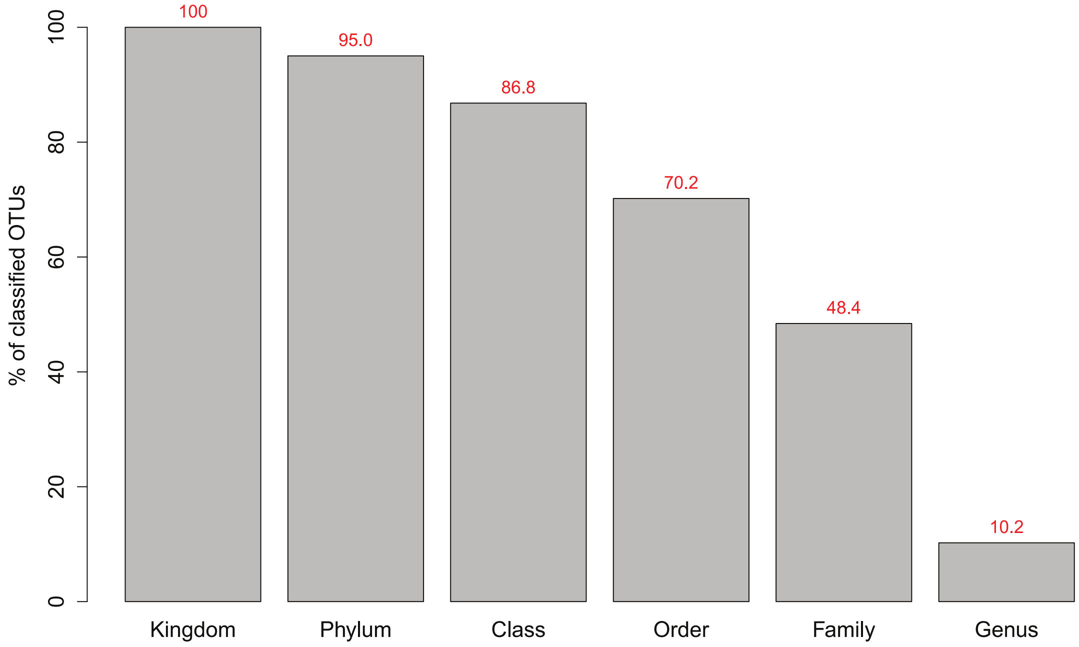


Figure S1 – Relative proportion of classified OTUs according to each taxonomic level.

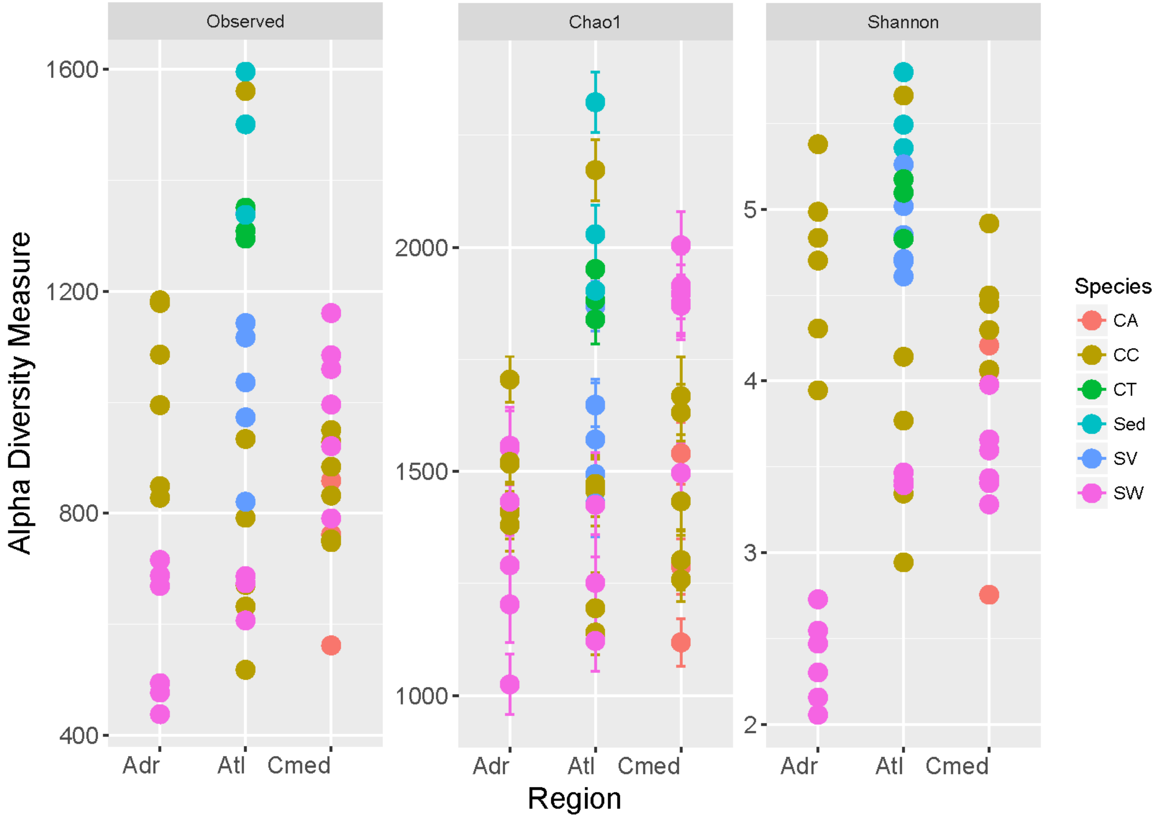
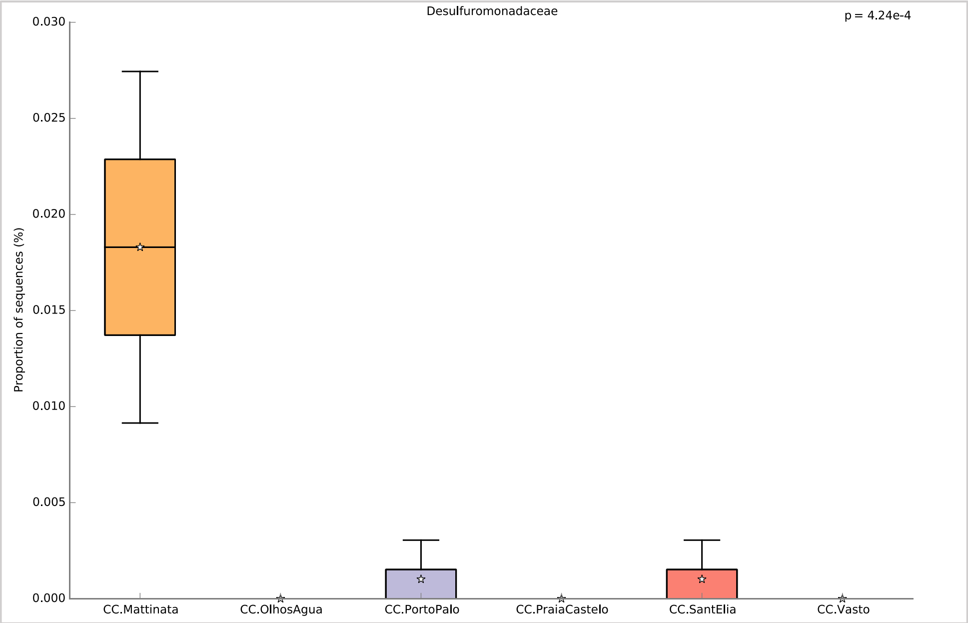
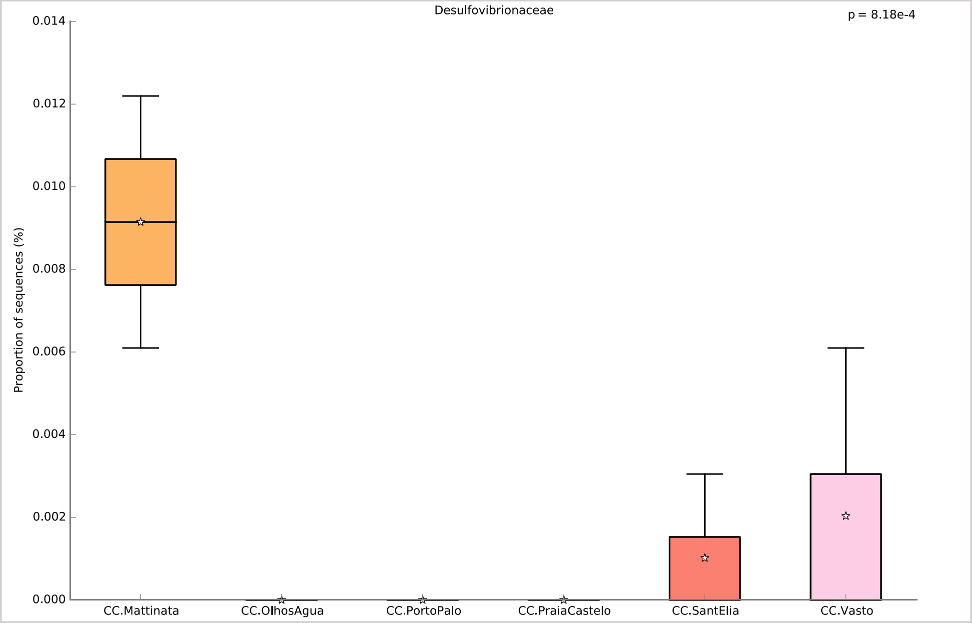


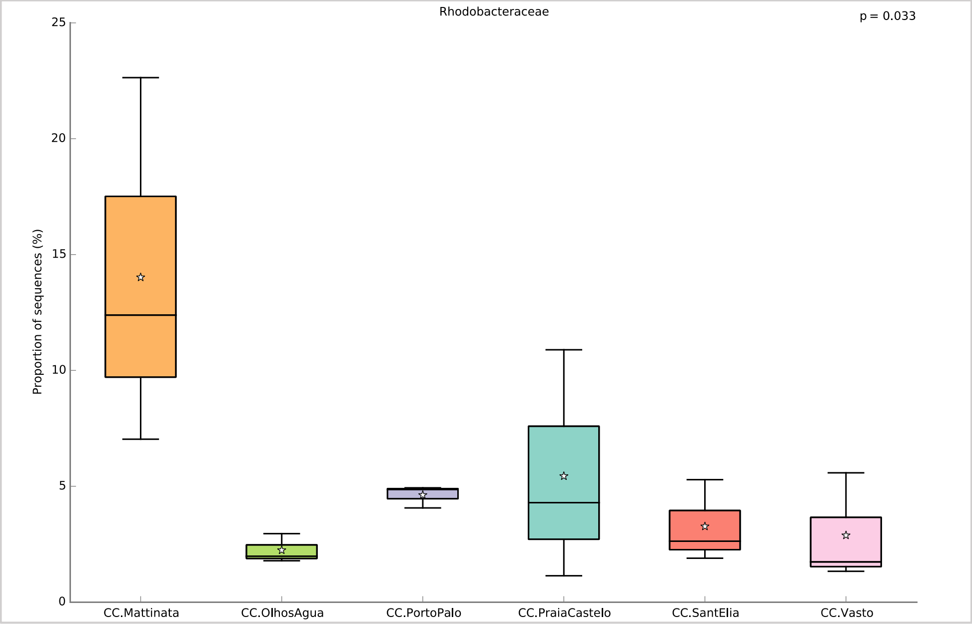
Figure S2 – Observed OTUs richness, Chao1 and Shannon Indexes of diversity for each region (Ard = Adriatic; Atl = Atlantic; Cmed = Central Mediterranean) and species combination. Observed richness is based on rarefied otu table while biodiversity indexes are estimated on the original not rarefied table. Ca = *Cystoseira amentacea;* Cc = *Cystoseira compressa;* Ct = *Cystoseira tamariscifolia;* Sed = sediment; Sv = *Sargassum vulgare;* SW = seawater a)


Desulfuromonadaceae
p = 4.24e-4
Proportion of sequences (%)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

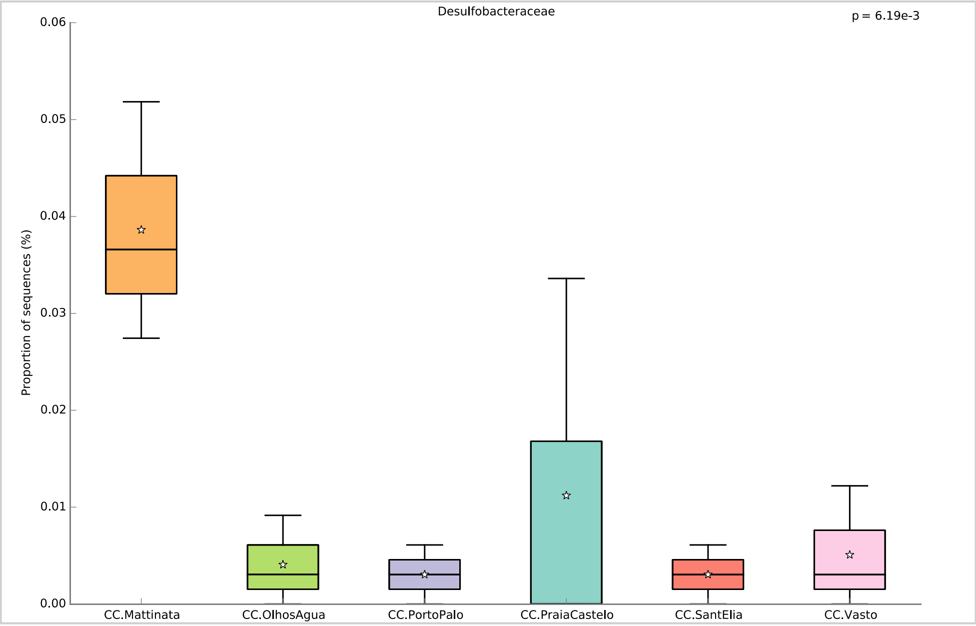
b)


Desulfovibrionaceae
p = 8.18e-4
Proportion of sequences (%)
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

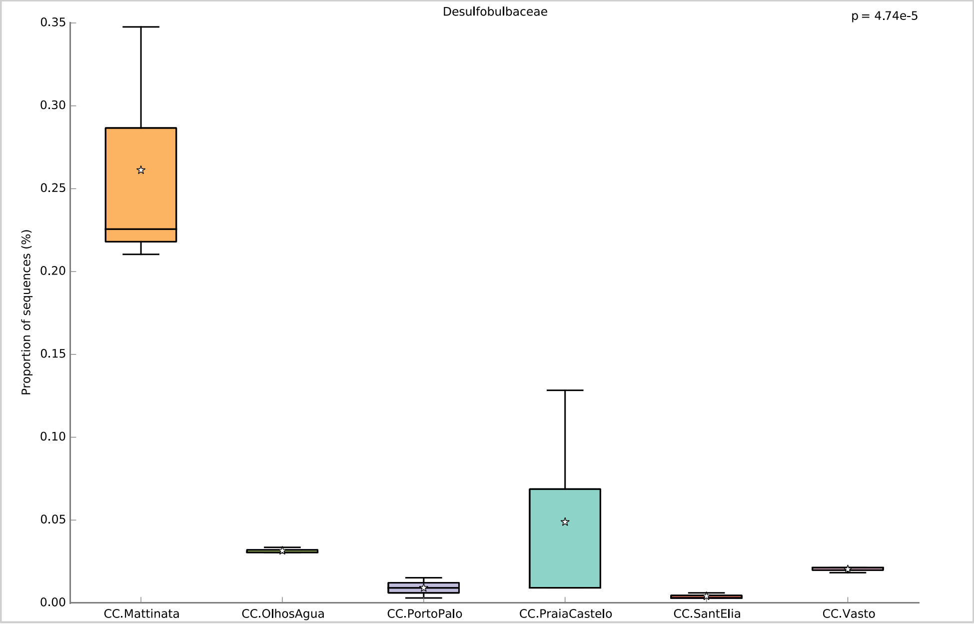
c)


Rhodobacteraceae
p = 0.033
Proportion of sequences (%)
25
20
15
10
5
0
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

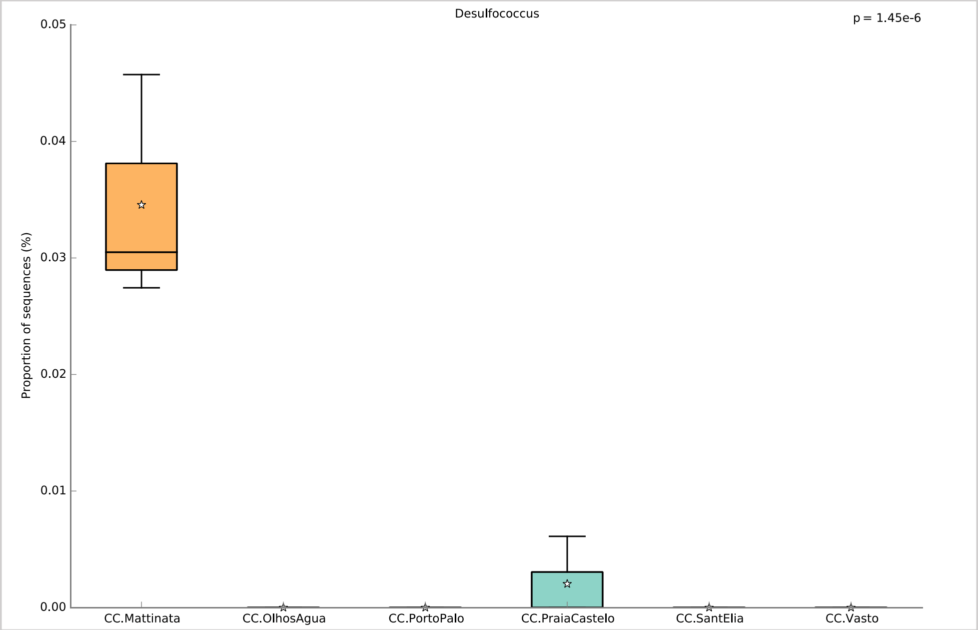
d)


Desulfobacteraceae
p = 6.19e-3
Proportion of sequences (%)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

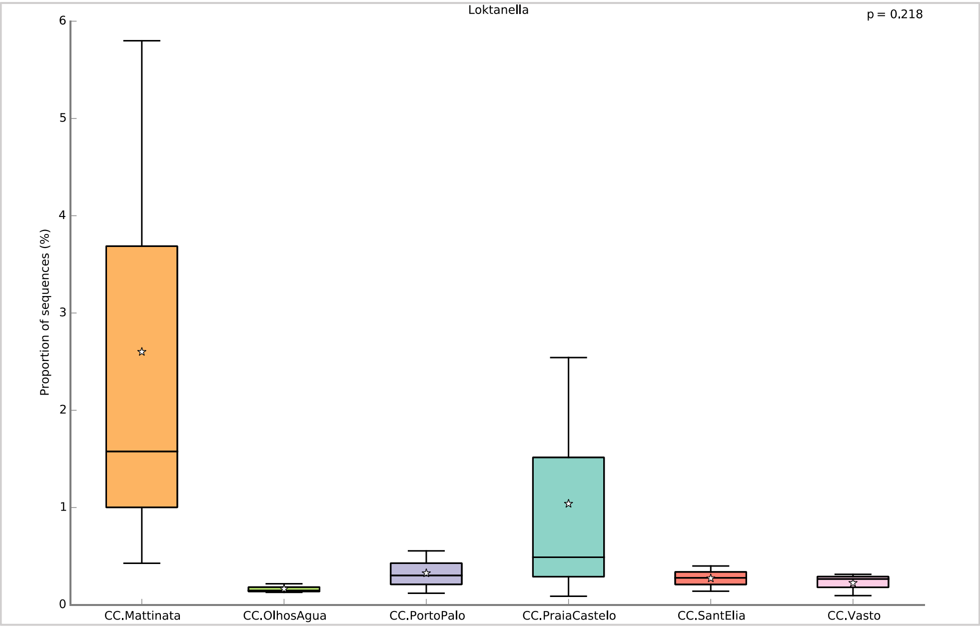
e)


Desulfobulbaceae
p = 4.74e-5
Proportion of sequences (%)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

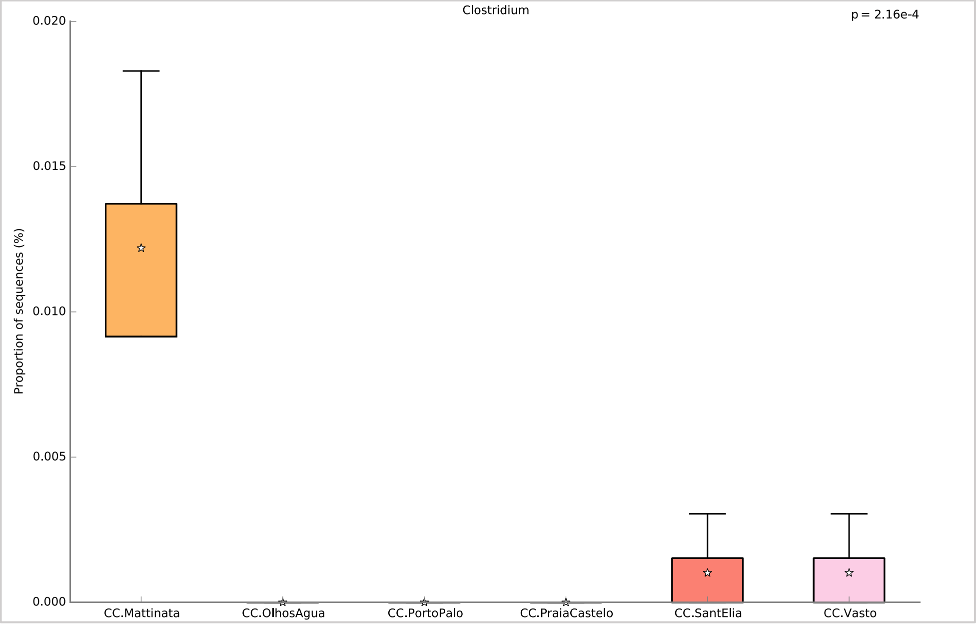
f)


Desulfococcus
p = 1.45e-6
Proportion of sequences (%)
0.05
0.04
0.03
0.02
0.01
0.00
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

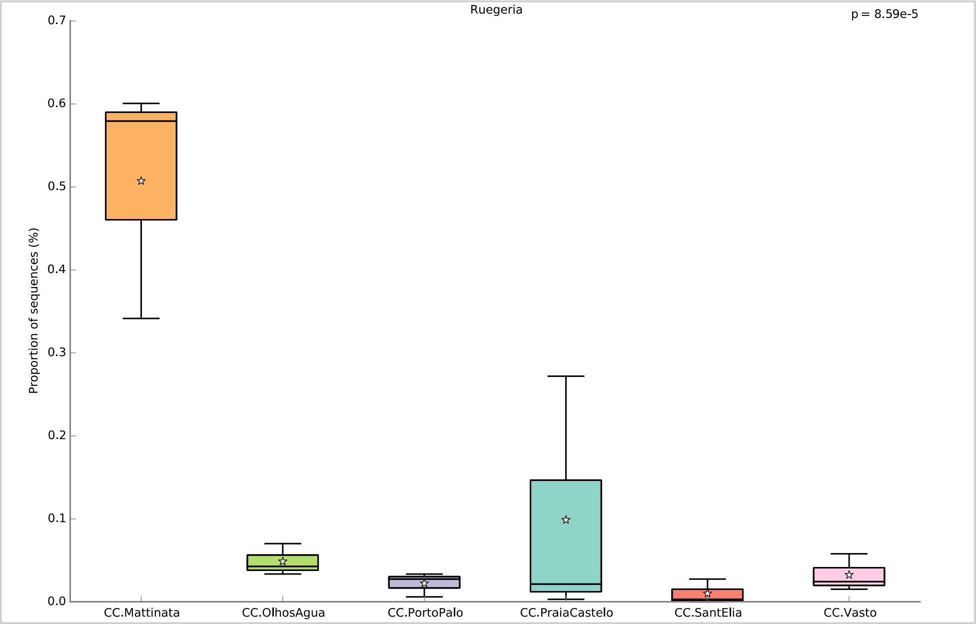
g)


Loktanella
p = 0.218
Proportion of sequences (%)
6
5
4
3
2
1
0
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

h)


Clostridium
p = 2.16e-4
Proportion of sequences (%)
0.020
0.015
0.010
0.005
0.000
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

i)


Ruegeria
p = 8.59e-5
Proportion of sequences (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CC.Mattinata
CC.OlhosAgua
CC.PortoPalo
CC.PraiaCastelo
CC.SantElia
CC.Vasto

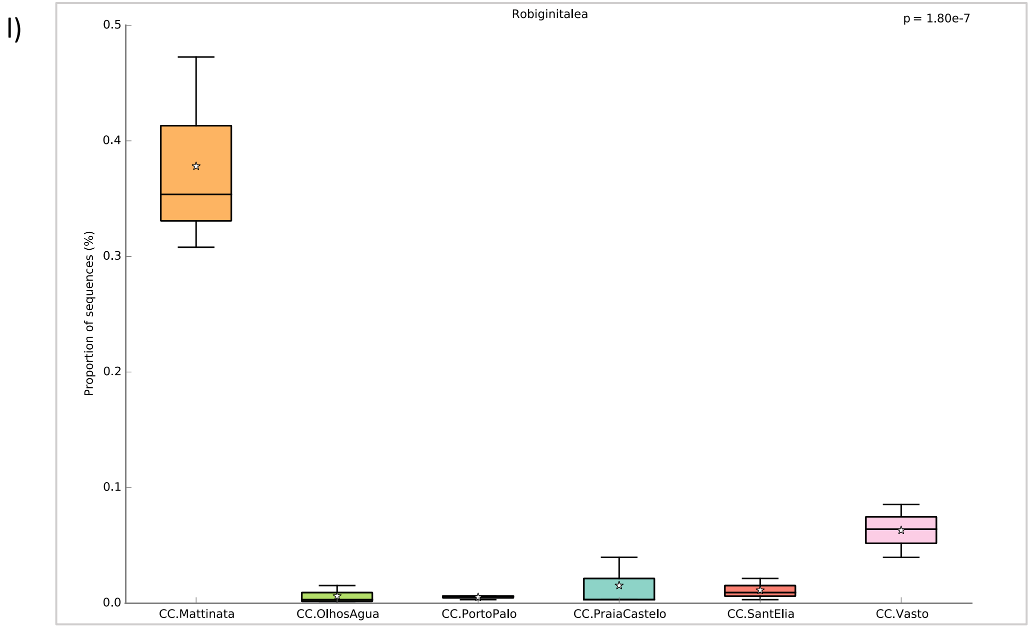


Figure S3 – Box plots showing relative proportion of sequences across locations of the main taxa of interest overrepresented in samples of *C. compressa* from Mattinata location. a) *Desulfuromonadaceae* b) *Desulfovibrionacae*; c) *Rhodobacteraceae*; d) *Desulfobacteraceae*; e) *Desulfobulbaceae*; f) *Desulfococcus*; g) *Loktanella*; h) *Clostridium*; i) *Ruegeria*; l) *Robiginitalea.* Boxplots shows median, the 25th and 75th percentile and the 95 percent confidence interval.

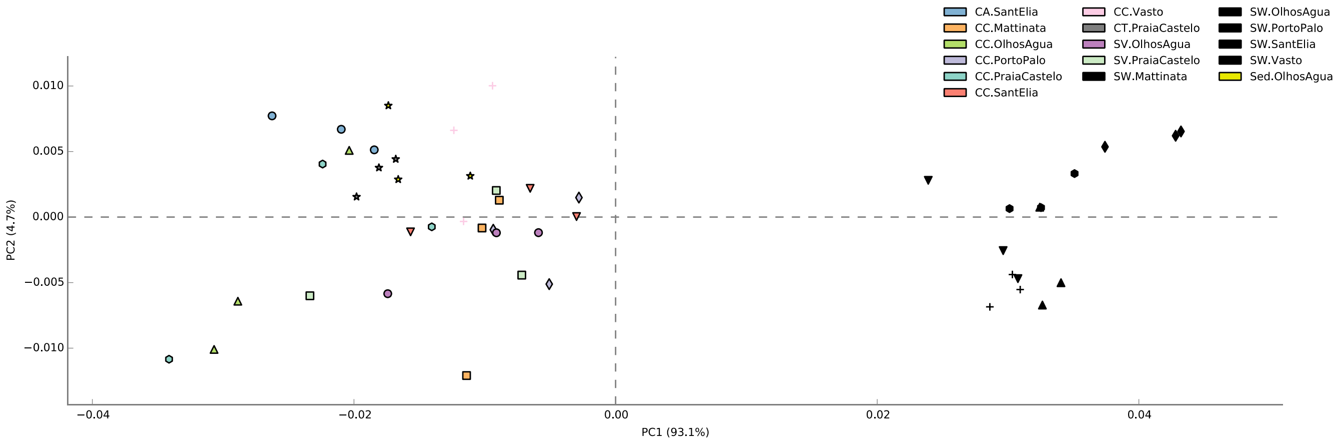


Figure S4 - PCA of PICRUSt functional assignment at the lowest KO category. CA = *C. amentacea;* CC = *C. compressa;* CT = *C. tamariscifolia;* SV = *S. vulgare;* SW = seawater; Sed = sediment.

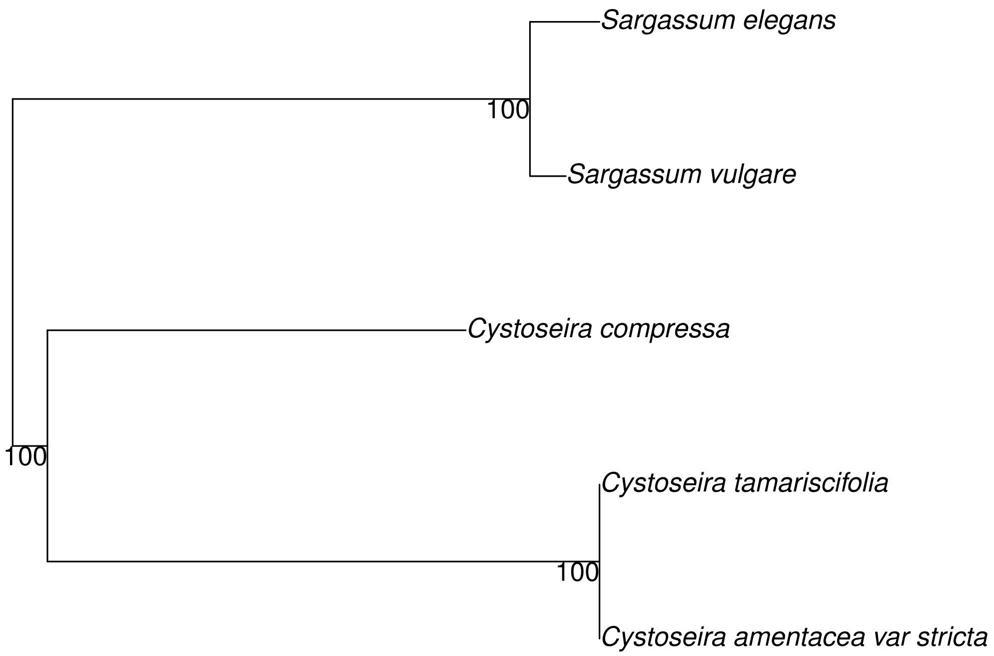


Figure S5 - Maximum-likelyhood phylogenetic tree based on the *psbA* DNA sequences data. Bootstrap support values are showed on the branches edges. *S. elegans* is also shown as reference with complete Sargassaceae tree in *Draisma et al. 2010.*

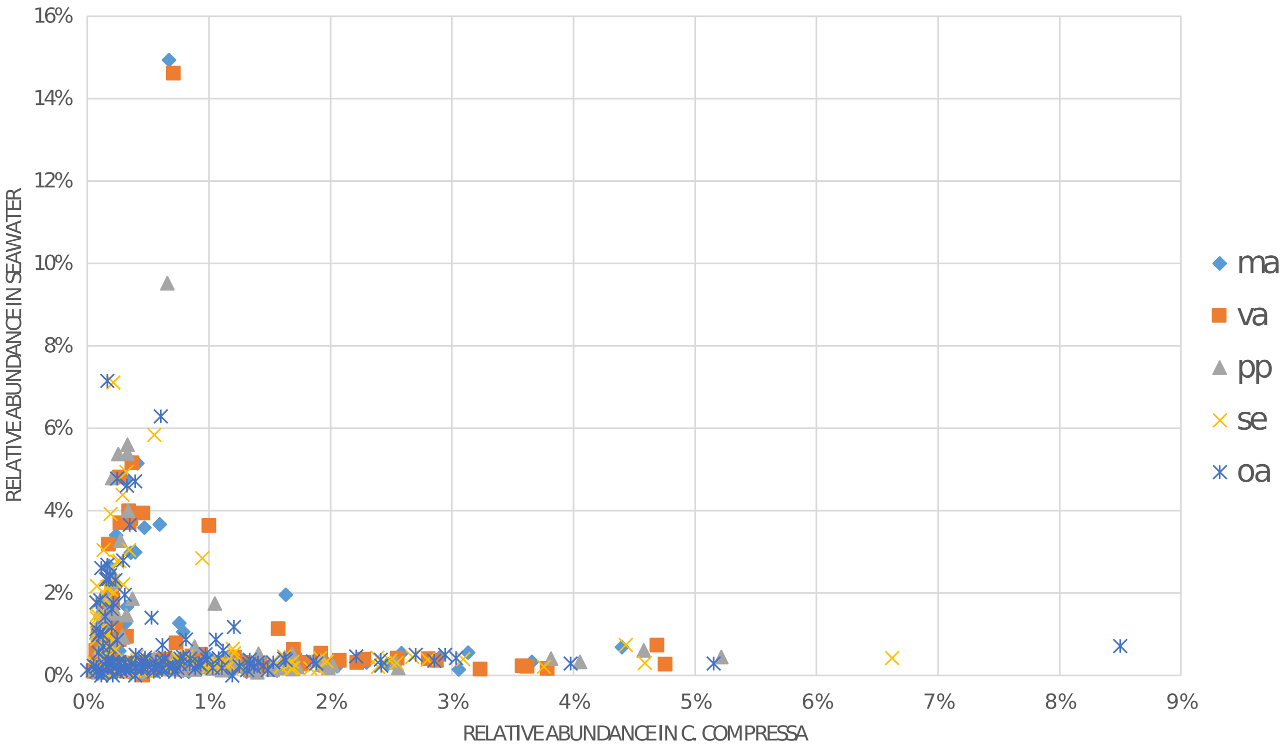


Figure S6 - Scatterplot between C. compressa and seawater relative abundance of each shared OTUs for every location. ma = Mattinata; va = Vasto; pp= Portopalo; se= Sant'Elia; oa = Olhos de Agua

CONCLUSIONS AND FINAL REMARKS

The major problems behind the topics of this thesis were the multiple anthropogenic pressures and climate instabilities that are causing canopy-forming seaweeds to decline (Steneck *et al.* 2002; Airoldi & Beck 2007; Gianni *et al.* 2013; Strain *et al.* 2014; Airoldi *et al.* 2015), raising serious concerns as they provide significant ecosystem services (Worm *et al.* 2006; Tinch & Mathieu 2009; Cheminée *et al.* 2013; Mineur *et al.* 2015). In this context, this thesis increased the understanding of the genetic background and the dynamics of marine canopies under global change, which is crucial knowledge to identify possible management scenarios limiting the loss and preserving these important habitats. This thesis focused on species of the genus *Cystoseira* that are some of the main canopy algae in the Mediterranean and adjacent Atlantic ocean, and was conceptually divided into three parts: 1) describe what is the genetic diversity and connectivity of those species across their distribution range; 2) understand how climate change will alter their distributional ranges and the consequences for each species gene pool, and 3) explore the association with bacterial communities (in a holobiont perspective) and their potential role in seaweed resilience and adaptation to changing environmental conditions.

This study represents an important step forward in understanding the genetic variability of *Cystoseira* species along their distributional ranges, as almost no previous genetic studies exist on these species (but see Susini *et al.* 2007b; Robvieux *et al.* 2012, 2013). The use of microsatellite markers allowed characterizing the genetic variability of each population and the connectivity among them. Information on the molecular ecology of these species also showed evidence of a potential loss of genetic diversity and decrease of populations' resilience in response to climate change. According to their climatic niche, we predicted extensive modifications of suitable habitat range for the three species of *Cystoseira* in the next future. Even the best-case scenario of a reduced greenhouse gasses (GHG) concentration predicts a remarkable decrease in habitat availability. The range shift will likely cause the loss of valuable genetic clusters, as dispersal and colonization in these species will hardly be able to cope with such fast and extensive climatic alteration. In the Mediterranean Sea, consequences of distributional changes would be particularly dramatic as they are not compensated by equally important expansion of species ranges and due to the absence of others structural species able to occupy the same ecological niche and functional role (see chapter 2). The models predicted that *C. amentacea* might be particularly exposed to extinction risk, with possible ecological consequences cascading on the coastal ecosystems. Furthermore, this species showed extremely low connectivity among populations and their connectivity level showed to be intrinsically related to the fragmentation of the natural habitat (see chapter 3). The predicted increase in modifications and fragmentation of rocky shores can potentially further increase isolation, reducing the connectivity and increasing the risk of losing evolutionary potential or facing genetic drift due to demographic reduction of populations.

This is also the first study addressing the specificity and spatial distribution of bacterial communities associated with different *Cystoseira* species. In the context of the hologenome theory (Zilber-Rosenberg and Rosenberg 2008), macroalgae and associated bacterial communities are connected by bidirectional chemical interactions and perturbation of this dynamic equilibrium can lead to changes in the fitness of the host. I found that significant differences can be linked to the spatial distribution of the collected samples, but that differences among species are often representing an equally great distinction factor. Species-specific bacterial community showed to be consistent at regional scale yet, its composition is modulated by geographical and environmental local conditions. However, a set of shared bacteria, forming the core community of *C. compressa*, was identified and assigned to potential key functions for the holobiont system. This characterization of the distribution and specificity of bacterial communities associated with *Cystoseira* could help identifying those factors that might enhance the resilience of these species to climatic changes and other stressors in the future.

The integration of these results will provide innovative insights and the right tools to help designing management projects for these structural species. In any sound species management plan, the restoration of good environmental quality should always be the first step. This is particularly important considering that experimental studies have shown that the reduction of local stressors can enhance the resilience of canopy-algae to global stressors such as climate change (Mancuso 2016; Strain *et al.* 2015).

One of the possible management strategies is the establishment of Marine protected areas (MPAs). MPAs have a strong potential for conservation and restoration of marine forests: both as a source of propagules and as priority sites for restoration activities. The establishment of MPAs potentially favors the recovery of macroalgae forests by increasing predation pressure on grazers (Babcock *et al.* 2010) or by eliminating sources of anthropogenic disturbance (Thibaut *et al.* 2016). Networks of MPAs should be designed to maintain or restore the connectivity among fragmented populations, therefore increasing resilience to future environmental disturbances (Frankham *et al.* 2002; Coleman *et al.* 2011). Thus, even if not providing direct protection from large-scale modifications, MPAs can improve local conditions and increase the ecosystems ability to buffer global impacts. Because some species have limited dispersal potential (as I have shown in the third chapter of this thesis), a network of MPAs should be specifically designed to restore and preserve critical dispersal pathways. Lack of connectivity among MPAs and habitat degradation along the coast may decrease the genetic variability and hamper the resilience and adaptation of these endangered habitat-forming species, possibly limiting the expected ecological and socio-economic benefits of protection.

Restoration of *Cystoseira* forests on both natural and artificial substrata is an alternative management strategy to counteract the local decline of these species (Falace *et al.* 2006; Susini *et al.* 2007a; Perkol-Finkel *et al.* 2012; Gianni *et al.* 2013). Again, such restoration interventions should be designed taking into account the connectivity between populations on short temporal scales, as for species such as *C. amentacea* this could be limited at very small distances. The loss of species from large, fragmented areas would be hardly reversible even by restoration, as limited dispersal would not allow recolonization in short time scales. This implies that restoration can still be effective to foster recovery if some natural populations are present in the area, and if adequate habitat patches can serve as stepping stones for migrants.

Based on the results obtained in this thesis, I suggest that the species target for reforestation should reflect the conservation priority (Figure 1). If the priority is to preserve the ecosystem functioning, then most of the times it could be more effective to choose a species that is able to guarantee the highest probability of long-term persistence and positive effects on the surrounding environment. Regrettably, restoration projects to restore natural environmental conditions are associated with high costs and uncertainties related to its success (Bayraktarov *et al.* 2016). Another possible application of my results is in eco-engineering of waterfronts and urbanized environments. There is growing interest in marine eco-engineering that combines the natural environment with infrastructures for the benefits of both nature and society (green-blue infrastructures). Because of their important ecosystem services canopy algae are some of the potential targets of such actions to elevate the ecological value of constructions/urbanization (e.g, Perkol-Finkel *et al.* 2012) and ecological knowledge can ensure eco-engineering objectives to be met. The choice of the species target and of the positioning to obtain the best possible outcome over a long period could be guided by the molecular, dispersal and predicted climatic suitability knowledge acquired here. In addition, extending our understanding of dynamics of microbial associated community could allow monitoring the progress of canopy algae and detecting early warning signals of stress. In conclusion, the results of this thesis can help in future selection of priority areas for conservation and restoration. It can also representan incentive to better planning, design and management of marine urbanized areas to mitigate habitat degradation and provide potentially suitable stepping stones (e.g. green-blue infrastructure) for threatened native populations.

References

Airoldi L, Ballesteros E, Buonomo R *et al.* (2015) Marine forests at risk: solutions to halt the loss and promote the recovery of mediterranean canopy-forming seaweeds. *5th Mediterranean Symposium on Marine Vegetation*, 28–33.

Airoldi L, Beck MW (2007) Loss, Status and Trends for Coastal Marine Habitats of Europe. *Oceanography and Marine Biology: An Annual Review*, 345–405.

Babcock RC, Shears NT, Alcala AC *et al.* (2010) Decadal trends in marine reserves reveal differential rates of change in direct and indirect effects. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 18256–61.

Bayraktarov E, Saunders MI, Abdullah S *et al.* (2016) The cost and feasibility of marine coastal restoration. *Ecological Applications*, **26**, 1055–1074.

Cheminée A, Sala E, Pastor J *et al.* (2013) Nursery value of Cystoseira forests for Mediterranean rocky reef fishes. *Journal of Experimental Marine Biology and Ecology*, **442**, 70–79.

Coleman MAM, Chambers J, Knott NA *et al.* (2011) Connectivity within and among a network of temperate marine reserves. *PloS one*, **6**, e20168.

Falace A, Zanelli E, Bressan G (2006) Algal transplantation as a potential tool for artificial reef management and environmental mitigation. *Bulletin of Marine Science*, **78**, 161–166.

Frankham R, Briscoe DA, Ballou JD (2002) Introduction to Conservation Genetics.

Gianni F, Bartolini F, Airoldi L *et al.* (2013) Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. *Advances in Oceanography and Limnology*, **4**, 83–101.

Mancuso (2016). Effects of local and global stressors on the status and future persistence of intertidal canopy-forming algae. PhD thesis, *Università di Bologna.*

Mineur F, Arenas F, Assis J *et al.* (2015) European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of Sea Research*, **98**, 91–108.

Perkol-Finkel S, Ferrario F, Nicotera V, Airoldi L (2012) Conservation challenges in urban seascapes: promoting the growth of threatened species on coastal infrastructures (M Cadotte, Ed,). *Journal of Applied Ecology*, **49**, 1457–1466.

Robvieux P, Valero MM, Aurelle MD *et al.* (2013) Docteur en Sciences.

Robvieux P, Videment J, Ribout C *et al.* (2012) First characterization of eight polymorphic microsatellites for Cystoseira amentacea var. stricta (Fucales, Sargassaceae). *Conservation Genetics Resources*, **4**, 923–925.

Steneck RS, Graham MH, Bourque BJ *et al.* (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, **29**, 436–459.

Strain EMA, Thomson RJ, Micheli F, Mancuso FP, Airoldi L (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology*, **20**, 3300–3312.

Strain EMA, van Belzen J, van Dalen J, Bouma TJ, Airoldi L (2015) Management of local stressors can improve the resilience of marine canopy algae to global stressors. *PloS one*, **10**, e0120837.

Susini ML, Mangialajo L, Thibaut T, Meinesz A (2007a) Development of a transplantation technique of Cystoseira amentacea var. stricta and Cystoseira compressa. In: *Biodiversity in Enclosed Seas and Artificial Marine Habitats*, pp. 241–244. Springer.

Susini M-L, Thibaut T, Meinesz A, Forcioli D (2007b) A preliminary study of genetic diversity in Cystoseira amentacea (C. Agardh) Bory var. stricta Montagne (Fucales, Phaeophyceae) using random amplified polymorphic DNA. *Phycologia*, **46**, 605–611.

Thibaut T, Blanfuné A, Boudouresque C-F *et al.* (2016) Unexpected Temporal Stability of Cystoseira and Sargassum Forests in Port-Cros, one of the Oldest Mediterranean Marine National Parks. *Cryptogamie, Algologie*, **37**, 61–90.

Tinch R, Mathieu L (2009) *Marine and Coastal Ecosystem Services*.

Worm B, Barbier EB, Beaumont N *et al.* (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science (New York, N.Y.),* **314**, 787–90.

Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews*, **32**, 723–735.

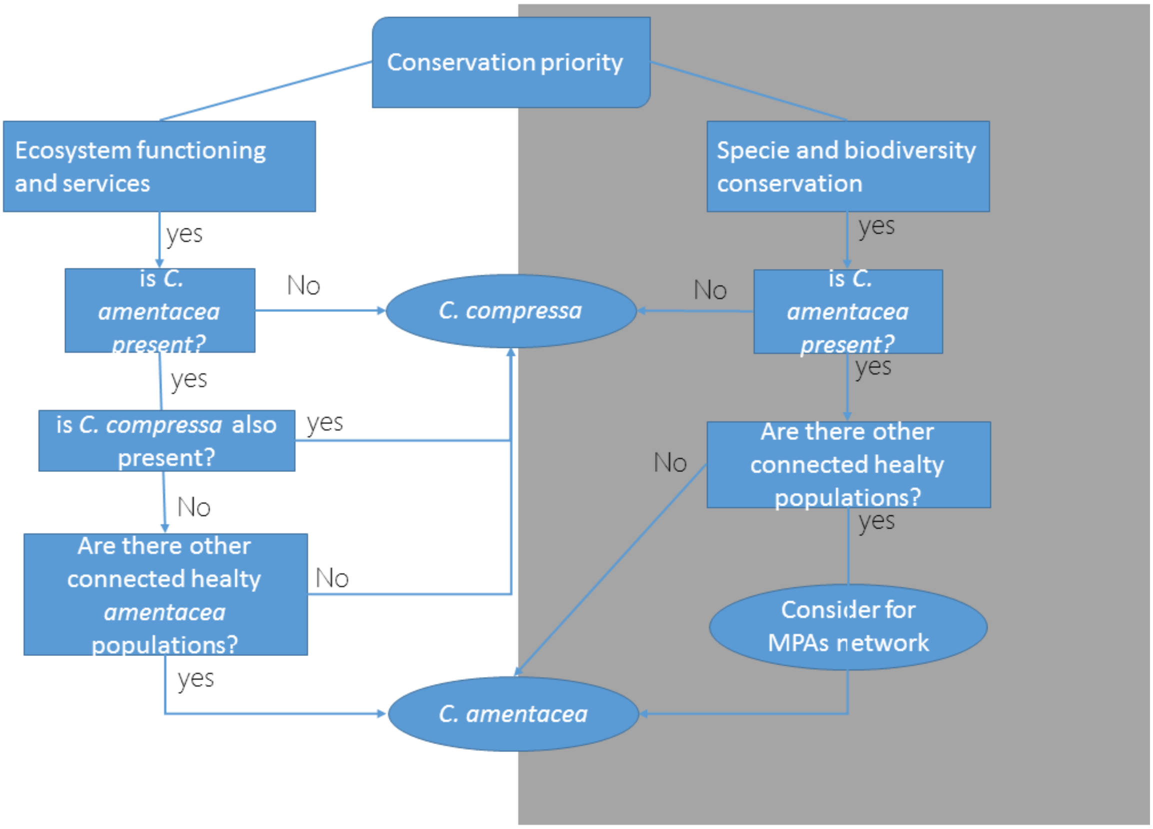


Figure 1 – Example of a reasoned choice flow chart for reforestation actions based on conservation priorities

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Roberto Buonomo

Università di Bologna
30 marzo 2017