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Kelps across Iberia: from patterns of abundance and distribution to top-down and bottom-up regulatory processes

João Nuno dos Reis Franco

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João Nuno dos Reis Franco
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Orientador
Iacopo Bertocci, Investigador, Stazione Zoologica Anton Dohrn, Itália

Coorientador
Isabel Sousa Pinto, Professor associado, Faculdade de Ciências da U.Porto

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João N. Franco, Thomas Wernberg, Iacopo Bertocci, Pedro Duarte, David Jacinto, Nuno Vasco-Rodrigues, Fernando Tuya. Herbivory drives kelp recruits into 'hiding' in a warm ocean climate. Marine Ecology Progress Series, 536:1-9 *Feauture Article*

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SUMMARY

Kelp are large brown macroalgae living mostly in shallow subtidal rocky habitats of temperate and arctic coastal regions. There, they are important foundation species due to their complex tri-dimensional structure and high productivity, which support a large diversity of associated organisms and provide a variety of ecosystem services of ecological and economic value.

Kelp forests are naturally resilient systems. Nevertheless, they are increasingly threatened by human and natural perturbations, including climate change, coastal anthropogenic development and herbivory. In general, shifts in patterns of distribution and abundance of kelp populations can be driven by a range of top-down and bottom up processes operating at multiple scales in space and time. Populations at their distributional range edges are expected to be firstly affected. Understanding patterns of distribution of kelps and their multiple drivers acting at local and global scales is crucial for predicting their responses under scenarios of current and expected environmental variation. This scientific information is essential for effective protection and management actions of these habitat formers and of the variety of associated biodiversity and ecosystem goods and services that rely on them.

This thesis aimed at assessing, by means of surveys, field and laboratory experiments conducted from 2011 to 2016, the patterns of distribution, abundance and diversity of kelp populations in the Iberian Peninsula, and understanding how abiotic and biological factors, acting separately or in complex interactions, shape their ecological and physiological responses.

In **Chapter I**, the interannual variability in the abundance and frequency of occurrence of subtidal kelp species and their major consumers was investigated across the north and west coast of Iberia during a six years field survey. Two perennial and three annual kelp species were recorded: *Laminaria hyperborea*, *Laminaria ochroleuca*, *Saccorhiza polyschides*, *Phyllariopsis brevipes* and *Phyllariopsis purpurascens*, respectively. Annual species dominated in terms of frequency (>80%), with *S. polyschides* being the most abundant and frequent (about 60%) of all kelps. Perennial species, however, were more abundant in the northern regions where *L. ochroleuca* was the most abundant kelp being sparse, or absent, in the southern regions. Consumers were more abundant in the southern, compared to the northern, regions. Herbivorous fishes, in particular, were more frequent, and about 40 times more abundant, in the southern regions, while sea-urchins were more frequent, but 6 times less abundant, in the northern regions compared to southern regions.

In **Chapter II**, the effects of herbivory on the performance of different life stages of *L. ochroleuca* sporophytes were assessed through an herbivore exclusion field experiment at central Portugal. Both the abundance and survival of all life stages increased when they were protected from herbivorous fishes and sea urchins. Concomitantly, blade linear and area growth of adult kelps displayed contrasting patterns, suggesting a compensatory growth mechanism suitable to buffer the negative impact of intense grazing.

In **Chapter III**, the effects of herbivory on the distribution and abundance of kelp recruits at two regions under contrasting ocean climate (‘cool’ vs. ‘warm’, north and central Portugal, respectively) and topography (‘open reef’ vs. ‘crevices’) were examined using a tethering field experiment. Grazing assays showed about 50 times higher rates of kelp biomass consumption, mainly by fishes, and null survival of kelp recruits in the ‘warm’ compared to the ‘cool’ region. Moreover, the abundance of kelp recruits was 3.9 times greater in the ‘cool’ region, where 85% of recruits were found in ‘open reef’ habitats. In contrast, 87 % of recruits in the ‘warm’ region were restricted to ‘crevices’.

In **Chapter IV**, using *L. ochroleuca* as a model species, a combination of mesocosm experiments and modelling (SDM: Species Distribution Model) was performed to examine the effects of crossed stressors, i.e. ocean temperatures and nutrient availability, on kelp physiological performance, to forecast the patterns of distribution of *L. ochroleuca* under climate change scenarios. Temperatures above 24.6 ºC were lethal irrespective of nutrients, while the optimal growth of juvenile sporophytes occurred between 12 °C and 18 ºC and no nutrient limitation. The SDM, where ocean temperature was the main predictor of kelp distribution consistently with temperature thresholds identified by eco-physiological responses, suggested a future expansion towards northern latitudes and a retreat from the southern limit/boundary of the current distribution of *L. ochroleuca*.

In conclusion, the overall trend of decline of kelps in the Iberian Peninsula reported by previous studies seems to be further supported by present findings. However, the documented large interannual variability in kelps’ patterns of distribution likely due to processes, or combination of processes, which were not controlled in present

surveys and experiments, calls for more prolonged monitoring and extended experimentation. These are essential to better quantify and separate natural variation and anthropogenic alterations, especially in a geographic area where both are strong, but data are relatively scarce. However, present results indicate herbivory as a key biological process regulating both the survival and performance of kelp, at least in the 'warm' region of the western coast of Iberia. Moreover, herbivory does not only affect the abundance of kelp recruits across latitude, but also their distribution at local scales, driving kelp recruits into 'hiding' in crevices under intense grazing pressure. This implies that where the recruitment success is compromised by herbivory, the persistence of kelps would depend on the availability of topographical refuges. Adding to herbivory, the expansion or retraction of *L. ochroleuca* along the European coast seems to be modulated mainly by temperature, although nutrient availability would be key to maintain optimal physiological performance. Identifying such an interactive effect was only possible by the complex study illustrated in Chapter IV, since it would have been necessarily overlooked using a single stressor experimental set – up. This emphasises the need for additional multiple - stressor studies, possibly extended to other species, to understand and predict responses of kelps to more and more realistic scenarios of complex environmental change. In this context, a future restriction of kelp populations to 'pockets' or 'islands' of suitable environmental variables, acting as refuges in the north western Iberia, is expected based on present data. The present thesis contributes to increase the current knowledge on distributional shifts and responses to perturbations of kelp species. This is crucial to design future studies specifically aimed at investigating how such changes may affect local patterns of associated biodiversity and abiotic and biological processes potentially having severe ecological and economic consequences.

RESUMO

Os kelps são algas castanhas que ocorrem normalmente habitats subtidais rochosos de pouca profundidade, nas regiões costeiras temperadas e árticas. Nestas regiões são espécies “fundadoras” importantes devido à sua complexa estrutura tridimensional e alta produtividade, uma vez que suportam uma vasta diversidade de organismos associados e providenciam diversos serviços de ecossistemas de elevado valor ecológico e económico.

As florestas de kelp são sistemas naturalmente resilientes. No entanto, estão cada vez mais ameaçadas por perturbações humanas e naturais, incluindo alterações climáticas, desenvolvimento antropogénico na zona costeira e herbivoria. Em geral, mudanças nos padrões de distribuição e abundância das populações de kelp decorrem de vários processos *top-down* e *bottom up* que operam a diferentes escalas espaciais e temporais. Estima-se que populações de kelp que ocorrem nos seus limites de distribuição sejam as primeiras a ser afetadas. A compreensão dos padrões de distribuição dos kelps e os múltiplos fatores que os influenciam, local ou globalmente, é crucial para prever as suas respostas a cenários de variação ambiental, atuais e previstos. Essas informações científicas são essenciais para uma proteção e gestão efetiva destes “construtores” de habitat e da variedade de bens e serviços de ecossistemas e biodiversidade associados que deles dependem.

Esta tese teve como objetivo avaliar, através de experiências em campo e laboratório efetuadas entre 2011 e 2016, os padrões de distribuição, abundância e diversidade das populações de kelp na Península Ibérica; e compreender como diferentes fatores abióticos e biológicos, atuando separadamente ou em interações complexas,

moldam as respostas ecológicas e fisiológicas das populações de kelp.

No **Capítulo I**, investigou-se a variabilidade interanual da abundância e frequência de ocorrência de espécies de kelp e os seus principais consumidores in situ ao longo da costa norte e oeste da Península Ibérica, durante seis anos. Foram registadas duas espécies perenes (Laminaria hyperborea e L. ochroleuca) e três espécies anuais (Saccorhiza polyschides, Phyllariopsis brevipes e Phyllariopsis purpurascens). As espécies anuais dominaram em termos de frequência (> 80%), tendo sido S. polyschides a mais abundante, com cerca de 60% da abundância total de todos os kelps nas zonas amostradas. As espécies perenes foram mais abundantes nas regiões a Norte, onde L. ochroleuca foi a mais abundante, sendo raras ou ausentes nas regiões Centro e Sul. Em geral, os consumidores de kelp foram mais abundantes no Centro e Sul, em comparação com as regiões do Norte. Em particular, os peixes herbívoros foram mais frequentes e cerca de 40 vezes mais abundantes nas regiões do Centro e Sul. Por outro lado, os ouriços-do-mar foram mais frequentes, mas 6 vezes menos abundantes nas regiões do Norte em relação às regiões do Sul.

No **Capítulo II**, avaliou-se o efeito da herbivoria na performance de diferentes fases de vida de esporófitos de L. ochroleuca, através de uma experiência de campo excluindo herbívoros. Tanto a abundância como a sobrevivência de todas as fases de vida aumentaram quando protegidas de peixes herbívoros e ouriços do mar. Simultaneamente, o crescimento (linear e área) da lâmina dos kelp adultos apresentou padrões opostos, sugerindo um mecanismo de crescimento compensatório capaz de atenuar o impacto negativo de herbivoria intensa.

No **Capítulo III**, investigou-se o efeito da herbivoria na distribuição e abundância de juvenis de kelp em duas regiões caracterizadas pelo

contraste do clima oceânico ("frio" /Norte versus "quente"/Centro) e topografia ("recife aberto" versus "fendas"), numa experiência de campo através de “tethering” (amarrar/ancorar juvenis de kelp ao substrato rochoso com um fio). Os resultados destes ensaios mostraram taxas 50 vezes maiores no consumo de biomassa de kelp, maioritariamente por peixes, e uma sobrevivência nula dos juvenis de kelp na região "quente" quando comparados com a região "fria". Além disso, a abundância de juvenis de kelp foi 3.9 vezes maior na região "fria", onde 85% dos juvenis foram encontrados em habitats de "recifes abertos". Em contraste, 87% dos juvenis na região "quente" restringiram-se a "fendas".

No **Capítulo IV**, usando L. ochroleuca como espécie modelo, realizou-se uma combinação de experiências de mesocosmos com modelação (SDM: Modelos de Distribuição de Espécies) para avaliar os efeitos cruzados de fatores de stress, i.e. temperaturas e disponibilidade de nutrientes, no desempenho fisiológico dos kelp e prever os padrões de distribuição de L. ochroleuca em diferentes cenários de alterações climáticas. As temperaturas acima de 24.6 º C foram letais, independentemente dos nutrientes. O crescimento ótimo dos juvenis ocorreu entre 12 ° C e 18 ° C e sem limitação de nutrientes. No SDM o principal preditor na distribuição dos kelp foi a temperatura do oceano, em concordância com os limites de temperatura identificados nas respostas eco-fisiológicas, sugerindo uma futura expansão desta espécie para as latitudes mais a norte e uma retração do limite/fronteira sul da atual distribuição de L. ochroleuca.

Em conclusão, os resultados obtidos nesta tese reforçam a tendência geral de declínio dos kelp na Península Ibérica, descrita em estudos anteriores. No entanto, a grande variabilidade interanual dos padrões de distribuição dos kelp observada, requer uma monitorização e experimentação mais prolongada, essenciais para melhor quantificar e separar a variação natural das alterações antropogénicas, especialmente numa área geográfica onde estas podem ser intensas, mas com escassez de dados registados. Adicionalmente, os resultados indicam a herbivoria como um processo-chave biológico regulador da sobrevivência e performance dos kelp, pelo menos na região ’quente’ da costa oeste da Península Ibérica. A herbivoria não só afeta latitudinalmente a abundância de juvenis de kelp, mas também a sua distribuição a uma escala espacial local, levando os juvenis de kelp a “esconderem-se” nas fendas sob herbivoria intensa. Isto implica que quando o sucesso do recrutamento é comprometido pela herbivoria, a persistência dos kelp depende da disponibilidade de refúgios topográficos. Além da herbivoria, a expansão ou retração na distribuição de L. ochroleuca parece ser modulada principalmente pela temperatura, embora a disponibilidade de nutrientes seja fundamental para manter um ótimo desempenho fisiológico. A identificação destes efeitos interativos foi apenas possível através dos resultados obtidos no complexo estudo realizado no Capítulo IV. A necessidade de estudos adicionais com múltiplos fatores de stress, e aplicados a outras espécies, foram enfatizados de modo a entender e prever as respostas de kelp em cenários cada vez mais realistas de alterações ambientais complexas. Neste contexto, e com base nos dados atuais, prevê-se uma futura retração das populações de kelp para ’bolsas’ ou ’ilhas’ no noroeste da Península Ibérica, que atuarão como refúgios por apresentarem condições ambientais adequadas. Esta tese contribui para aumentar o conhecimento atual dos kelp em relação a alterações na sua distribuição e as suas respostas a diferentes perturbações. Este conhecimento é essencial para dar suporte a estudos futuros orientados no sentido de investigar como estas mudanças podem afetar os padrões locais da biodiversidade associada a kelps e processos abióticos e biológicos, com potencial impacto ecológico e económico.

CONTENTS

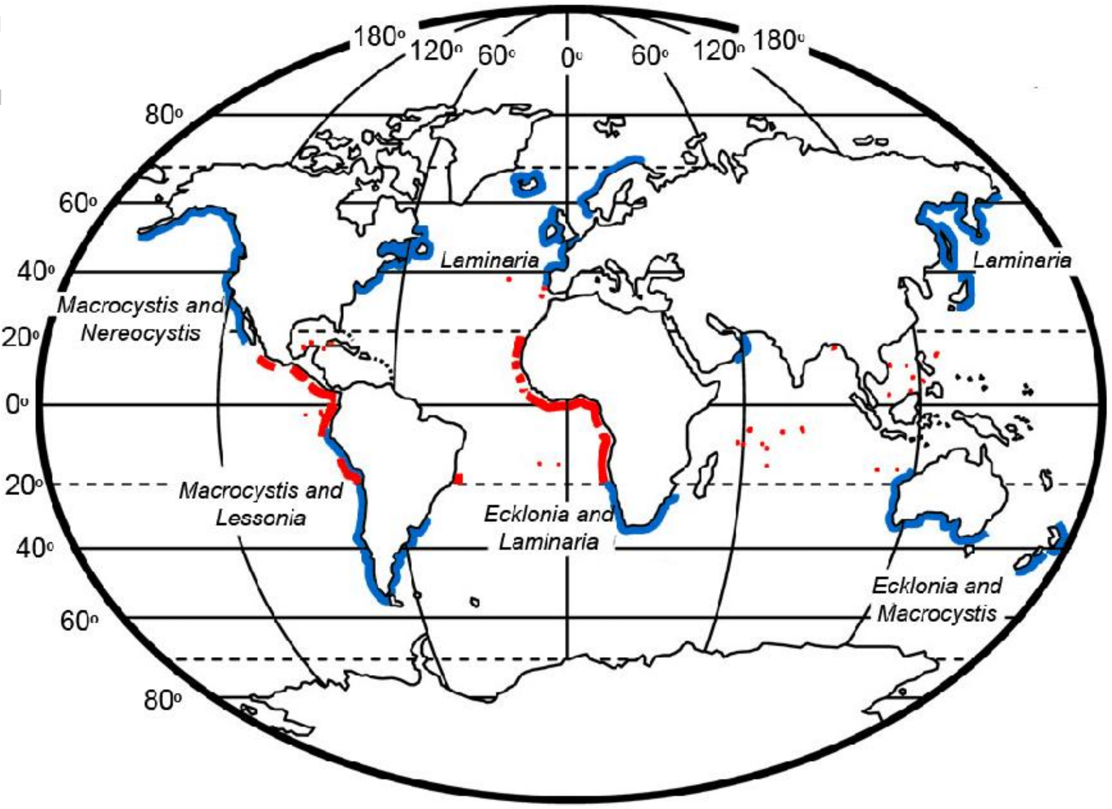
GENERAL INTRODUCTION

Figure General Introduction View of seafloor at Montanha de Camões, where *Laminaria ochroleuca* is present. This plateau is located at 22 Km West off Cabo da Roca (45 m depth). Photograph by R. Guerra.

KELPS: GLOBAL OCCURRENCE AND DISTRIBUTION IN EUROPEAN WATERS

Kelps are large brown algae typically belonging to the order Laminariales. Although kelps are technically restricted to this order, several species of large canopy-forming brown algae, such as those belonging to the order Tilopteridales (split from the order Laminariales), are often referred to as kelp. Kelps largely occur in shallow subtidal (< 30 m depth) rocky habitats in most temperate and high latitude coastal areas of the world (Dayton 1985). However, under suitable conditions, such as very clear water, some species of kelp may occur at much greater depths (60 - 200 m) in other geographic areas, including tropical regions, where they are known to form extensive deep-water forests (Graham et al. 2007). Most of kelp forests are made of five dominant genera: *Laminaria*, *Ecklonia*, *Lessonia*, *Nereocystis* and *Macrocystis* (Fig. 1). *Laminaria* is the dominant genus in the eastern and western Atlantic Ocean and the western Pacific Ocean; *Ecklonia* is prevalent in Austral Asia and South Africa; *Nereocystis* is common along the Pacific coast of North America; the giant kelp *Macrocystis* dominates along the Pacific coasts of North and South America, Southern Australia and New Zealand (Lüning 1990; Mann 2000; Guiry and Guiry 2017).

Figure 1.
Geographic distribution of kelp forests in surface (bl
and deep (red lines)
Adapted from Santel
(2007)



In most coastal areas of Europe, provided the suitable hard substratum (continuous rock, boulders, cobbles, artificial structures) and adequate water quality, one or more species of kelp may be found. There are 13, including two aliens, confirmed species of kelp in European waters (Table 1), eight of which are present in Iberian coastal waters (Araújo et al. 2003; Assis et al. 2009; Gallardo et al. 2016).

Table 1. Kelp species (order Laminariales and Tilopteridales) and their distribution in European waters (according to Lüning 1990, Guiry and Guiry 2017, and references therein).

Kelp species	Distribution
Alaria esculenta (Linnaeus) Greville	Spitzbergen; Murmansk to southern Brittany
Chorda filum (Linnaeus) Stackhouse	Spitzbergen; Novaya Zemlya to northern Spain
Laminaria digitata (Hudson) Lamouroux	Spitzbergen; Murmansk to Brittany
Laminaria hyperborea (Gunnerus) Foslie	Spitzbergen; Murmansk to northern Portugal
Laminaria japonica [a] Areschoug	Mediterranean
Laminaria ochroleuca Bachelot de la Pylaie	Bristol Channel - Morocco; Mediterranean
Laminaria rodriguezii Bornet	Mediterranean
Laminaria solidungula J. Agardh	Spitzbergen, Novaya Zemlya
Phyllariopsis brevipes (C. Agardh) Henry et South	Southern Bay of Biscay to Morocco; Mediterranean
Phyllariopsis purpurascens (C. Agardh) Henry et South	Northern Spain to Morocco; Mediterranean
Saccharina latissima (Linnaeus) C.E. Lane	Spitzbergen; Murmansk to northern Portugal
Saccorhiza dermatodea (de la Pylaie) J. Agardh	Spitzbergen; Novaya Zemlya to mid-Norway
Saccorhiza polyschides (Lightfoot) Batters	Mid-Norway to Ghana; parts of Mediterranean
Undaria pinnatifida [a] (Harvey) Suringar	Channel coasts to Portugal; Mediterranean

[a] introduced species

BACKGROUND OF KELP ECOSYSTEMS IMPORTANCE

The majority of our planet is covered by oceans with a capacity to sustain life vastly larger than that of their terrestrial counterparts. It has been estimated that aquatic plants (*sensu lato)* provide roughly half of the global primary production of organic matter (Falkowski and Raven 2007). Since salty seas occupy globally much larger areas than freshwater lakes and rivers, there is no doubt that the majority of aquatic productivity relies on primary producers of the oceans, especially from coastal ecosystems (Beer et al. 2014). Indeed, kelps are among the most representative primary producers on rocky coasts of temperate regions. Due to their high productivity and complex tri-dimensional structure, kelps are important foundation species (*sensu* Dayton 1985) (Fig. 2).

Figure 2. Overview above (A) and below (B) the canopy of a *Saccorhiza polyschides* kelp forest in the Iberian coast. Photographs by N.V. Rodrigues

The high growth rate of kelps is responsible for the high rates of primary production recorded for kelp forests, which rank as one of the most productive ecosystems on Earth (Mann 1972, 1973; Duarte et al. 2013). As such, they constitute the nutrient pool for the next ecosystem level, i.e. secondary producers, hence fuelling food webs (Duggins et al. 1989; Fredriksen 2003; Page et al. 2008). In fact, the large primary production generated within kelp systems can have impacts far beyond the relatively narrow coastal stretch they occupy.

For instance, large detached pieces of kelp commonly reach, as 'wrack', to coastal zones, where they become food for detritivores and microbes (Lucas et al. 1981). Such a process makes kelp carbon available to coastal communities of suspension feeders, as well as to herbivores and detritivores feeding directly on kelps, eventually leading to its decomposition, recycling or export out of the system (Duggins et al. 1989; Spalding et al. 2003; Bernardino et al. 2012). Concomitantly, the export of kelp material to offshore areas can benefit benthic organisms in the deep through 'kelp falls' (Bernardino et al. 2012) and pelagic organisms through dissolved organic material (Abdullah and Fredriksen 2004). Terrestrial ecosystems can also benefit as kelp washes up on beaches (Bradley and Bradley 1993; Krumhansl 2012) (Fig. 3).

Figure 3.
Sandy beaches benefit
from imported kelp wrack from the
nearby kelp forest in northern Portugal.
Photograph by J.N. Franco

Overall, carbon cycling in kelp forests is characterized by rapid biomass turnover that can be as high as 10 times per year (Mann 1972).

Kelps are known as hotspots of biodiversity, which is extraordinarily high in comparison with other algal communities. For example, in some kelp forests the invertebrate fauna in 1 m^2 can often exceed 10^5 individuals, and a single kelp individual can impressively host >100 different species and 8000 individual organisms (Christie et al. 2003). The tri-dimensional structure of kelp provides substrata and microhabitats for a broad spectrum of macro- and micro- fauna and flora by increasing habitat volume, heterogeneity and complexity, and through directly providing food and shelter (Steneck et al. 2002; Smale et al. 2013; Teagle et al. 2017). Moreover, kelp canopies serve as ecologically important nursery grounds (Holbrook et al. 1990; Tegner, MJ & Dayton 2000), and offer protection to several fishes, crustaceans and molluscs, including species of commercial and conservation importance (Steneck et al. 1993, 2002; Norderhaug et al. 2005; Smale et al. 2013; Bertocci et al. 2015). On a broader scale, kelps play a crucial role as biological engineers (Jones et al. 1994) due to their ability to alter the environment and resources available to other organisms. Specifically, kelp canopies affect light (Connell 2003a), sedimentation (Connell 2003b), physical abrasion (Irving and Connell 2006), flow dynamics (Eckman et al. 1989), space availability and nature (Christie et al. 2007), and food quantity and quality (Krumhansl and Scheibling 2011; Krumhansl 2012). In addition, kelp species themselves are harvested for a wide range of uses, such as for food, food additives, pharmaceutical and cosmetic applications, animal feed, and biofuel production (Smit 2003; Kim et al. 2017). Therefore, not surprisingly, large amounts of kelp are grown commercially in marine farms in many parts of the world, where they are harvested mainly for human and animal consumption (Gutierrez et al. 2006). Finally, kelp ecosystems have high recreational and social value by providing preferred spots for fishing or diving, as well as opportunities for education on environmental issues relating to the ocean and its resources. All together, the provisioning, regulatory, habitat, social and cultural services provided by kelp ecosystems are valued in the range of billions of dollars annually (Vásquez et al. 2013; Smale et al. 2013; Bennett et al. 2016).

CLIMATIC, NON-CLIMATIC AND BIOLOGICAL FORCES IN KELP ECOSYSTEMS

There is general scientific consensus that climate is rapidly changing. Although this is of increasing concern since marine ecosystems are centrally important for the biology of the planet, a comprehensive understanding of how they respond to anthropogenic climate change is still poorly developed (Hoegh-Guldberg and Bruno 2010). However, modifications of ocean temperature, biogeochemistry, salinity, sea level, UV radiation and circulation patterns have all been detected within the last few decades and are expected to continue (IPCC 2007). Increases in extreme meteorological events is also expected, including intensification and rise in the frequency, intensity and temporal variance of severe storms (e.g. Wolff et al. 2016) and heat waves (Wernberg et al. 2016). Moreover, the rising of sea temperature is accompanied by changes in patterns and strength of winds and ocean currents, upwelling and associated nutrient supply, ocean stratification, sea level and rainfall dynamics. All such physical and chemical perturbations interplay to potentially impact marine organisms, including kelps, at any level of biological organization, with direct and indirect effects which can become apparent as changes in physiology (e.g. growth and reproduction rates), abundance, distribution, phenology, ontogeny and trophic interactions (Harley et al. 2006; Brierley and Kingsford 2009; Wernberg et al. 2011; Gao et al. 2012; Richardson et al. 2012).

Kelps, analogously to other primary producers, are critically controlled by the physical environment, which determines how well plants can survive in a particular area. For example, temperature is one of the most important factors controlling the geographic distribution of seaweeds, so that their tolerance to high (summer maxima) and low (winter minima) temperatures generally define their biogeographical boundaries (Lüning 1984; Adey and Steneck 2001). Once these basic requirements are met, the availability of resources (e.g., light and nutrients) dictates how fast plants grow. In the absence of disturbance, these factors determine the standing biomass of actively growing vegetation, and ultimately the amount of material fixed by photosynthesis that becomes available to higher trophic levels (Duggins et al. 1989). In parallel with this bottom-up flow of primary production driven by the abiotic environment, top-down consumptive forces operate to alter producer biomass (Terborgh and Estes 2010). Primary producers are consumed by grazers, which in turn are fed upon by their predators. When such trophic interactions are strong, the balance between predators and grazers can effectively determine the amount and type of plant biomass and resulting levels of primary production (Pace et al. 1999). However, the balance between bottom-up and top-down regulatory processes is modulated by various forms of physical and/or chemical stressors and/or direct anthropogenic disturbances, such as overfishing (Tegner et al. 1996) or declining of water quality (Matsunaga et al. 1999). These factors can contribute to alter the biomass of producers and consumers to varying degrees depending on the type of disturbance and the structure of the community prior to its occurrence (White and Jentsch 2001), ultimately making kelp forest ecosystems globally and locally dependent on complex interactions of biotic, climatic and non-climatic factors.

Kelp ecosystems are naturally highly variable on temporal (seasonal and interannual) and spatial scales (Gagné et al. 1982; Cavanaugh et al. 2011; Reed et al. 2011). Different processes have been indicated as responsible for such variation, including (i) environmental and biological drivers, such as currents, temperature, substrate, depth, nutrient availability, swell intensity, size of kelp patches, grazing and structure of assemblages within a given system (Dayton 1985; Dayton et al. 1992); and (ii) variation in the ability of kelp to resist to (Ghedini et al. 2015) or recover from small- and large-scale disturbances (Edwards 2004; Scheibling et al. 2013). In general, recent evidence suggests that the capacity of kelp forests to recover from disturbance may be eroding (Wernberg et al. 2010; Ling et al. 2014). Drastic declines of kelp forests up to their replacement with completely different systems have been documented in many regions in response to a range of climatic, non-climatic and biological stressors (Steneck et al. 2002; Connell et al. 2008; Johnson et al. 2011; Bennett et al. 2015; Filbee-Dexter et al. 2016; Wernberg et al. 2016) (Fig. 4).

Figure 4. Canopy-forming algae characterise most temperate coasts (e.g. *L. ochroleuca* kelp forests pictured on the top), but some forests have been replaced by extensive covers of turf-forming algae (e.g. Peniche coast). Photographs by T. Wernberg and J.N. Franco

STUDY REGION: IBERIAN PENINSULA

The Iberian Peninsula is included in the marine ecoregion of South European Atlantic Shelf within the Lusitanian province (Spalding et al. 2007). This classification is based on relative homogeneity in terms of main ecosystems, organisms and environment. However, regional studies have demonstrated local discrepancies at all these levels, especially along the northern and western coasts of the Iberian Atlantic Peninsula. For example, differences in mean seawater temperature are observed along this region, decreasing from south to north and increasing from west to east (Lemos and Pires 2004; Gómez-Gesteira et al. 2008). In parallel, different macroalgal assemblages and/or individual species typically associated with temperature gradients can be observed from north to south along the western coast (Lima et al. 2007; Assis et al. 2009, 2016; Tuya et al. 2012) and from west to east in northern Iberia (Fernandez 2011; Borja et al. 2013; Voerman et al. 2013; Piñeiro-Corbeira et al. 2016; Assis et al. 2017). A notable case among these is the introduced kelp *Undaria pinnatifida*, which is found in many parts of the Iberian coast. Adding to such documented variation in abiotic and biological characteristics, further climate-related changes, such as increase of water temperature, upwelling relaxation, increase of wave heights and storminess (Lemos and Pires 2004; Borja et al. 2013; Sydeman et al. 2014), are described and predicted for the near future in this geographic area, with expected direct and/or indirect influences on ecologically relevant macroalgae and associated biodiversity.

The present PhD project was carried out at five regions of the Atlantic Iberian Peninsula (Fig. 5). Observational surveys and manipulative experiments were performed across all regions or within a subset of them depending on the specific hypotheses under examination.

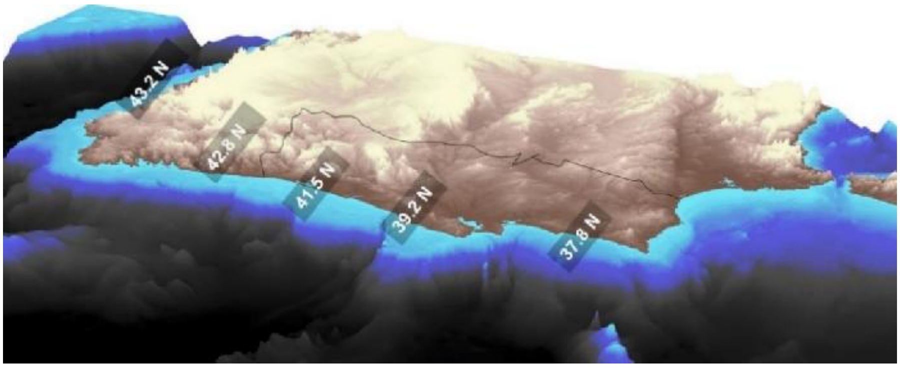


Figure 5.
Partial view of the Atlantic Iberian Peninsula showing the five study regions of the present PhD Project

GENERAL AIM AND THESIS STRUCTURE

As previously illustrated, ongoing and future global environmental changes may play a key role in shaping biogeographic patterns, primary productivity and biological relationships of ecologically and economically important kelp species and associated habitats. Assessing and predicting such modifications is a main topic of current ecological research, to which the present work aimed at contributing by focusing on kelp systems occurring in the Iberian Peninsula. A lack of information in this area about kelp forests is generally acknowledged. In fact, despite the unequivocal importance of Iberian kelps at all levels, only a few empirical and quantitative studies have addressed their spatial and temporal variation and clarified the mechanisms underlying their dynamics and possible decline. In order to fill these gaps, a range of descriptive and manipulative approaches were adopted, including the collection and analysis of data on kelps and their main consumers (fishes and sea urchins) from surveys carried out across the Iberian Peninsula over six years to assess their interannual variation. Such data were complemented with *in situ* experiments aimed at exploring the effects of herbivory on kelp performance at varying life stages. Mesocosm experiments combined with modelling aimed at assessing kelp physiological limits and forecasting potential range shifts in their distribution under varying climate change scenarios. Specifically, the present thesis is framed around four chapters; the main objective of each is:

Chapter 1: To document the interannual variability of kelps and their consumers in the Iberian Peninsula (manuscript to be submitted).

Chapter 2: To investigate how herbivory affects the growth and survival of different life stages of kelp (paper accepted in Marine Biology).

Chapter 3: To investigate how herbivory affect kelp recruits in regions under contrasting ocean climate and their small-scale distribution (paper published in Marine Ecology Progress Series 536: 1-9).

Chapter 4: To assess the physiological responses of kelp (*Laminaria ochroleuca*) to varying temperature and nutrients and, through the combination with Species Distribution Models, to forecast its possible distributional range shifts under varying climate change scenarios (paper in press in Journal of Ecology. doi:10.1111/1365-2745.12810).

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CHAPTER I

INTERANNUAL VARIABILITY OF KELPS AND THEIR CONSUMERS IN IBERIA

Figure Chapter I. Scientific diver folding up the belt transect after counting kelp individuals at Peniche. Photograph by N.V. Rodrigues

João N. Franco[1,2], Thomas Wernberg[3], Iacopo Bertocci[1,4], David Jacinto[5], Jesus Troncoso[6], Brezo Martinez[7], Nuno Vasco Rodrigues[8], Francisco Arenas[1], Isabel Sousa Pinto[1,2], Fernando Tuya [9]

[1] CIIMAR/CIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal.

[2] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4150-181 Porto, Portugal

[3] School of Plant Biology & UWA Oceans Institute (M470), University of Western Australia, Crawley WA 6009, Australia

[4] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy

[5] MARE - Marine and Environmental Sciences Centre, Laboratório de Ciências do Mar, Universidade de Évora, apartado 190, 7520-903 Sines, Portugal

[6] ECIMAT, Station of Marine Sciences of Toralla, Department of Ecology and Animal Biology, University of Vigo, Spain

[7] Rey Juan Carlos University, Calle Tulipán sn., 28933 Móstoles, Madrid, Spain

[8] MARE –Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria, Leiria, Portugal

[9] IU-ECOAQUA, Grupo en Biodivesidad y Conservación, Marine Sciences Faculty, Universidad de Las Palmas de Gran Canaria, 35017, Las Palmas, Canary Islands, Spain

ABSTRACT

Kelps are foundation species in many near-shore temperate areas, where there is wide evidence that their patterns of abundance are modulated by climatic and non-climatic drivers. We analysed, between 2011 and 2016, the interannual variability of occurrence and abundance of kelp species and their major consumers (fishes and sea urchins), as well as interannual patterns of ocean climate (seawater temperature and chl a), at five regions along a seven degrees' latitudinal gradient encompassing the north and west coast of Iberian Peninsula. Sea surface temperature showed a significant correlation with latitude (mean variation of 0.5 - 2ºC), while chl *a* oscillated irregularly among the studied regions. Two perennial and three annual kelp species were recorded: *Laminaria hyperborea*, *L. ochroleuca*, *Saccorhiza polyschides*, *Phyllariopsis brevipes* and *Phyllariopsis purpurascens*, respectively. Kelp spatial and temporal variation were species-dependent, but annual species dominated in terms of frequency of occurrence (>80%). *Saccorhiza polyschides* was the most abundant species, representing ca. 60% of total abundance of kelps. Perennial species were relatively more abundant in the northern regions and sparse, or absent, in the southern regions. *Laminaria ochroleuca* was (ca. 2 times) the most abundant perennial species. Consumers were more abundant in the southern, compared to the northern, regions. Herbivorous fishes were more frequent in the southern regions and about 40 times more abundant there compared to the northern regions. Conversely, the frequency of occurrence of sea-urchins in the northern regions was lager compared to herbivorous fishes, but in the southern regions sea urchins were 6 times more abundant. The large variability of abundance of these habitat-formers reinforce the need of adequate monitoring programs to properly assess the apparently decline of kelps particularly in regions where is recognized the lack of data, such as in the Iberian Peninsula subtidal coast.

KEYWORDS

abundance; Atlantic Ocean; fishes; grazers; Iberian Peninsula; Laminariales; latitude; macroecology; rocky reefs; urchins

INTRODUCTION

Kelps are large brown macroalgae occurring along most cold-temperate rocky marine coasts and have a widely recognized ecological importance as foundation species, providing food and shelter for many organisms (reviewed by Wiencke and Bischof 2012; Ólafsson 2015; Schiel and Foster 2015). Kelp systems are highly dynamic and their spatial and temporal patterns are generally driven by the biology of the main constituent species (Dayton et al. 1992). As other primary producers, abiotic variables such as temperature and/or nutrients (Bartsch et al. 2008), and biological interactions such as herbivory (Poore et al. 2012), play a crucial rolein shaping population dynamics by affecting the physical structure, reproduction and productivity of kelps.

A total of 13 kelp species are described for European coastal waters. These belong to the orders Laminariales and Tilopteridales and show patterns of distribution typically related to their specific temperature tolerance (Lüning 1990). Along the Atlantic coast of southern Europe, seven kelp species are recorded (Bárbara et al. 2005; Assis et al. 2009), including the introduced *Undaria pinnatifida*. These species are morphologically distinct and show different growth strategies, i.e. annual (e.g. *Saccorhiza polyschides*, *Phyllariopsis brevipes*) and perennial (e.g. *Laminaria ochroleuca*, *Laminaria hyperborea*). In general, annuals are normally characterized by rapid nutrient uptake, fast growth and low nutrient storage capabilities, which make them being often considered as opportunistic species (Littler and Arnold 1980). On the contrary, perennials tend to grow slowly and to store large quantities of nutrients that can provide a sort of reserve during periods of nutrient shortage (Carpenter 1990). In the Iberian Peninsula, both types of kelp co-occur; interannual variability is expected depending on the species and the surrounding habitat. Indeed, the causes for shifts in the abundance and distribution of kelps in Europe are reported as being species-dependent and regionally variable (Araújo et al. 2016; Krumhansl et al. 2016). It is recognized, however, that the general paucity of quantitative data from many European coasts, especially in subtidal rocky reef habitats, may prevent detecting crucial ecological changes at relevant spatial scales (Smale et al. 2013). This is particularly evident for the southern Atlantic European coasts (Araújo et al. 2016). Nevertheless, recent studies have shown a declining trend of kelps in different regions along the Iberian Peninsula associated with seawater temperature increase (Fernández 2011, 2016; Assis et al. 2013; Voerman et al. 2013) and high herbivory (Franco et al. 2015). The present study was aimed at describing inter-annual patterns in the abundance of kelp species and their major consumers, i.e. herbivorous reef fishes and sea urchins, at five regions along a 7° latitudinal gradient in the northern and western coast of the Iberian Peninsula.

MATERIALS AND METHODS

STUDY REGIONS

This study was conducted at five regions in the north-western part of the Iberian Peninsula, from Galicia in Spain to the Portuguese Atlantic coast along the southern European coastline (Fig.1). The regions were Sines (SIN), Peniche (PEN), Viana do Castelo (VIA), Vigo (VIG) and Coruña (COR), each including a stretch of coast between 10 and 20 km long. The Portuguese Atlantic coastline is rectilinearly oriented from north to south, with a similar overall exposure to dominant NW and W swells among regions. The shore is characterized by extensive sandy beaches interspersed with limestone, sandstone, shale or granitic reefs in both the intertidal and the shallow subtidal zone (Tuya et al. 2012). The Galician coastline is extremely rugged with open coast sections interrupted by sheltered “rías” i.e. coastal inlets formed by a partial submergence of an unglaciated river valley, and an alternation of hard substrata and sandy beaches (Bárbara et al. 2005; Piñeiro-Corbeira et al. 2016). The annual sampling in the Portuguese and the Galician regions started in 2011 and 2014, respectively, and finished at all regions in 2016.

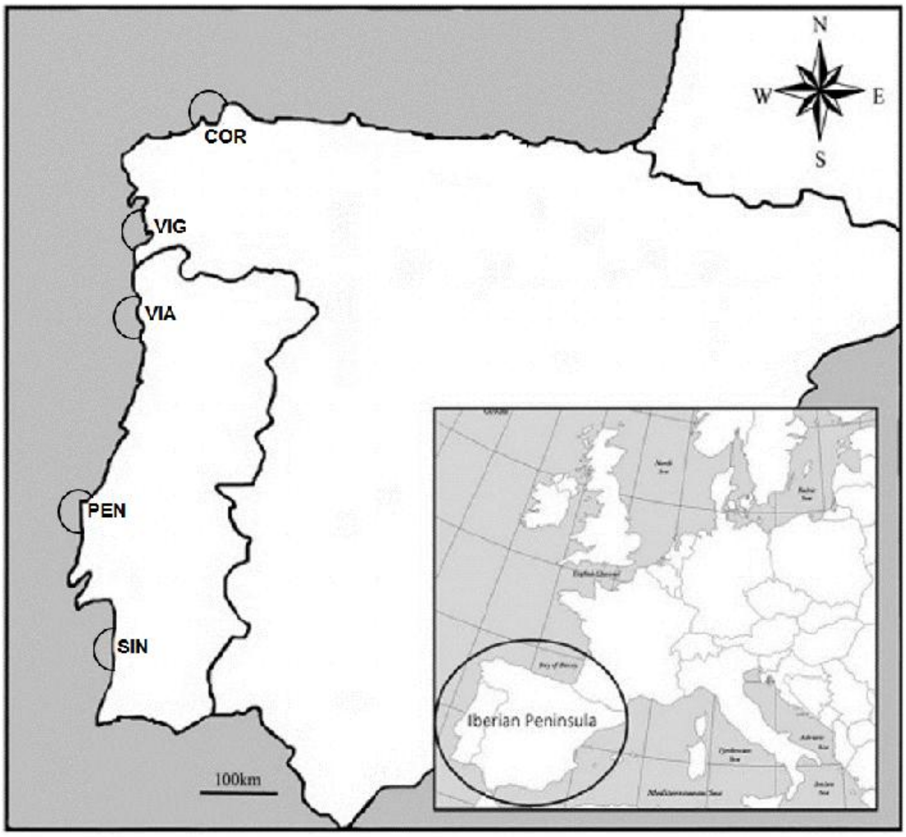


Figure 1.
Map of Iberian Peninsula showing the location of the 5 study regions: COR = Coruña (43.2° N), VIG = Vigo (42.8° N), VIA = Viana do Castelo (41.5° N), PEN = Peniche (39.2° N) and SIN = Sines (37.8° N).

COLLECTION OF DATA ON OCEAN CLIMATE

Sea surface temperature (SST) and chlorophyll (chl a) concentration were extracted, throughout 2011 to 2016, from monthly Level-3 Standard Mapped Image files generated by the [MODIS-Aqua] sensor available from NASA Earth observation satellites through the Interactive Online Visualization and Analysis Infrastructure (http://giovanni.gsfc.nasa.gov/) (Acker and Leptoukh 2007). Data were extracted from pixels that encompassed all reefs within each study region from monthly images produced from January of 2011 to December 2016.

COLLECTION OF DATA ON KELP ABUNDANCE

Within each region, five rocky reefs, 1 to 5 km apart, at 6 to 11 m depth, were selected randomly. All sampling was done annually during summer months (June -July). At each reef within each region, the abundance of kelp species was estimated along five 25 × 2 m haphazardly located belt transects. All individuals of each kelp species were counted along each transect by SCUBA divers. Densities of each kelp species were expressed as the number of individuals per 50 m^2. Pooled annual and perennial kelp species were expressed as frequency of occurrence over the total abundance of kelps from each region per year.

COLLECTION OF DATA ON GRAZERS ABUNDANCE

At the same reefs, the number of individuals of different herbivorous fishes and sea urchins was assessed, respectively along five 25 × 4 m and five 25 × 2 m haphazardly located belt transects. Fishes were categorized according to their trophic affinities (Henriques et al. 2013; www.fishbase.org). Herbivorous fishes were considered as those able to consume algae, thus also including omnivorous species (Franco et al. 2015). Densities of urchins was expressed as the number of individuals per 50 m^2 and herbivorous fishes as the number of individuals per 100 m^2 and as frequency of occurrence over the total abundance of grazers from each region at each sampled year.

STATISTICAL ANALYSES

Pearson correlation (r) was used to describe the relationship between the mean values of oceanographic variables per region (SST and chl a) and latitude. Differences in the abundance of each kelp species, herbivorous fishes and sea urchins through the years and among regions were tested by 3-way ANOVA. Separate analyses were performed for the three Portuguese regions (SIN, PEN and VIA) sampled during the period 2011-2016 (SP1) and for all the five regions (SIN, PEN, VIA, VIG and COR) sampled during the period 2014-2016 (SP2). Therefore, the first ANOVA included the factors: Year (5 levels, random), Region (3 levels, fixed, crossed with Year), Reef (5 levels, random and nested within both Year and Region), with five transects in each reef providing the replicates. The second ANOVA bas based on the same model, but with Year and Region characterized by three and five levels, respectively. Before each ANOVA, the assumption of homogeneity of variances was checked through Cochran's *C* test. Only the abundance of the herbivorous fishes and that of urchins in SP1 had to be ln(x+1)-transformed to achieve homogeneous variances. When relevant, post-hoc comparisons were carried out using pairwise Student-Newman-Keuls (SNK) tests (Underwood 1997).

RESULTS

OCEAN CLIMATE CONDITIONS

The five regions showed a year-round latitudinal gradient in mean SST of ~ 0.5 to 2°C (Fig. 1A, $r = 0.99$, $n = 5$, $p < 0.001$), but chl *a* did not show a significant correlation with latitude (Fig. 1B, $r = 0.48$, $p < 0.001$).

INTERANNUAL PATTERNS OF KELP ABUNDANCE

Five kelp species were identified during the study, including two perennial (*Laminaria hyperborea* and *Laminaria ochroleuca*) and three annual (*Saccorhiza polyschides*, *Phyllariopsis purpurascens* and *Phyllariopsis brevipes*) species. However, the last two species were pooled together as *Phyllariopsis* spp. due to the impossibility of consistently discriminating between them underwater. Significant inter-annual and/or inter-regional variability of each kelp species was documented in the two periods (Figs. 2, 3 and Table 1).

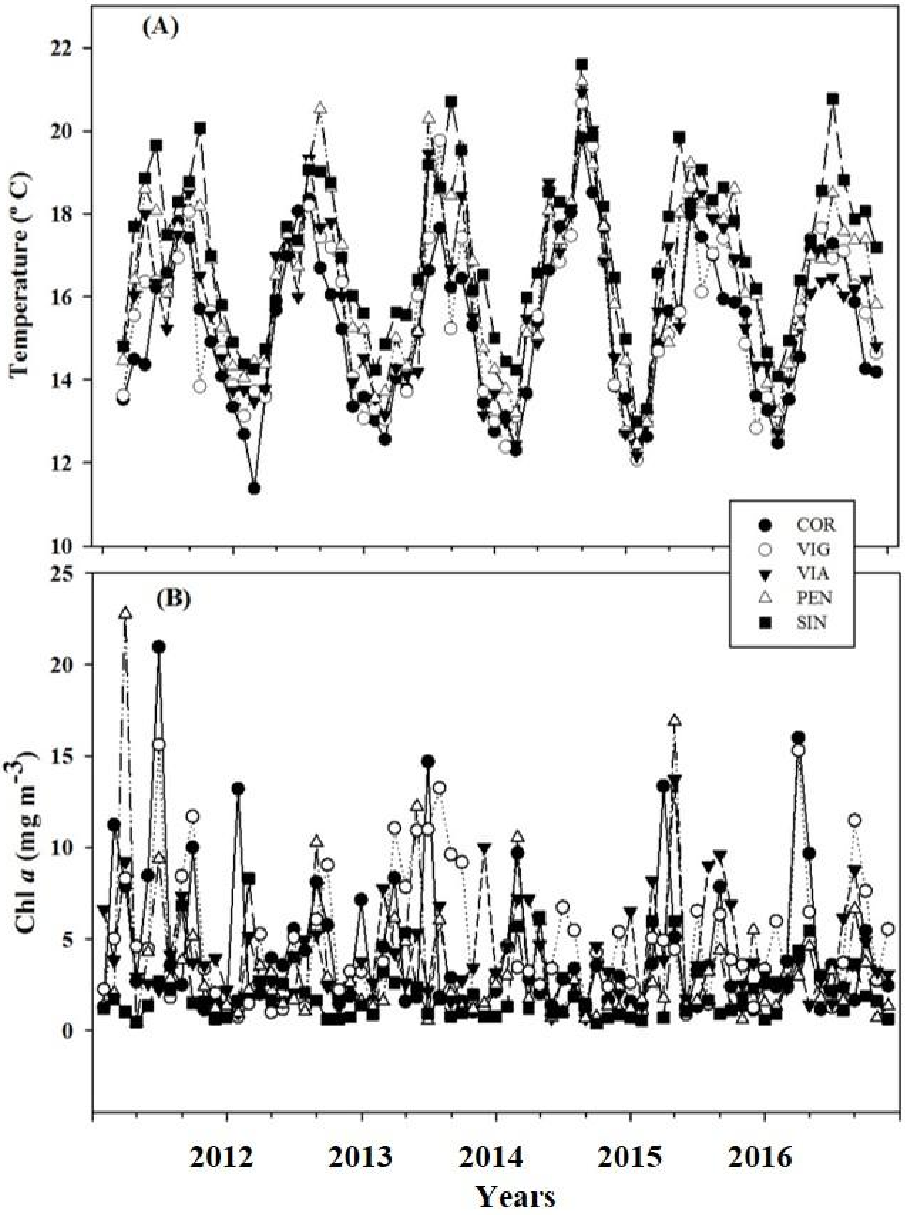
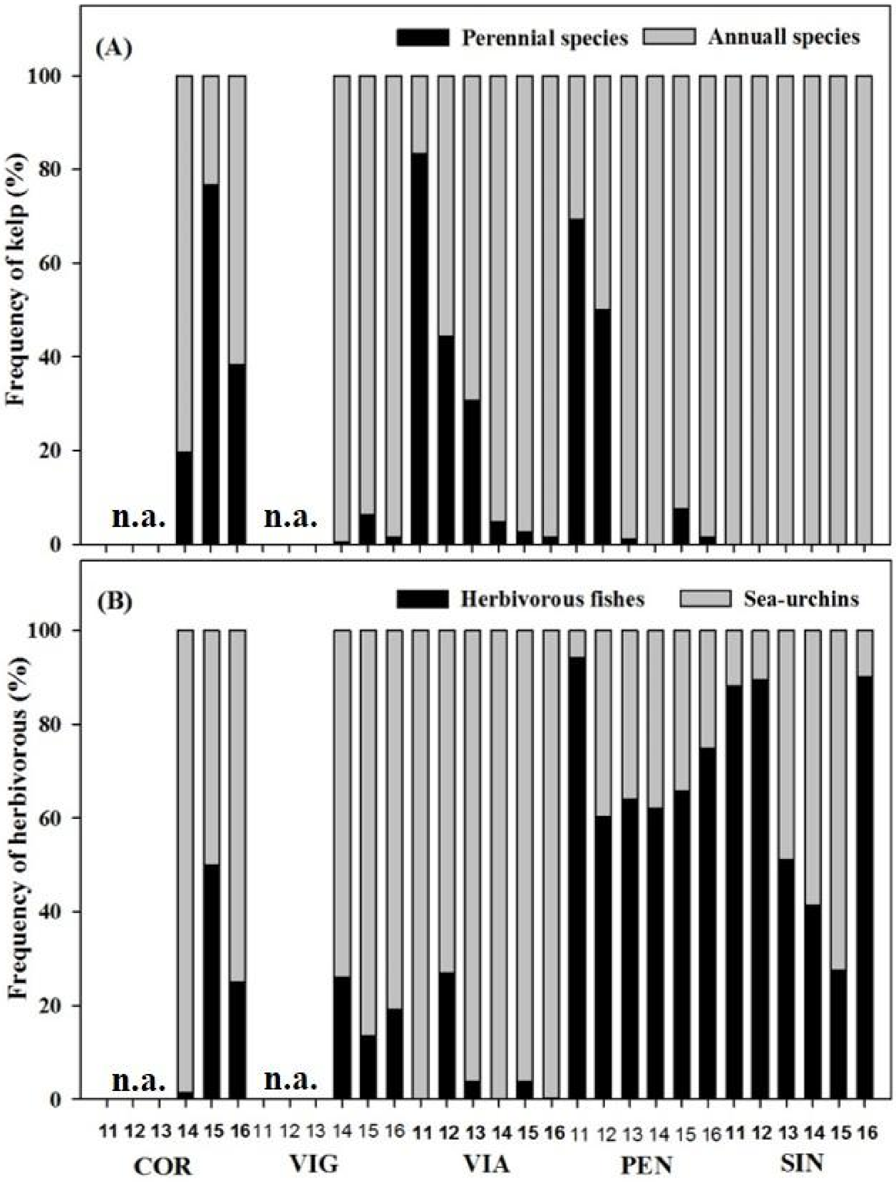


Figure 1.
Ocean climate patterns at 5 study regions along a latitudinal gradient in NW of Iberian Peninsula: (A) sea surface temperature (SST, n = 70 monthly values); (B) surface chl a concentration (n = 70 monthly values). COR=Coruña, VIG=Vigo, VIA=Viana do Castelo, PEN=Peniche and SIN=Sines.

Figure 2.
Frequency of occurrence of kelp species pooled as perennial and annual over the total abundance of kelps (A) and herbivores (B) for each of the 5 study region through sampled years (n.a. = non available data). COR=Coruña, VIG=Vigo, VIA=Viana do Castelo, PEN=Peniche and SIN=Sines.



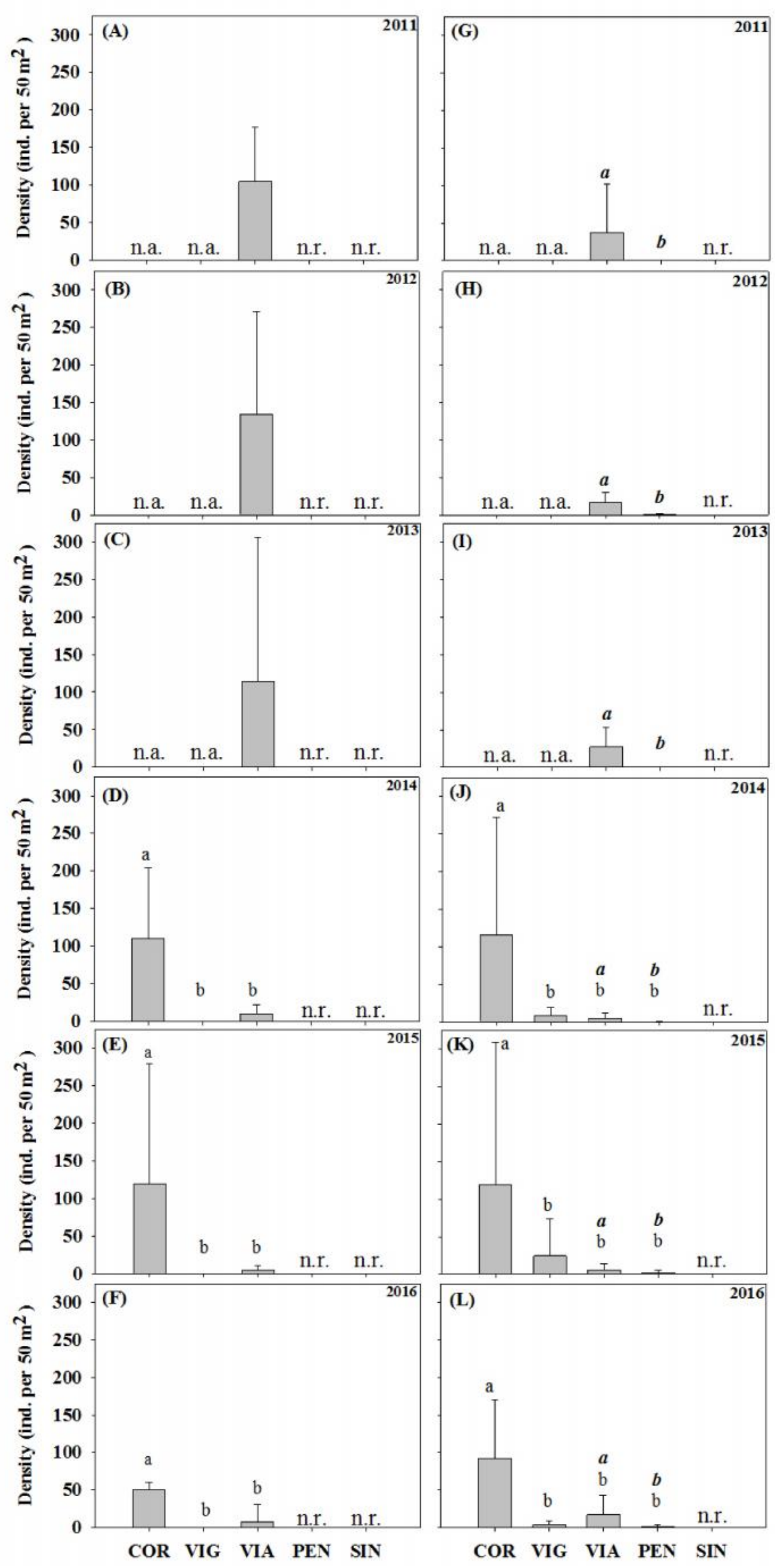


Figure 3. Abundance of *Laminaria hyperborea* (A-F) and *L. ochroleuca* (G-L) at the five regions through time (mean + SE, n = 5). n.a. = non available data, n.r.= no records. Different letters above bars represent significant differences of the means between regions (*italic* and regular font for SP1: 3 regions: 2011-2016 and SP2: 5 regions: 2014-2016)

Table 1. Results of ANOVA testing for differences in the abundance of kelp species at three regions (Sines = SIN, Peniche = PEN and Viana do Castelo =VIA) during the period 2011-2016 (SP1), and at five regions (SIN, PEN, VIA, Vigo = VIG and Coruña = COR) during the period 2014-2016 (SP2). Significant effects are reported in bold.

	(SP1) 3 regions: 2011-2016				**(SP2) 5 regions: 2014-2016**			
Source	DF	MS	*F*	P	DF	MS	*F*	P
Laminaria hyperborea								
Year	5	31294.39	2.23	**0.060**	2	21574.47	2.02	0.141
Region	2	193566.42	6.19	**0.017**	4	85677.25	3.93	**0.047**
Reef (Year × Region)	72	14025.59	9.57	**0**	60	10665.86	13.68	**0**
Year × Region	10	31294.39	2.23	**0.025**	8	21789.70	2.04	**0.056**
Residual	360	1465.73			300	779.39		
SNK tests	All years: VIA>PEN= SIN				All years: COR > VIG=VIA=PEN=SIN			
Laminaria ochroleuca								
Year	5	1244.99	0.8	0.549	2	1782.87	0.12	0.888
Region	2	14029.88	8.53	**0.006**	4	162570.34	76.62	**0**
Reef (Year × Region)	72	1546.85	15.79	**0**	60	14996.26	5.33	**0**
Year × Region	10	1645.59	1.06	0.401	8	2121.73	0.14	0.996
Residual	360	97.97			300	2812.59		
SNK tests	All years: VIA>PEN=SIN				All years: COR > VIG=VIA=PEN=SIN			
Sacchoriza polyschides								
Year	5	1067350.87	7.61	**0**	2	373323.61	3.24	**0.046**
Region	2	4377663.79	4.94	**0.032**	4	579818.63	1.3	0.346
Reef (Year × Region)	72	140271.77	4.76	**0**	60	115398.48	21.12	**0**
Year × Region	10	886820.72	6.32	**0**	8	444556.68	3.85	**0.001**
Residual	360	29491.94			300	5463.77		
SNK tests	2011, 2012, 2015: VIA=PEN=SIN 2013, 2014, 2016: VIA>PEN=SIN				2014: COR= VIG> VIA, PEN, SIN 2015:COR=VIG=VIA=PEN=SIN 2016: VIA>COR=VIG=PEN=SIN			
***Phyllariopsis* spp.**								
Year	5	945842.69	5.92	**0.0001**	2	856859.12	4.44	**0.0159**
Region	2	262385.66	0.5	0.6193	4	652558.97	0.98	0.4702
Reef (Year × Region)	72	159674.82	8.85	**0**	60	193031.94	8.84	**0**
Year × Region	10	521854.66	3.27	**0.0016**	8	666585.28	3.45	**0.0025**
Residual	360	18047.69			300	21840.96		
SNK tests	2011, 2012, 2013, 2014, 2015: VIA=PEN=SIN 2016: VIA>PEN=SIN				2011, 2012, 2013, 2016: VIA > COR=VIG=PEN=SIN 2014, 2015: COR=VIG=VIA=PEN=SIN			

Overall, the annual species dominated in frequency of occurrence (>80%) over the perennial species (Fig. 2A). *Laminaria hyperborea* was found only at COR and VIA in both sampling periods. In SP1, at VIA, this species showed a significant decrease of 16% from 2011-2013 to 2014-2016 (Table1, P<0.05); in SP2, *L. hyperborea* was 11 times more abundant in COR compared to VIA (Fig. 3 and Table 1). *Laminaria ochroleuca* was always absent in the southernmost region (SIN) and showed different patterns of abundance at the other regions in both sampling periods. In SP1, in particular, *L. ochroleuca* was 13 times more abundant in VIA compared to PEN, while in SP2 COR had the largest abundance of this species compared to VIG, VIA and PEN (9, 14 and 47 times less, respectively) (Fig. 3 and Table 1). *Saccorhiza polyschides* was always absent in the southernmost region (SIN), while at PEN it was only recorded since 2013 onwards. This species also showed large annual and regional variability in both sampling periods (Fig. 4 and Table 1), though representing, in general, the most abundant kelp during both sampling periods (51 and 68% of total kelp abundance, respectively). *Phyllariopsis* spp. also showed a large annual and regional variability during both sampling periods (Fig. 4 and Table 1), but it was the kelp with the most widespread spatial distribution, being the only one observed at all regions. This was the second most abundant kelp in both SP1 and SP2 sampling periods (37 and 22% of total kelp abundance, respectively).

INTERANNUAL PATTERNS OF GRAZERS ABUNDANCE

Among the total 33 fish species recorded during the study, only 6 were considered as grazers: *Sarpa salpa*, *Diplodus sargus*, *D. cervinus*, *D. vulgaris*, *Oblada melanura* and *Spondyliosoma cantharus*. In SP1, the southernmost regions (PEN and SIN) showed an overall 50 times higher abundance compared to VIA. The same pattern was confirmed in SP2, where the southernmost regions showed 32 times higher abundance compared to all three northernmost regions (VIA, VIG and COR) (Fig. 5, Table 2). Overall, the frequency of occurrence of herbivorous fishes was more than 65% higher than that of sea-urchins in PEN and SIN, while this pattern was reversed in the three northernmost regions, where the frequency of occurrence of sea-urchins was higher (>80%, Fig 2B). *Paracentrotus lividus* was the dominant sea-urchin species at all regions and was always more abundant in the two southernmost regions compared to VIA (where it was ~4 times less abundant) during SP1, and to VIA, VIG and COR (where it was ~7.5 less abundant) during SP2 (Fig. 5, Table 2).

Table 2. Results of ANOVA testing for differences in the abundance of kelp consumers at three regions during the period 2011-2016 (SP1), and at five regions during the period 2014-2016 (SP2). Symbols and abbreviations as in Table 1.

	(SP1) 3 regions: 2011-2016				**(SP2) 5 regions: 2014-2016**			
Source	DF	MS	F	P	DF	MS	F	P
Herbivorous fishes								
Year	5	14.16	8.09	**0**	2	548.13	5.23	**0.008**
Region	2	186.88	17.47	**0.005**	4	1873.50	5.73	**0.017**
Reef (Year × Region)	72	1.75	3.09	**0**	60	104.89	3.12	**0**
Year × Region	10	10.69	6.11	**0**	8	327.19	3.12	**0.005**
Residual	360	0.56			300	33.62		
SNK tests	2011, 2013: PEN>SIN>VIA 2012, 2016: SIN>PEN>VIA 2014, 2015: PEN=SIN>VIA				All years: SIN=PEN>VIA=VIG=COR			
Sea urchins								
Year	5	58.88	10.88	**0**	2	4989.82	2.81	0.068
Region	2	33.76	2.9	**0.011**	4	14741.26	11.09	**0.002**
Reef (Year × Region)	72	5.41	12.3	**0**	60	1775.17	4.7	**0**
Year × Region	10	11.64	2.15	**0.030**	8	1329.27	0.75	0.648
Residual	360	0.44			300	377.88		
SNK tests	All years: PEN=SIN>VIA				All years: SIN=PEN>VIA=VIG=COR			

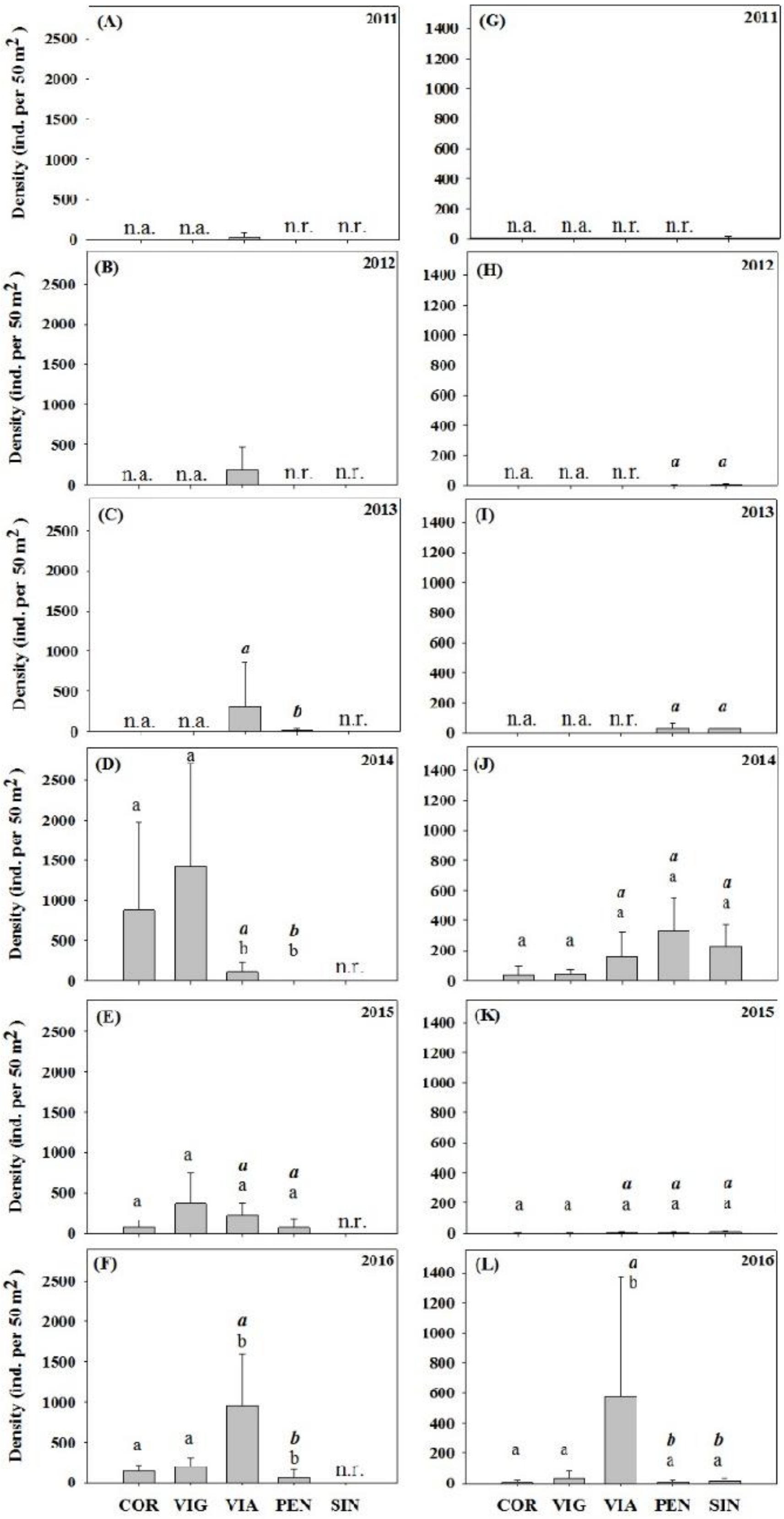
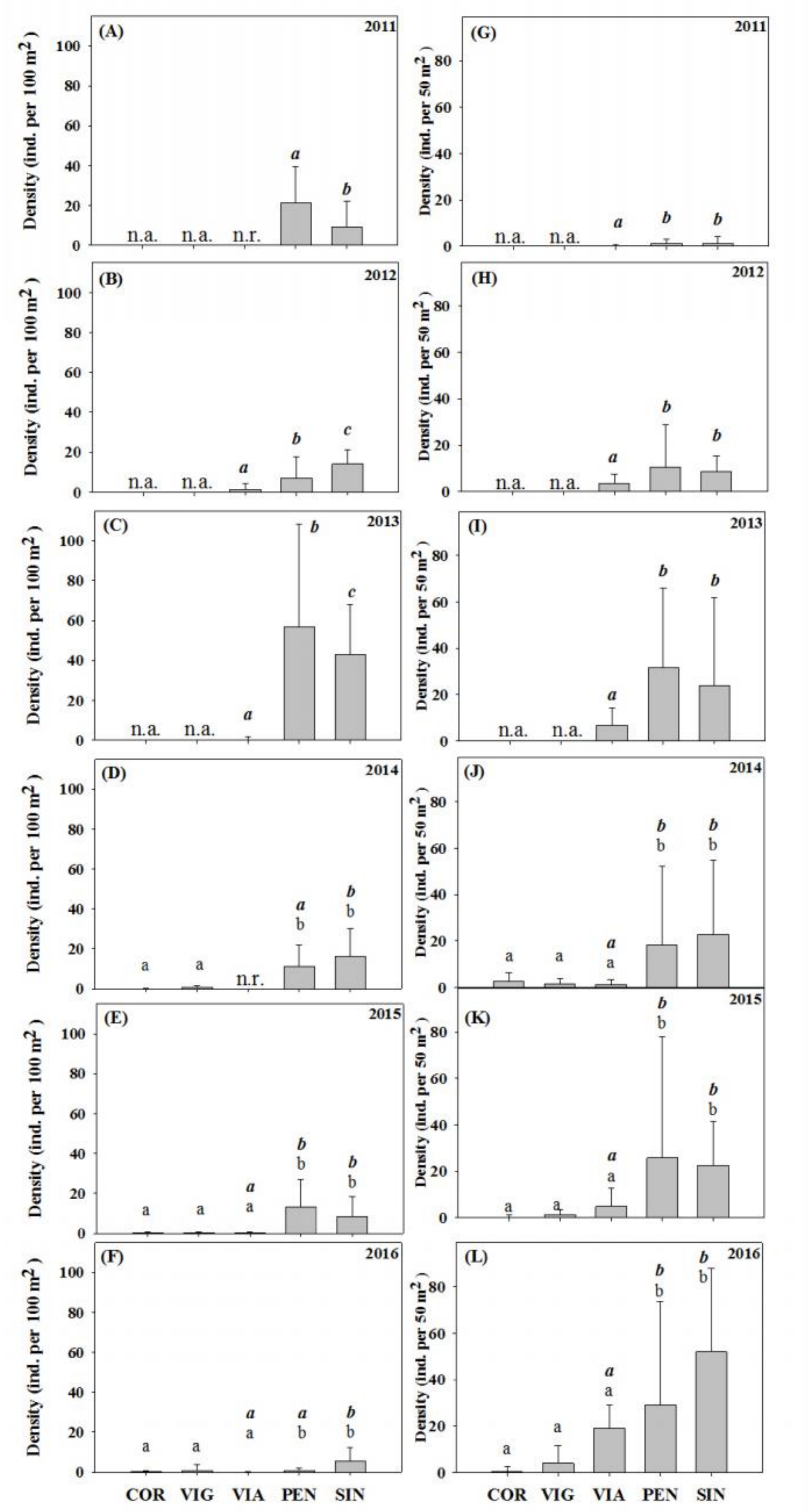


Figure 4. Abundance of *Saccorhiza polyschides* (A-F) and *Phyllariopsis* spp. (G-L) at the five regions through time (mean + SE, n = 5). n.a. = non available data, n.r.= no records. Different letters above bars represent significant differences of the means between regions (*italic* and regular font for SP1: 3 regions: 2011-2016 and SP2: 5 regions: 2014-2016)

Figure 5.
Abundance of herbivorous fishes (A-F) and sea urchins (G-L) during the sampling period at the different regions (mean + SE, n = 5). n.a. = non available data. Different letters above bars represent significant differences of the means between regions (italic and regular font for SP1: 3 regions: 2011-2016 and SP2: 5 regions: 2014-2016)



DISCUSSION

This study was aimed at filling some gaps in the current knowledge on patterns of distribution, abundance and diversity of kelp populations and their major consumers in southern Europe, which prevent an accurate and effective assessment of the status of such important habitat-formers (Smale et al. 2013; Araújo et al. 2016). To our knowledge this is the first study of the patterns of abundance of the most representative Iberian kelp species, at subtidal rocky reefs, covering a relatively large spatial scale of 7º of latitude.

Two perennial species, namely *L. hyperborea* and *L. ochroleuca*, and three annual species, namely *S. polyschides*, *P. brevipes* and *P. purpurancens*, were found during this study. Although not sampled in present surveys, two more kelp species are described for the same geographic area. These are *Saccharina latissima* and the introduced *Undaria pinnatifida*, although relatively less representative and with very discrete distributions (Bárbara et al. 2005; Assis et al. 2009). Present findings indicate that *L. hyperborea*, a kelp with cold-water affinity, suffered a considerable reduction of abundance in northern Portugal in the last three years (2014-2016) and, concurrently retracted its southern distribution limit from central to northern Portugal (http:\\ macoi.ci.uc.pt - Portuguese Seaweeds Website, Lima et al. 2007; Assis et al. 2009). On the contrary, kelps with a warm-water affinity, such as *Laminaria ochroleuca* and *S. polyschides*, showed larger abundances at the three northernmost regions. *Phyllariopsis* spp. were present at all regions, with reduced abundances in the two northernmost regions compared to the others. Overall, throughout the entire study, it was also evident a trend of dominance of annual species at all regions, while perennial species were essentially present in the northernmost regions only.

The retraction of kelp species from southern Europe is usually associated to abiotic factors, such as an increase in seawater temperature. Such an environmental change, in particular, has been invoked as a main driver of the retraction of *L. ochroleuca* and *Saccorhiza polyschides* in southern Portugal (Franco et al. in press; Tuya et al. 2012; Assis et al. 2013) and of *L. ochroleuca*, *L. hyperborea* and *S. polyschides* eastern of Coruña towards the Gulf of Biscay (Fernández 2011, 2016). Nevertheless, species distributions are not always linear with latitude and distribution ‘pockets’ or ‘islands’ can occur irrespectively of the overall putative latitude-related limits due to particular combinations of habitat conditions (Helmuth et al. 2002).

Present results also reported a larger presence or grazers, especially fishes, in the two southernmost regions (Peniche and Sines), suggesting the local potential impact of biological interactions (herbivory) on kelp populations. This hypothesis is reinforced by recent studies which have shown detrimental effects of consumers, mainly fishes, on kelp in southern Europe. For example, Franco et al. (2015) reported that kelps from warmer, southern, locations along the Portuguese coast are exposed to more intense grazing pressure than kelps from colder, northern, locations. Moreover, despite the general lack of previous quantitative data allowing robust comparisons and assessments of actual shifts of kelp distribution in southern Europe, it is widely recognized by common people and scientific communities that kelps are declining there (Assis et al. 2009). As reported elsewhere, such declines are likely to lead to drastic changes of the diversity and structure of whole kelp-associated communities (Graham 2004; Wernberg et al. 2016).

In conclusion, this study represents an important step towards an accurate and effective assessment of trends of kelp populations in a geographic area where kelps have a crucial ecological, social and economic importance. Present data are not only relevant for the monitoring purpose of determining whether and to what extent kelps and their supported assemblages are changing in space and time, but also to contribute to identify their most likely drivers, including natural and anthropogenic factors and processes. Future monitoring programs should be supported by scientists and the government, and specifically designed studies are recommended to correctly track and examine the simultaneous and possibly interactive role of multiple factors in causing changes of patterns of distribution of kelps and of the diverse organisms and services which on them rely in the Iberian Peninsula.

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CHAPTER II

MODULATION OF DIFFERENT KELP LIFE STAGES BY HERBIVORY: COMPENSATORY GROWTH VERSUS POPULATION DECIMATION

Figure Chapter II. Kelp indiduals growing in natural environment at Peniche. Kelps were previouls seeded in artificial discs at laboratory (microscopic stage). Photograph by J.N. Franco

João N. Franco[1,2], Thomas Wernberg[3], Iacopo Bertocci[1,4], David Jacinto[5], Paulo Maranhão[6], Tânia Pereira[1], Brezo Martinez[7], Francisco Arenas[1], Isabel Sousa-Pinto[1,2], Fernando Tuya[8]

[1] CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal.

[2] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4150-181 Porto, Portugal

[3] School of Biological Sciences & UWA Oceans Institute (M470), University of Western Australia, Crawley WA 6009, Australia

[4] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy

[5] MARE - Marine and Environmental Sciences Centre, Laboratório de Ciências do Mar, Universidade de Évora, apartado 190, 7520-903 Sines, Portugal

[6] MARE - Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria, 2520-641 Peniche, Portugal

[7] Rey Juan Carlos University, Calle Tulipán sn., 28933 Móstoles, Madrid, Spain

[8] IU-ECOAQUA, Grupo en Biodivesidad y Conservación, Marine Sciences Faculty, Universidad de Las Palmas de Gran Canaria, 35017, Las Palmas, Canary Islands, Spain

ABSTRACT

Partitioning the effects of herbivory on different life stages of primary producers is key to understanding the population-wide consequences of herbivory. We assessed the performance of microscopic (MiS<1 mm) juveniles, macroscopic (MaS) juveniles and adult kelp (*Laminaria ochroleuca*) under contrasting herbivory regimes through a herbivore exclusion field experiment. The abundance of MiS and the survival of MaS decreased by 67% and 63%, respectively, when herbivorous fishes and sea urchins were present. Blade growth (linear and area) of adult kelp displayed contrasting patterns under herbivore pressure: a 60% increase and a 46% decrease, respectively. These results indicate that while herbivory severely reduces juvenile survival, it may also induce compensatory growth (measured as linear growth) in adult kelp. In summary, we here demonstrate how herbivory affects all sporophyte life stages of the kelp *L. ochroleuca*. This is likely to have important implications for situations where historical patterns of herbivore presence and herbivory are changing, such as is increasingly the case in many temperate regions due to warming around the world.

KEYWORDS

Atlantic Ocean; climate; exclusion; grazing; kelp forests; *Laminaria ochroleuca;* Portugal; plant-herbivore interaction; range limit; southern Europe

INTRODUCTION

Plant-herbivore interactions play a critical role in the dynamics of populations and assemblages across different habitats (Burkepile 2013) and productivity gradients (Proulx and Mazumder 1998). Green food webs, where herbivore–plant interactions play a significant role in energy transfer, are ubiquitous (e.g. Gaines & Lubchenco 1982, Duffy & Hay 2000, Moles et al. 2011) and the strength of such interactions shapes the patterns of distribution and abundance of many species involved (Maron & Crone 2006). Consequently, changes in the direction, frequency, and intensity of herbivory can ultimately lead to substantial shifts in the structure of whole ecosystems (Pace et al. 1999; Bruno and O'Connor 2005; Vergés et al. 2014; Hanley and La Pierre 2015). This is of particular concern since ongoing global pressures, including climate change and over-fishing, may impair biotic interactions through direct effects on both primary producers (e.g. reducing net productivity and/or diversity) and herbivores (e.g. changing abundance, behavior and/or diversity).

In producer-based food webs, understanding plant-herbivore interactions is a challenging requirement for addressing a number of relevant questions, from predicting ecological dynamics to managing ecosystems under natural and anthropogenic pressures. In this context, it is important to understand the impact of herbivory over the full lifecycle of marine plants (Russell et al. 2012), which can be exposed to different levels of herbivory as they develop trough different life stages. As a result, the impact of herbivores on plant resistance traits can vary with plant ontogeny, promoting changes in the amount and type of resistance traits during the development of the plant to minimize the impacts of herbivory (Boege and Marquis 2005). These changes can also be shaped by demographic priorities such as establishment, growth, or reproduction of the individuals (Boege and Marquis 2005). Intraspecific variation in resistance to herbivory, including the different life stages of primary producers, should be tackled as an important element of plant–herbivore interactions. However, the vast majority of studies currently focus on effects on a single species and at a single ontogenetic stage; very few studies examine effects on multiple life stages of habitat-forming species, leading to population-level extrapolations that might be misleading or incomplete (Boege and Marquis 2005; Vergés et al. 2008).

Kelp are foundation species (Dayton 1972) in many near-shore temperate areas, where there is wide evidence that their patterns of abundance, distribution and functioning may be critically shaped by herbivores (Franco et al. 2015; Vergés et al. 2016; Zarco-Perello et al. 2017). Kelp possess natural defences against herbivores through a range of mechanisms, e.g. physiological adaptation, physical and associative traits, chemical defence and compensatory growth (Targett and Coen 1992; Gagnon et al. 2003; Cerda et al. 2009;

Biskup et al. 2014). However, ‘extreme’ changes in herbivory levels may compromise the resilience of whole communities supported by these seaweeds (Steneck et al. 2002; Bennett et al. 2015; Vergés et al. 2016). Notable examples of this include the effects of sea-urchins which, through their intense grazing activity, may lead to the eradication of erect algae and their replacement with ‘barren’ areas (Ling et al. 2010; Filbee-Dexter and Scheibling 2014). Negative effects of herbivory on kelp are also reported for tropical fishes currently expanding into temperate regions (Vergés et al. 2014, 2016; Bennett et al. 2015; Zarco-Perello et al. 2017).

Declines in abundance and induced herbivore- and/or temperature-driven phase shifts of kelp at regional or local scales have been extensively documented (Ling et al. 2014; Filbee-Dexter et al. 2016; Wernberg et al. 2016). In Europe, the causes for changes in kelp abundance are generally reported as being species-dependent and regionally variable (Araújo et al. 2016). For example, declines in northern and central Europe have mainly been attributed to human overharvesting of kelp and/or overgrazing by sea urchins (Sivertsen 2006; Raybaud et al. 2013). In southern Europe, increasing ocean temperature is a likely driver of declining kelp forests (Fernandez 2011; Voerman et al. 2013; Assis et al. 2016). In this context, Franco et al. (2015) recently reported that kelp from warmer, southern, locations along the Portuguese coast are exposed to more intense herbivory pressure than kelp from colder, northern, locations, suggesting indirect effects of temperature through herbivory could also play a key role in southern Europe.

The Portuguese coast spans more than 800 kilometres and is recognized as an important transitional zone between north-eastern Atlantic warm-temperate and cold-temperate species, which makes this coast an area of great sensitivity to the effects of climate change (Tuya et al. 2012; Teixeira et al. 2014). A large number of cold- and warm-water species have their southern, or northern, distributional range edges along the west coast of the Iberian Peninsula. These include the cool-water kelp *Laminaria hyperborea* and *Saccharina latissima* (Lima et al. 2007). The number of records of fish and algal species with sub-tropical affinities extending northwards, relative to their usual distributional range, or increasing in abundance, have become more frequent in the last decade (Lima et al. 2007; Bañón and Mucientes 2009; Rodrigues et al. 2012; Piñeiro-Corbeira et al. 2016).

The present study aimed at examining the influence of herbivory on the 'golden kelp', *Laminaria ochroleuca*, in central Portugal. This warm-water species is currently expanding its distributional range northwards, locally competing with native *L. hyperborea* (Smale et al. 2015). We excluded macro-herbivores (fishes and sea urchins) from experimental plots and, subsequently, estimated the abundance, survival and growth of *L. ochroleuca* compared to control plots under herbivory. The responses of *L. ochroleuca* were examined for different development stages, including the abundance of microscopic (<1 mm) juveniles, the survival of macroscopic (between 3.5 and 4.5 cm, lamina length) juveniles and the growth of adult (> 25 cm, lamina length) individuals. This allowed partitioning the effects of herbivory on three life stages of a kelp. Specifically, we have tested the hypothesis that herbivory would exert a negative impact on L. ochroleuca at different life stages, but that such an effect could be, at least in part, counteracted by compensatory growth of adult individuals. Moreover, the experiment was replicated at each of two randomly selected sites (~ 6 km apart, at 5-7 m depth) representative of the region and characterized by similar rocky substrate and topography. This allowed to test whether the examined responses to experimental treatments were generalizable across the region, a relevant issue given the likely between-sites variation in uncontrolled potentially relevant factors such as the abundance of herbivores in the system (Franco et al. 2015).

MATERIALS AND METHODS

STUDY REGION AND ABIOTIC CHARACTERIZATION

The study was carried out at Peniche, central Portugal (39.2º N, 9.2º W). This is considered a warm-temperate region along the Portuguese western coast (Tuya et al. 2012), with recent evidence of intense herbivore pressure on kelp (Franco et al. 2015). Macroalgal assemblages in the study region include perennial kelp species, which were once abundant and now show a sparse distribution, such as L. ochroleuca, or annual species, such as *Phyllariopsis sp*. (Assis et al. 2009; Tuya et al. 2012; Pinho et al. 2015). For abiotic characterization of the study area a set of data loggers were deployed throughout the course of the experiment to measure sea water temperature (ºC) and illuminance (Lux) (HOBO data-loggers Pendant Temp-Light, Onset Computer Corporation), and water movement (m s^{-2}) using gravitational data loggers (HOBO Pendant G, Onset Computer Corporation), following (Figurski et al. 2011). The loggers were set to record the temperature and illuminance at 15 min intervals, and acceleration at 6 min intervals. For light, loggers were cleaned on a weekly basis to avoid fouling that would interfere with measurements. Mean levels of illuminance between 08:00 to 17:00 (n =1,878 measurements) were extracted. For water movement (WM), all values (n=26,160 measurements) were extracted. Water samples were collected, at six occasions (two randomly dates within every 30 days), water samples (n = 4), approximately 1 m above the bottom, and immediately filtered and stored on ice until return to land. Samples were then frozen (−20°C) until chemical analysis for NO3 and PO4, which was performed through a colorimetric auto-analyzer (Skalar® SAN Plus Segmented Flow Analyser).

MACRO-HERBIVORES ABUNDANCE IN STUDY REGION

Macro-herbivore abundances were visually assessed in July 2014 and July 2015 at five rocky reefs chosen at random within the study region. On each reef, all adult and sub-adult fishes were counted along five, randomly oriented, 25 x 4 m belt transects (Tuya et al. 2012). Fishes were categorized according to their trophic affinities (according to Henriques et al. 2013; fishbase.org). Herbivorous fishes were considered as those able to consume algae, thus also including omnivorous species (Franco et al. 2015). The number of *Paracentrotus lividus*, the most common sea-urchin in continental Portugal (Jacinto et al. 2013), was counted along five 25 x 2 m transects.

EXPERIMENTAL DESIGN

A caging experiment was set up to assess the effect of the exclusion of macro-herbivores on the survival and growth of *L. ochroleuca* at two sites within the study region. At each site, steel cages (50 x 50 x 50 cm) were attached to the bottom through stainless steel eyebolts (Fig. 1). At each site, six cages were randomly allocated to each of three experimental conditions, for a total of 36 cages: exclusion of macro-herbivores (full cage: F), procedural control (partial cage with a roof and two half-sides, thus allowing access to herbivores: P), and unmanipulated control (50 x 50 cm plots just marked at corners with eyebolts: C). The cages (plots) were covered by polyethylene net (2 cm mesh size). Cages were randomly assigned to plots to ensure any small-scale effects of illuminance and water movement did not confound herbivore exclusion. Moreover, both illuminance (HOBO data-loggers Pendant Temp-Light, Onset Computer Corporation) and water movement (HOBO Pendant G, Onset Computer Corporation) were compared between a randomly chosen experimental replicate allocated to the F, P and C treatments. Measurements of illuminance and water movement across all treatments showed no cage artefacts (Fig. S1a, S1b, S2a, S2b, methodological details and data in supplementary material).

SET-UP AND SAMPLING OF *L. OCHROLEUCA* ABUNDANCE, SURVIVAL AND GROWTH

Three different life stages of *L. ochroleuca* were placed in each experimental plot (n=6, Fig. 1): (a) two adult individuals (total length >25cm), fastened by the holdfast to a 16 x 4 cm PVC tile using a rubber-protected cable tie that allowed the natural movement of each kelp; (b) four epoxy surface discs (total disk area = 2460 mm2) screwed to a PVC tile with microscopic (< 1 mm) sporophytes (MiS) (440 ± 50, mean ± SE, n=144 discs); (c) 10 macroscopic (lamina length between 3.5 and 4.5 cm) juvenile sporophytes (MaS) distributed on a 15 cm long cotton rope attached to a PVC tile.

All adult individuals were collected at Mindelo (41.1°N, 8.74º W) and individually tagged with a numbered cable tie around the stipe. The blade area of each individual was measured, before the start of the experiment, by taking a picture over a blank scaled surface where the blade was stretched by an acrylic transparent board. The same procedure was repeated at the end of the experiment. Using Image J (Muth 2012), the contrast analyses (before vs. after) of kelp laminas provided, for each individual, the growth during the course of the experiment. The linear growth of adult L. ochroleuca was estimated using the hole-punch method (Parke 1948). At the deployment day, two holes were punched in the centre of the lamina, at 2 and 4 cm above the main meristem at the junction between the stipe and the main blade. Thallus extension was assessed in situ every month after deployment (July, August and September 2014) by measuring the distance between the two holes and the meristem and subtracting the initial 4 cm distance. The total linear growth was calculated as the sum of all months.

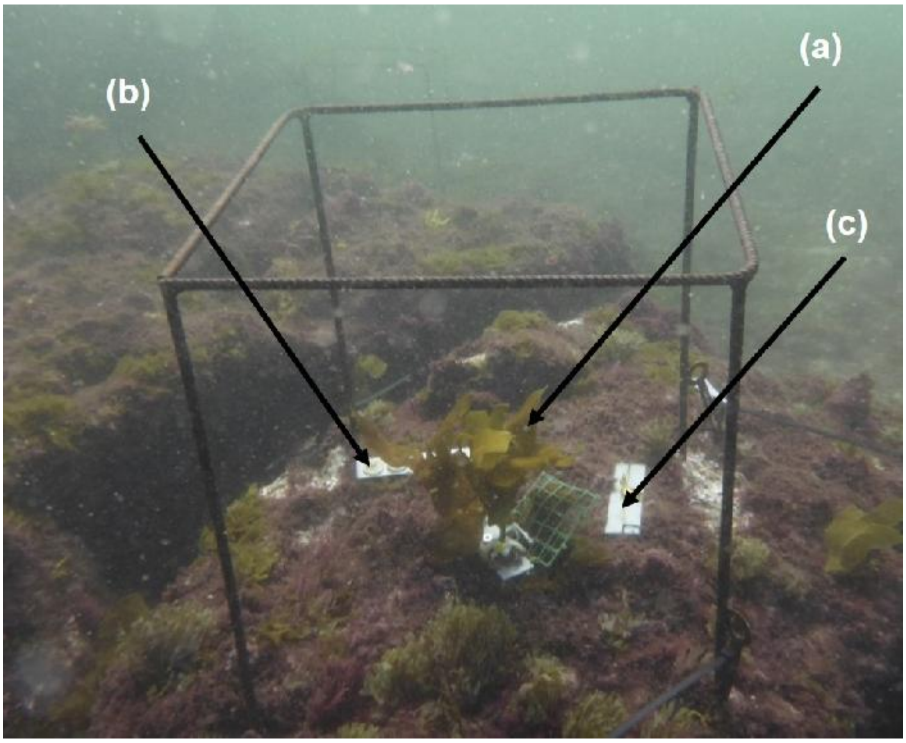


Figure 1.
(a) Adults, (b) Microscopic (MiS) juveniles and (c) macroscopic (MaS) juveniles of *Laminaria ochroleuca* within the cage before closure to exclude herbivores.
Photograph by J. N. Franco

Four weeks before the experiment started, gametophytes of *L. ochroleuca* from adults collected at Mindelo were cultivated in the laboratory (adapted from guidelines described in Pereira et al. 2011). The gametophyte solution resulting from the cultivation was then left in contact with sterile epoxy discs to allow them to become homogenized, fix to the disk and develop into MiS. The individuals growing on the seeded discs were counted in situ at the end of the experiment. It is worth noting that an observed reduction in the number of MiS between the beginning and the end of the experiment could have been due, in principle, by other (than herbivory) factors, including for instance environmental conditions, characterizing the experimental units. However, our experimental setting was such as the most obvious, potentially relevant uncontrolled factors (e.g. water movement, light) were likely to analogously affect all experimental units. Therefore, the relative effects of treatments ascribed to herbivory could be quantified in an unconfounded way. The same procedure was performed to obtain MaS, but cultivation started earlier to allow the growth until the desired size; a cotton rope was used instead of epoxy discs. The number of living individuals were counted in situ at the end of the experiment. All the biological material was kept in an aerated 500 L outdoor tank at 15 ºC until deployment. The experiment ran from 14 June to 30 September 2014, including weekly visits for cleaning, maintaining and opening cages when needed for measurements.

STATISTICAL ANALYSES

The abundance of herbivorous fishes and the sea-urchin *P. lividus* was analyzed by 2-way ANOVA, including: Year (2 levels, random) and Reef (5 levels, random and nested in years).The total linear growth, the total blade area growth, the abundance of MiS and the survival of MaS, at the end of the experiment, were analysed by 2-way ANOVA, including the crossed factors ‘Site’ (2 levels, random) and ‘Herbivore exclusion’ (3 levels: F, P and C, fixed). The same model was used to analyse differences in the linear growth of L. ochroleuca separately for each of three sampling times. Before each ANOVA, the assumption of homogeneity of variances was checked with Cochran’s C test. The percentage of survival of MaS was Arcsin transformed to achieve homogeneous variances. When relevant, post-hoc comparisons of significant means were carried out with Student-Newman-Keuls (SNK) tests.

RESULTS

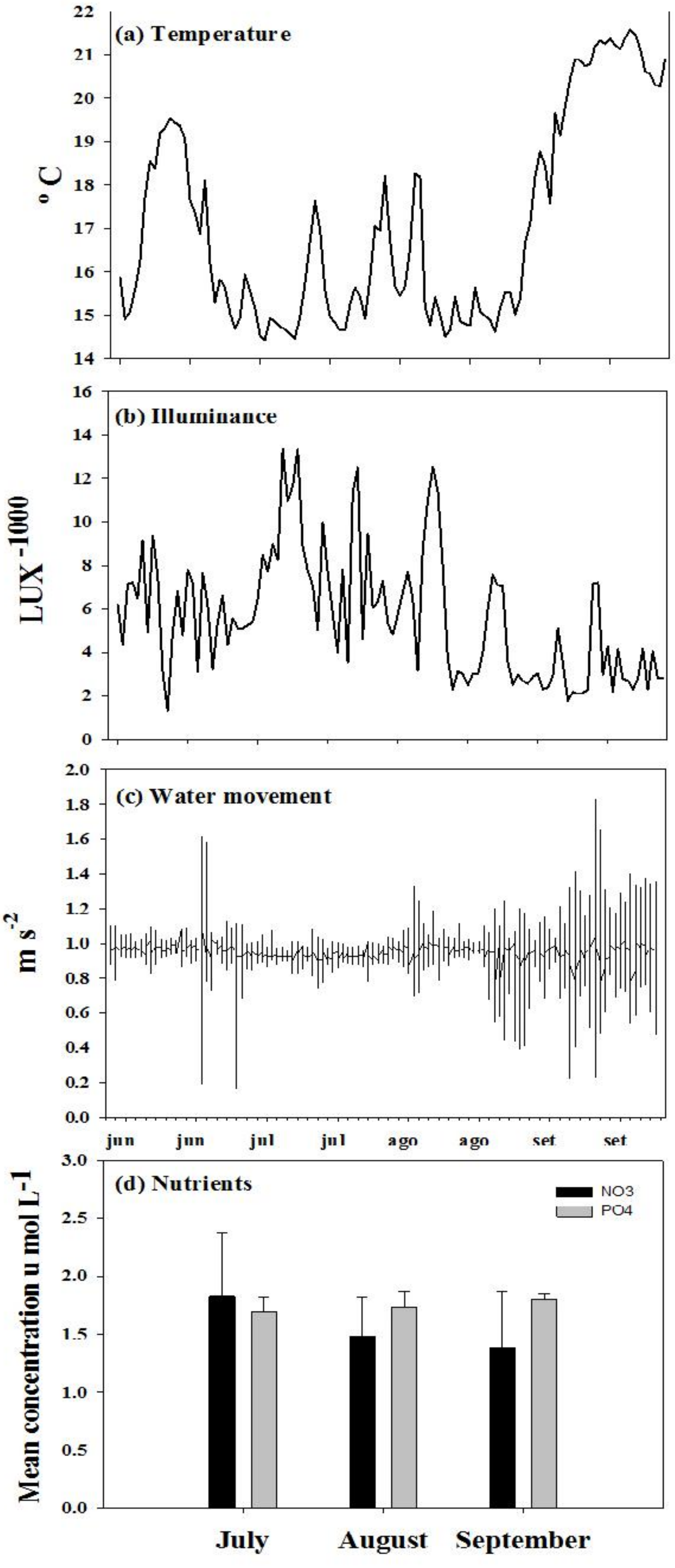
ABIOTIC CHARACTERIZATION AND ABUNDANCE OF MACRO-HERBIVORES

During the experiment (July to September 2014), the water temperature ranged between 14.4 and 21.5 ºC (Fig. 2a), the mean illuminance at the bottom between 1,296 and 13,395 Lux (Fig. 2b) and the water movement between 0.16 and 1.82 m s-2 (Fig.2c). The concentration of nitrate varied from 1.82 to 1.38 µmol L-1, while that of phosphate between 1.69 and 1.80 µmol L-1, respectively (Fig.2d).

Abundance of herbivorous fishes was not different between reefs or among surveys (Table 1, Fig. 3a), while abundance of sea urchins varied significantly between reefs but not among surveys (Table 1, Fig 3b). Macro-herbivores including omnivorous species able to consume algae, were dominated by four fish species: *Sarpa salpa*, *Oblada melanura*, *Diplodus vulgaris* and *Diplodus sargus* (out of 13 recorded fish species; Table S1 in the supplementary material) and one sea urchin, *P. lividus* (Fig. 3b).

Figure 2.

Abiotic characterization of the study region. (a) Temperature daily mean values, (b) mean illuminance between 8:00-17:00h, (c) water movement every 6 minutes, during the experimental period, and (d) mean nutrient concentration at three random times through the course of the experiment.



(a) Temperature

(b) Illuminance

(c) Water movement

(d) Nutrients

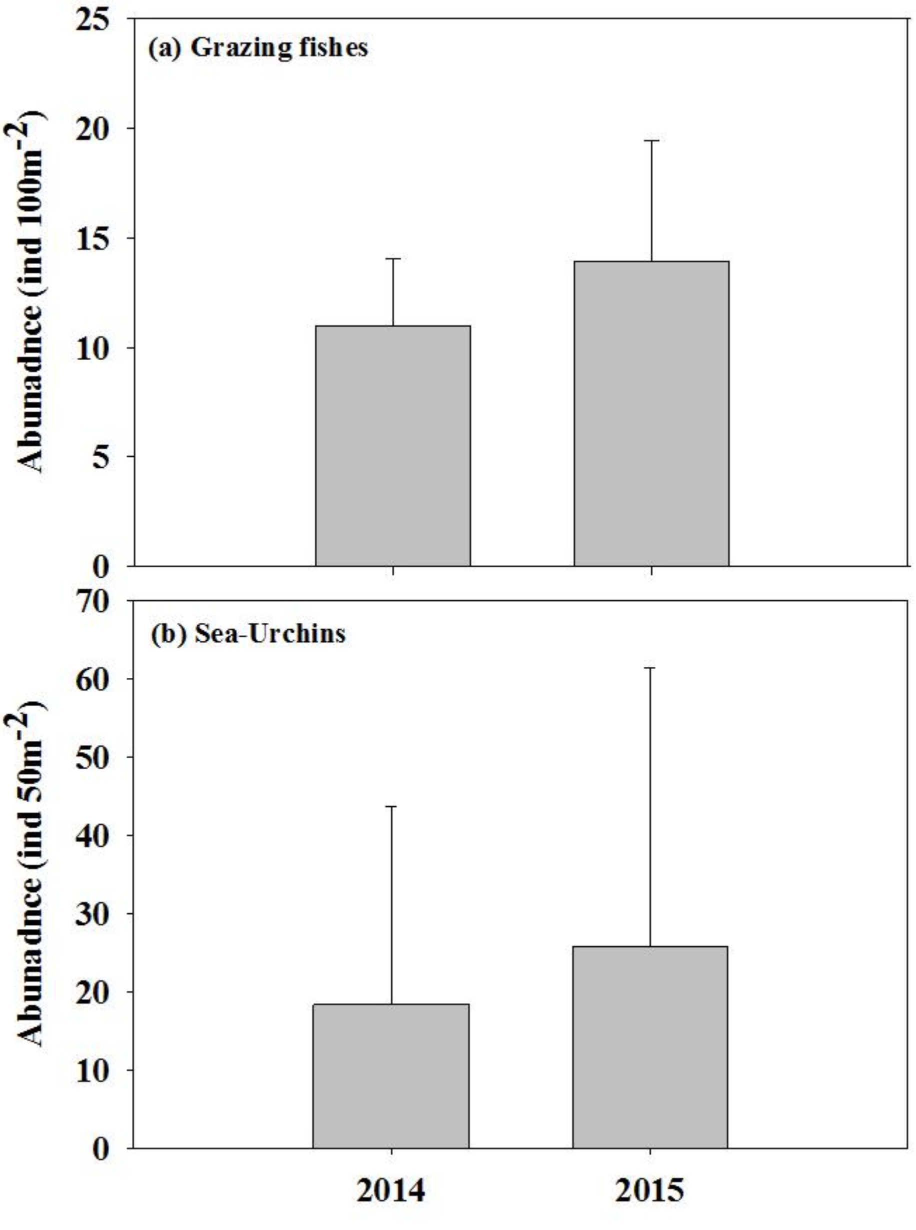


Figure. 3.
(a) Abundance of grazing fishes and (b) sea urchins in 2014 and 2015 (mean + SE, n = 5).

ABUNDANCE OF MIS AND SURVIVAL OF MAS

The abundance of MiS was, on average, 60 and 73% times greater within full cages compared to procedural and the unmanipulated controls (Table 1, Fig. 4a), respectively. The survival of MaS was, on average, 68 and 72% higher where herbivores were excluded relative to procedural and unmanipulated controls, respectively (Table 1, Fig. 4b).

Figure 4.
(a) Abundance of microscopic sporophytes and (b) survival of macroscopic sporophytes (mean + SE, n=6 and n=6, respectively) for each experimental treatment at the end of the study. Different letters above bars indicate significantly different means according to SNK test.

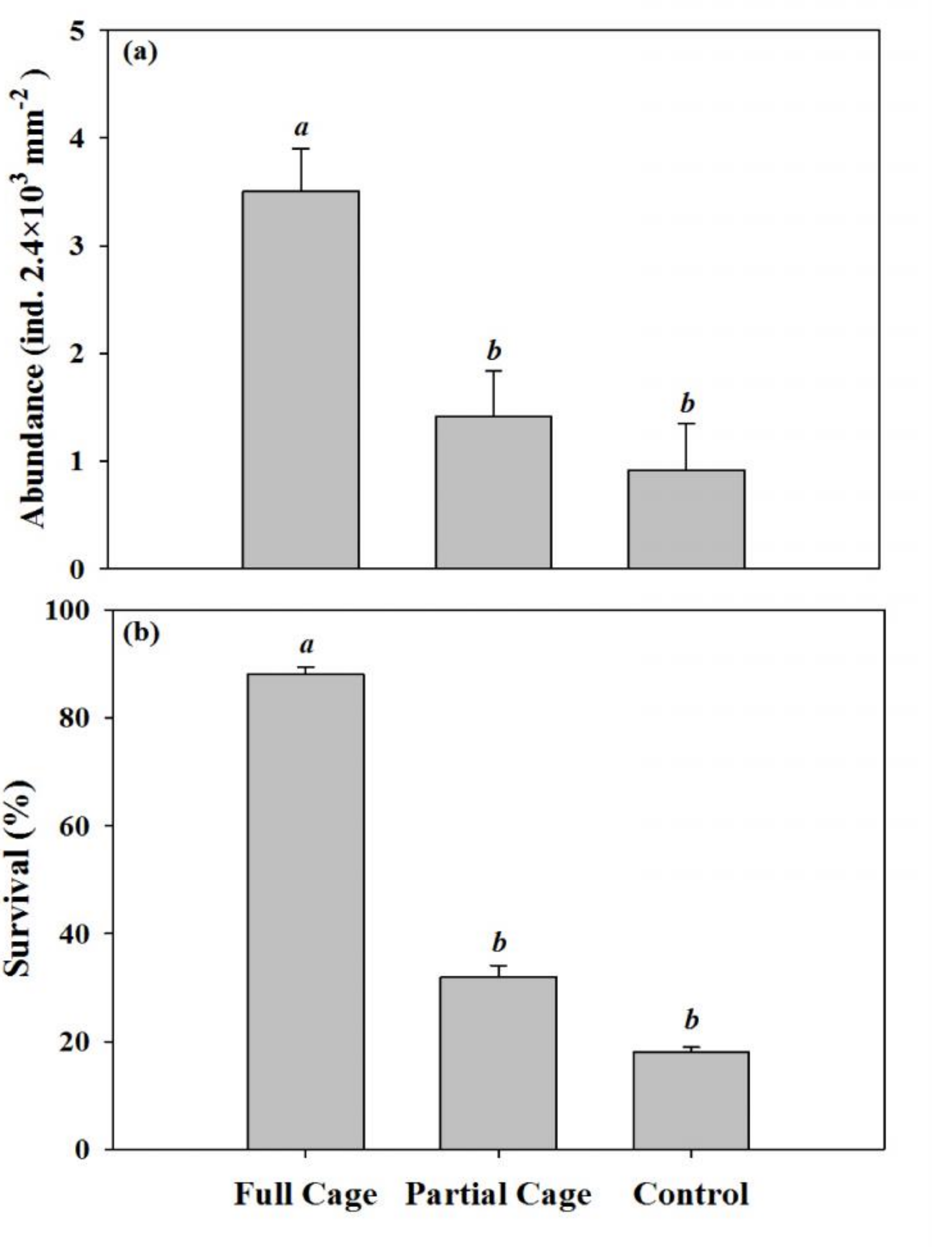


Table 1. Results of ANOVAs on the abundance of herbivorous fishes and sea urchins according to years (2014 *vs*. 2015) and reefs, and on effect of exclusion of herbivores at two reef sites on the abundance of microscopic sporophytes (MiS), the survival of macroscopic sporophytes (MaS), the total blade area growth, the total linear growth and the monthly linear growth of *Laminaria ochroleuca*.

	Transformation	Cochran's test	df	MS	*F*	P
Abundance of grazing fishes	none	*C* = 0.119 (Ns)				
Year			1	69.62	0.55	0.479
Reef (Year)			8	126.22	0.76	0.639
Residual			40	165.90		
Abundance of sea-urchin	Ln(X+1)	*C* = 0.291 (Ns)				
Year			1	11.4317	0.85	0.3832
Reef (Year)			8	13.4276	11.11	**<0.001**
Residual			40	1.2091		
Abundance of MiS	none	*C* = 0.330 (Ns)				
Site			1	2500.00	1.45	0.238
Cage treatment			2	56319.44	38.62	**0.025**
Site × Cage treatment			2	1458.33	0.85	0.439
Residual			30	1722.22		
Survival of MaS	ArcSin %	*C* = 0.302 (Ns)				
Site			1	51.32	3.15	0.0861
Cage treatment			2	494.16	70.7	**0.013**
Site × Cage treatment			2	6.99	0.43	0.655
Residual			30	16.29		
Total blade area growth	none	*C* = 0.413 (P < 0.01)				
Site			1	7545.04	0.17	0.680
Cage treatment			2	1540214.40	112.28	**0.009**
Site × Cage treatment			2	13717.62	0.31	0.733
Residual			66	43957.98		
Total linear growth	none	*C* = 0.2193 (Ns)				
Site			1	690.68	2.11	0.152
Cage treatment			2	239936.10	752.12	**0.001**
Site × Cage treatment			2	319.01	0.97	0.384
Residual			66	328.02		
July: Linear growth	none	*C* = 0.214 (Ns)				
Site			1	2	0.01	0.9275
Cage treatment			2	68065.71	83.34	**0.011**
Site × Cage treatment			2	816.69	3.41	0.0389
Residual			66	239.42		
August: Linear growth	none	*C* = 0.286 (Ns)				
Site			1	288	3.93	0.5165
Cage treatment			2	51411.88	455.2	**0.002**
Site × Cage treatment			2	112.94	1.54	0.2217
Residual			66	73.27		
September: Linear growth	none	*C* = 0.283 (Ns)				
Site			1	34.72	0.73	0.3968
Cage treatment			2	43874.06	12523	**<0.001**
Site × Cage treatment			2	3.5	0.07	0.9293
Residual			66	47.73		

BLADE AREA AND LINEAR GROWTH

Total blade area growth in the herbivory-excluded treatment was larger compared to both procedural and unmanipulated controls, with, respectively, an increase by 101.7 ± 26.2% and a decrease by 32.5 ± 9.3% and 58.5 ± 8.7% relative to the initial blade area (Table 1, Fig. 5). Conversely, the total linear growth, at the end of the experiment, was significantly reduced within full cages compared to procedural and unmanipulated controls (59.6 ± 1.2 and 60.7 ± 1.1%, respectively, Table 1, Fig. 5).

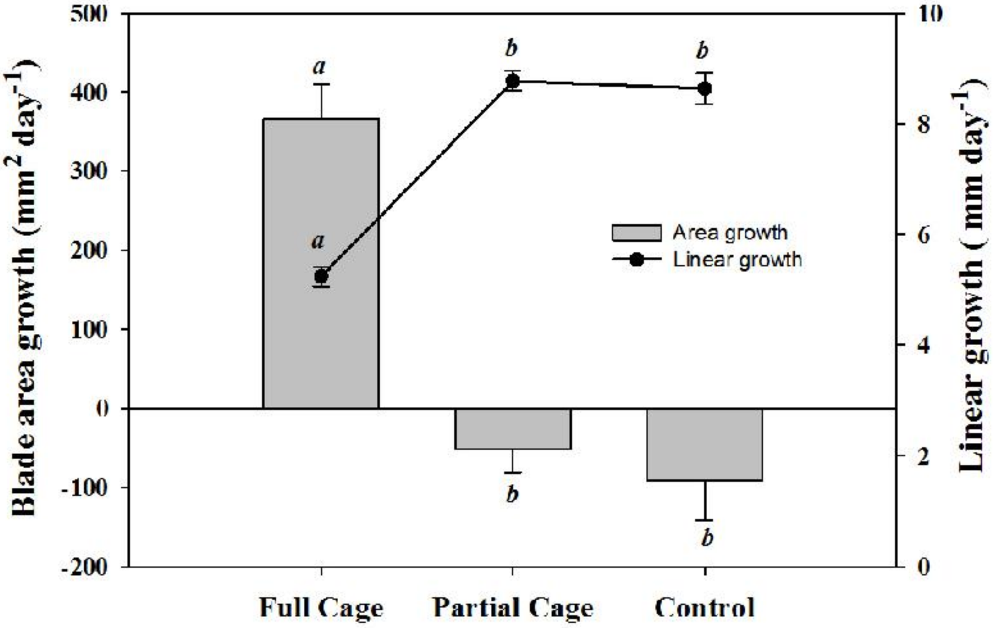


Figure 5.
Total blade area and linear growth of adult sporophytes (mean + SE, n=6) for each experimental treatment at the end of the study. Different letters above/below bars and dots indicate significantly different means according to SNK test.

The mean monthly linear growth was, across the three measured times, also significantly reduced within full cages compared to procedural and unmanipulated controls (60.3 ± 1.1 and 61.0 ± 1.6%, respectively, Table 1, Fig. 6).

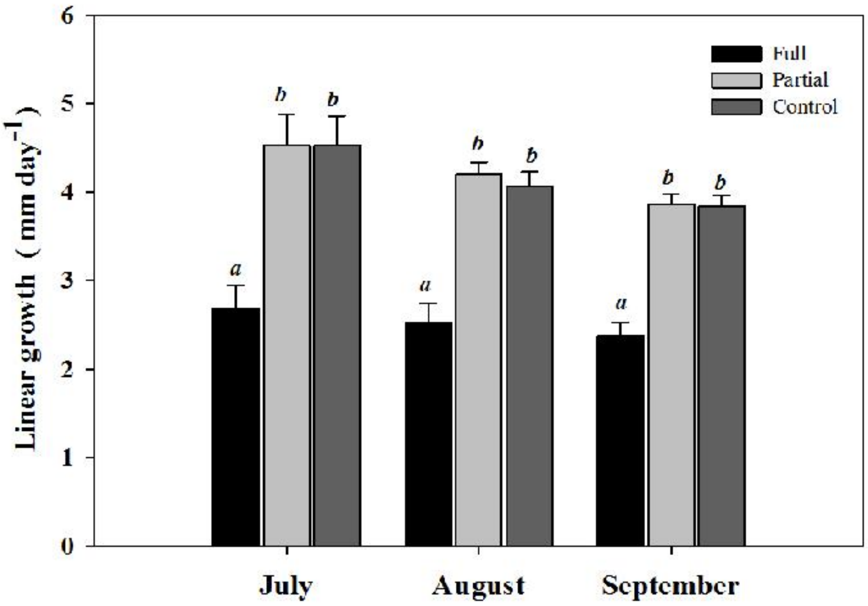


Figure 6. Monthly linear growth of adult sporophytes (mean + SE, n=6) for each experimental treatment. For each month, different letters above bars indicate significantly different means according to SNK test.

DISCUSSION

Our findings indicate that the survival of both microscopic and macroscopic juveniles and the lamina extension of adult *L. ochroleuca* were considerably increased when released from grazing by sea urchins and fishes. This agrees, in general, with a previous study indicating that intense herbivory could contribute to limit kelp distribution and abundance in southern Europe (Franco et al. 2015). Herbivory is often selective and its effects may vary depending on life-stages of both algae and grazers. Herbivores might change preference for specific life stages over the life cycle of the algae and a species representing a preferred food for juvenile herbivores is not necessarily a preferred food for adults. Differences between juvenile and adult algae in a number of physical and chemical properties, including texture, deterrent compounds and nitrogen concentration, are major causes affecting herbivore preference (Van Alstyne et al. 1999). Even though we were not able to quantify the variation of herbivory among different kelp life stages, our study indicated a considerable impact of herbivory on each of the studied life-stages of *L. ochroleuca*, as suggested by previous research involving other kelp species (e.g. Van Alstyne et al. 2001). Herbivorous sea urchins and fishes may reduce the population-level biomass of *L. ochroleuca* through both the consumption of blades of adult individuals and the elimination of small-sized sporophytes. Such biological disturbance is likely to compromise the future of this habitat-forming species which, once apparently abundant and widespread across Portugal, is already sparse in central and southern Portugal (Assis et al. 2009; Tuya et al. 2012; Pinho et al. 2015). It has been reported that damage induced by herbivorous fishes and sea urchins on adult kelp may lead to a critical reduction in their physiological performance and/or extent of reproductive tissue, which can ultimately cause death (Davenport and Anderson 2007). In extreme situations, overgrazing can provoke the collapse of entire kelp forests (Filbee-Dexter and Scheibling 2014; Ling et al. 2014; Vergés et al. 2016). At the same time, the consumption of kelp recruits can prevent the establishment of new 1adult populations (or recovery of old populations lost to other processes, (Bennett et al. 2015)), although such an adverse effect can be mitigated by the occurrence of natural refuges, e.g. crevices in the substratum, providing protection to juvenile kelp (Franco et al. 2015). Moreover, the effects of large herbivores can be further exacerbated by mesoherbivores, such as gastropods, amphipods and isopods, which were not examined in this study. In some cases, it has been shown that mesoherbivores may disproportionately compromise the individual fitness and the structure of whole populations of kelp (Poore et al. 2014).

The evidence for strong kelp-herbivore interactions is especially relevant in transition zones such as the study region in Portugal, where temperature increments can have profound implications for local marine communities (Lima et al. 2007), analogously to other geographic areas (Wernberg et al. 2016). Warming temperatures can strengthen plant-herbivore interactions (O'Connor 2009), particularly at the tropical-temperate boundary (Vergés et al. 2014; Zarco-Perello et al. 2017). For example, a complete shift from a temperate kelp forest to a system dominated by tropical and sub-tropical species was described in western Australia following a marine heat-wave (Wernberg et al. 2016). Sea-water temperature has recently been indicated as an important driver of changes in patterns of distribution and growth of *L. ochroleuca* (Franco et al. in press). In addition, climate-driven range expansions of herbivorous fishes, and increasing abundance of already locally occurring herbivores, have been reported along the Portuguese coast (Franco et al. 2015)). However, since sea-water temperature was within the optimal range (< 20 ºC, see Franco et al. in press) throughout the experiment, present responses could be reasonably ascribed mainly to grazing effects, although potentially interacting with other biotic and abiotic processes.

Kelp have natural responses against herbivores, including physical and/or chemical defenses (reviewed by Bartsch et al. 2008). Compensatory growth is considered an alternative strategy, which has been widely reported for terrestrial plants, but only in a few cases demonstrated for macroalgae (Hay et al. 2011), including kelp (Cerda et al. 2009, Gao et al. 2013). In this experiment, adult kelp exposed to herbivores displayed higher elongation rates of the meristematic tissue (i.e. linear growth). To our knowledge, this ability has never been shown for *L. ochroleuca* and, as far as we are aware, was previously described only for two other kelp species, *Macrocystis pyrifera* (Cerda et al. 2009) and *Undaria pinnatifida* (Gao et al. 2013).

The development of reproductive structures (sori) in *L. ochroleuca* occurs through the transition from the vegetative to the reproductive stage (Bartsch et al. 2008) and this mechanism may allow to compensate the loss of blade tissue. The same was reported for *Lessonia nigrescens*, a kelp species with similar reproductive structures as *L. ochroleuca* (Pansch et al. 2008). However, the effectiveness of this compensation mechanism may vary through the year. Gao et al. (2013) demonstrated increased growth of *U. pinnatifida*, compensating artificial excision during the growing season, but not during the maturation season encompassed within the annual life cycle. In this experiment, *L. ochroleuca* showed a reduction of linear growth over the experimental period, which started in June and finished in September, corresponding to its growing and early maturation season, respectively. It is worth noting, that our experimental procedures could only control for the exclusion of herbivory, but not for its intensity. Therefore, it cannot be excluded that, analogously to other Laminariales, *L. ochroleuca* is unable to compensate extreme losses of blade biomass at very high herbivory pressure (Cerda et al. 2009).

In conclusion, the present study showed compensatory growth of adult *L. ochroleuca* in response to herbivory. However, this ability was counterbalanced by negative effects on juvenile kelp, including their micro and macroscopic phases. Negative effects of simultaneous herbivory pressure at different life stages are likely expected to be more evident in sparse kelp populations and/or in regions where patterns of herbivory are more prone to change (e.g. central Portugal). In conjunction with environmental perturbations, such as changes in seawater temperature this herbivore pressure might contribute to the loss of these important habitat formers and, consequently, the loss of associated ecological and economic goods and services.

SUPPLEMENTARY MATERIAL

Table S1. Trophic guilds (inv: invertebrate feeders, ma: macrocarnivores, om: omnivores, her: herbivorous, according to Henriques et al. 2013 and www. fishbase.org) and abundances (individuals x 100 m^{-2}, mean and SE, n = 25) of fish species in each year.

Species	**Trophic guild**	**2014**		**2015**	
		Mean	SE	Mean	SE
Boops boops	om	0.00	0.00	4	19.60
Ctenolabrus exoletus	inv	0.56	0.90	0.56	0.80
Coris julis	inv	0.08	0.39	1	2
Ctenolabrus rupestris	inv	0.28	0.72	0.00	0.00
Diplodus sargus	om	2.00	4.40	1.52	4.19
Diplodus vulgaris	om	7.40	7.09	6.32	6.28
Gobiusculus flavescens	inv	3.40	9.87	0	0
Labrus bergylta	inv	0.88	1.53	1.36	1.83
Oblada melanura	om	0.16	0.54	0	0
Sarpa salpa	her	2	5	5.52	11.35
Serranus cabrilla	ma	0	0	0.64	0.89
Symphodus spp.	inv	0.20	0.69	0.60	0.80
Trisopterus sp.	inv	0.04	0.20	0.16	0.37

EFFECT OF CAGING ON ILLUMINANCE AND WATER MOVEMENT

Two data loggers were deployed in the centre of one randomly chosen full, partial and control plot to test for differences among treatments. Illuminance availability (Fig. S4) and water movement (Fig. S5) were measured and averaged, respectively, at six (n=150) and two times (n=50) every minute over 25 minutes in newly assembled cages and in cages with fouled mesh-nets, just before the weekly cleaning. Differences of illuminance and water movement among treatments were analysed by 1-way ANOVA.

Figure. S1.

(a) Mean illuminance in newly assembled cages, and (b) in cages with fouled mesh-nets, before the weekly cleaning, according to experimental treatments (mean + SE, n=150). Same letters above bars indicate non-significant differences.

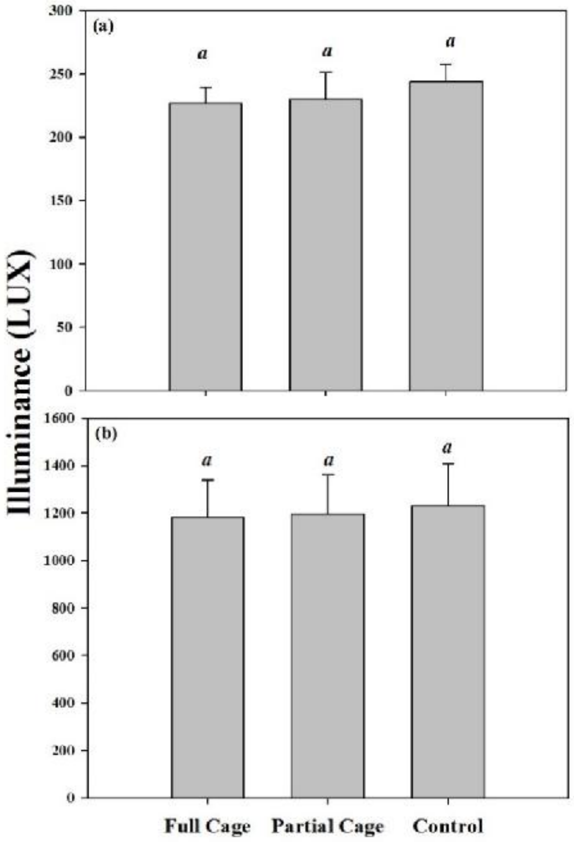
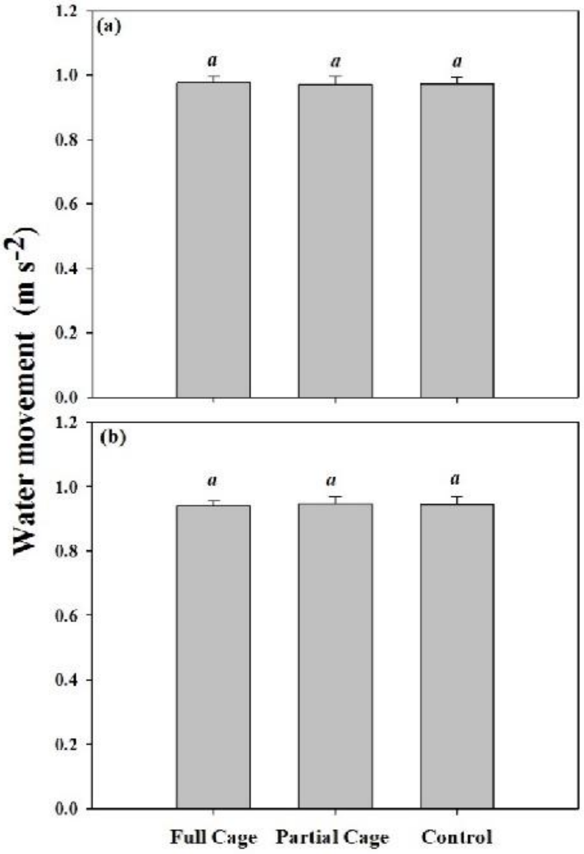


Figure. S2.

(a) Mean water movement in newly assembled cages, and (b) in cages with fouled mesh-nets, before the weekly cleaning, according to experimental treatments (mean + SE, n=50). Same letters above bars indicate non-significant differences.



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CHAPTER III

HERBIVORY DRIVES KELP RECRUITS INTO ‘HIDING’ UNDER A WARM OCEAN CLIMATE

Figure Chapter III. Kelp *Laminaria ochroleuca* growing inside a crevice in Peniche. Crevices are physical refuges from grazing fishes. Photograph by J.N. Franco

João N. Franco[1,2], Thomas Wernberg [3], Iacopo Bertocci[1], Pedro Duarte[4], David Jacinto[5], Nuno Vasco-Rodrigues[6], Fernando Tuya[7]

[1] CIIMAR/CIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas 289, 4050-123 Porto, Portugal.

[2] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4150-181 Porto, Portugal

[3] School of Plant Biology & UWA Oceans Institute (M470), University of Western Australia, Crawley WA 6009, Australia

[4] Norwegian Polar Institute, Fram Centre, N-9296 Tromsø, Norway

[5] MARE – Marine and Environmental Sciences Centre, Laboratório de Ciências do Mar, Universidade de Évora, Apartado190, 7521-903 Sines, Portugal

[6] GIRM – Marine Resources Research Group, Polytecnic Institute of Leiria, Campus 4, Santuário Nª Sª dos Remédios, 2520 - 641 Peniche, Portugal

[7] Grupo en Biodiversidad y Conservación, Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain

ABSTRACT

Assessing effects of herbivory under varying ocean climate scenarios and over small spatial scales is crucial for understanding its influence on primary producers. Effects of herbivory on the distribution and abundance of kelp recruits were examined experimentally at two regions under contrasting ocean climate. Specifically, the abundance and survivorship of kelp recruits and the abundance of macro-herbivores were compared between a 'cool' and a 'warm' region in northern and central Portugal. At each region, the abundance of kelp recruits and the intensity of grazing were compared between areas of contrasting topography within reefs ('open reef' *vs*. 'crevices'). Compared to the warm region, the abundance of kelp recruits was 3.9 times greater in the cool region, where 85% of recruits were found in 'open reef' habitats. In contrast, 87 % of recruits in the 'warm' region were restricted to 'crevices'. The 'warm' region had 140 times greater abundances of sea urchins, 45 times more herbivorous fish and 4.1 times more grazing marks on kelp recruits than the 'cool' region. Grazing assays showed *ca*. 50 times higher rates of kelp biomass consumption, mainly by fishes, and null survivorship of kelp recruits in the 'warm' relative to the 'cool' region. Consequently, herbivory does not only affect the abundance of kelp recruits across latitude, but also their distribution at local scales, driving kelp recruits into 'hiding' in crevices under intense herbivory. Where net recruitment success is compromised by herbivory, the persistence of kelps is contingent on availability of topographical refuges.

KEYWORDS

Atlantic Ocean; Portugal; habitat complexity; climate; kelp forests; grazing; range limit

INTRODUCTION

Herbivory is a key ecological process shaping the structure and dynamics of biological communities (Pace et al. 1999, Schmitz et al. 2000, Poore et al. 2012). Herbivory affects primary producers in all systems, including lakes, forests, grasslands, kelp forests, coral reefs and marine pelagic systems (Schmitz et al. 2000, Shurin et al. 2002, Steneck et al. 2002), altering their biomass, productivity, physical structure and diversity over a range of spatial and temporal scales (Chesson 2000, Stachowicz et al. 2007). With grazing rates up to three times greater than in terrestrial systems (Cyr & Pace 1993), herbivory is particularly influential in driving patterns of community structure in aquatic systems. In addition, the strength of predator-prey interactions that alter the abundance, biomass and productivity of primary producers indirectly via trophic cascades is stronger in aquatic compared to terrestrial systems (Shurin et al. 2002).

The intensity of herbivory is hypothesized to decrease from tropical to temperate regions of the world's oceans (Gaines & Lubchenco 1982, Bolser & Hay 1996). Support for this hypothesis includes decreasing abundances and diversity of herbivores towards colder waters (Horn 1989, Ebeling & Hixon 1991, Meekan & Choat 1997, Floeter et al. 2005), stronger chemical defenses of algae at lower latitudes (Cronin et al. 1997, Craft et al. 2013), effects of water temperature on herbivore-plant interactions (see below) and herbivore digestive physiology due to reduction of the digestion efficiency (Gaines & Lubchenco 1982). Warmer water temperatures increase the metabolic processes favoring higher consumption rates of herbivores and growth of primary producers, although consumption rates might increase faster than production (Sanford 2002, Brown et al. 2004), potentially resulting in a negative balance between production and consumption (O'Connor 2009). Studies aimed at empirically testing whether rates of herbivory change across latitude are, however, limited, particularly in subtidal environments (Moles et al. 2011). Yet, while some results support the progressive decrease in herbivory intensity with increasing latitude (Wootton & Oemke 1992, Schemske et al. 2009, González-Bergonzoni et al. 2012), a recent meta-analysis of 613 field experiments (Poore et al. 2012) revealed that, at a global scale, the impact of marine herbivores on primary producers does not change with latitude. Not surprisingly, such inconsistent findings has prevented the formulation of general and widely accepted explanations for the importance and nature of such biotic interactions and for their changes with latitude (Cahill et al. 2014). Moreover, Poore et al. (2012) emphasized the importance of producer traits, including both the identity and morphology, as responsible for very variable grazing impacts on different organisms. In this context, large brown seaweeds, such as kelps, are amongst the most impacted species by herbivores within temperate marine vegetation.

Kelps are 'foundation' species (Dayton 1975) on many shallow rocky coasts at polar and temperate latitudes, supporting highly diverse associated assemblages and providing essential, and exceptionally valuable, ecosystem services (Schiel & Foster 1986, Steneck et al. 2002, Crain & Bertness 2006, Bolton 2010). In many systems, herbivory plays a major role in driving patterns of abundance and distribution of kelps, with subsequent consequences for the ecological performance of kelp forests (Anderson et al. 1997, Moles et al. 2011, Harley et al. 2012).

There is wide-spread evidence that herbivores (e.g. sea-urchins) can eradicate kelps at both large and small spatial scales (Hart &

Scheibling 1988, Watanabe & Harrold 1991, Anderson et al. 1997, Sivertsen 2006, Ling et al. 2009, 2010), producing large 'barren' areas that replace entire kelp forests, or creating gaps within kelp canopies. Moreover, there is a growing body of literature showing that tropical fish communities are expanding to temperate regions with negative ecological impacts (Graham 2010, Nakamura et al. 2013, Wernberg et al. 2013, Vergés et al. 2014).

Kelp distribution and abundance can also be influenced by biotic and abiotic processes operating at small, local, scales, i.e. from a few centimeters to tens of meters, through alterations in reef architecture. In spite of the documented importance of reef topography for structuring reef-associated assemblages (e.g. fishes) on temperate reefs (Tuya et al. 2011), little is known about its influence on macroalgal assemblages, kelps in particular (Toohey et al. 2007). Habitats or environmental factors that convey spatial and temporal resistance and/or resilience to communities exposed to biophysical disturbances can be considered as 'refugia' (Sedell et al. 1990). In this context, substratum topography offers protection to different organisms against multiple types of disturbance, as demonstrated by accumulating studies from terrestrial (Reader 1992) and aquatic systems (Bergey 2005, Bennett et al. 2010, Brandl et al. 2014). For example, encrusting algae of the genus *Hildenbrandia* escapes herbivory by propagules starting to grow from small cracks and crevices inaccessible to grazers (Underwood, 1980). Analogously, Bergey (2005) described the importance of crevices to increase algal recovery after disturbance, as well as Brandl et al. (2013) indicated the crucial role of crevices for early life stages of both coral and macroalgal populations. Nevertheless, to our knowledge, no study has tested the role of topographical refugia for kelp recruit survival.

The coast of continental Portugal extends for more than 800 km and is a recognized interface between cold-temperate and warm-temperate species (Lima et al. 2007, Tuya et al. 2012). Recently, Tuya et al. (2012) described a biogeographic discontinuity in the composition and structure of reef-associated algal and fish assemblages between northern (a 'cool' region) and central and southern Portugal (a 'warm-temperate' region). Importantly, kelps (*Laminaria ochroleuca*, *Laminaria hyperborea* and *Saccorhiza polyschides*) have shown abrupt latitudinal clines in their abundances across continental Portugal, being common in the north, at very low densities in central Portugal, and practically absent in southern Portugal (Tuya et al. 2012, Assis et al. 2013). A latitudinal gradient in ocean climate, particularly sea-water temperature (mean annual difference of ~1 to 2 ºC) and primary production all year-round (Lemos & Pires 2004) has been proposed as the primary cause of these macroecological patterns (Lima et al. 2007, Tuya et al. 2012).

In this study, we took advantage of the environmental contrasting in ocean climate across continental Portugal to assess the influence of herbivores on the abundance of juvenile kelps at warmer compared to cooler regions. This could modify the importance of habitat topography (i.e. the local availability of refuges) as a driver of the local distribution of kelp recruits through protection against herbivores. Specifically, we tested the hypotheses that (i) the abundance of macro-grazers and the intensity of herbivory (quantified in terms of bite marks and consumption of juvenile kelps) would be lower in northern than central Portugal; (ii) kelp recruits would be more abundant in northern than central Portugal; (iii) rates of herbivory would be lower in crevices than in open reef habitats. Overall, the integration of these hypotheses led to the prediction that herbivory might not only provide a major contribution to macroecological differences in the abundance of early life-stages of kelps at large spatial scales, but also represent a key driver of their reef-scale distribution.

MATERIALS AND METHODS

STUDY AREA

This study was conducted at Peniche (PEN, 39.2ºN, 9.2º W) and Vila do Conde (VIL 41.2º N, 8.45º W), separated by *ca*. 250 kilometers and *ca*. 2º of latitude, in central and northern Portugal, respectively.

This coastline is almost rectilinearly oriented from north to south, maintaining a similar overall exposure to dominant NW and W swells. The shore is characterised by extensive sandy beaches interspersed with limestone, sandstone, shale or granitic reefs in both the intertidal and the shallow subtidal. There are clear differences in ocean climate conditions between these two regions: VIL has a mean annual SST of ~1 to 2 ºC lower than PEN, while differences in chlorophyll *a* and nutrient concentrations are minor (Tuya et al. 2012). Within each region, replicate rocky reefs (~ 1 km apart) were selected randomly at 6-11 m depth. Herbivore (sea-urchins and fishes) surveys and grazing experiments were carried out between the 16th and 18th July and between the 6th and 8th August 2012 in PEN and VIL, respectively, and again between the 8th and 12th July 2013 for herbivore surveys.

KELP RECRUIT ABUNDANCE AND GRAZING MARKS

The abundance of kelp (*Laminaria* spp. and *Saccorhiza polyschides*) recruits was estimated along ten transects (1 x 0.2 m) at each of five reefs within each region. Sampling at each reef was stratified according to 'open reef' and 'crevices' to encompass these two contrasting habitats of reef topography (Vanderklift & Kendrick 2004). Since different species of juvenile kelp sporophytes are morphologically indistinguishable, particularly underwater, we were unable to establish the species identity of recruits. A total of 234 kelp recruits – 10 to 25cm total length - were collected (69 from 'crevices' and 165 from 'open reef' habitat) for a semi-quantitative analysis of grazing marks. In the laboratory, recruits from each reef were photographed, the presence of grazing marks was quantified and each recruit was assigned to one of three categories: no grazing, light grazing (< 25% of tissue affected by grazing) and heavy grazing (> 50% of tissue affected by grazing) (Fig. 1).

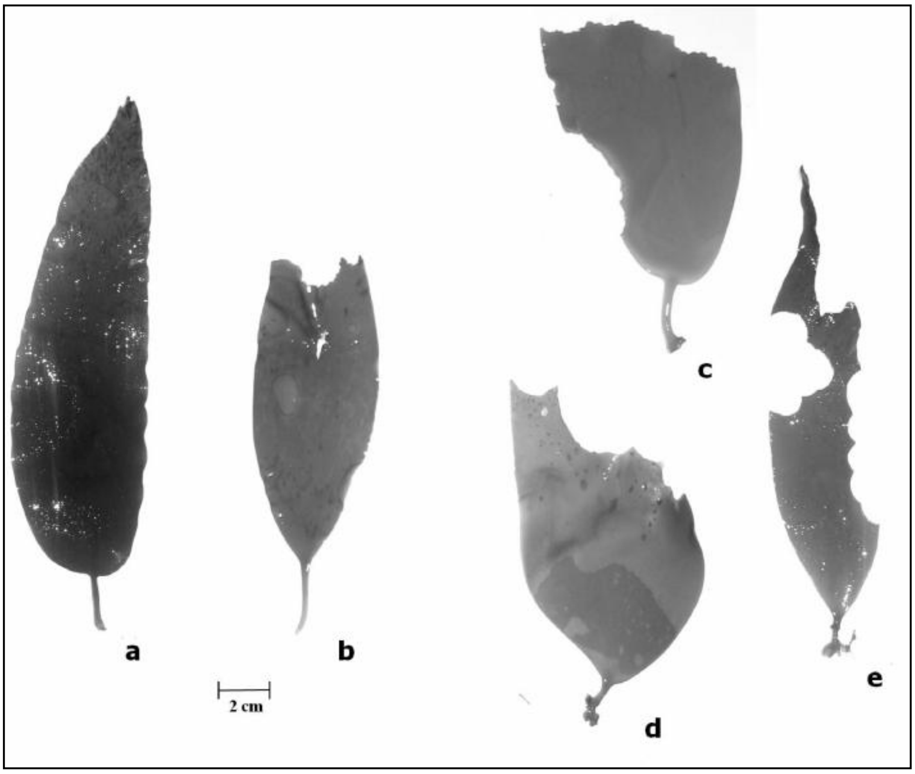

Figure 1.
Examples of different grazing marks on kelp recruits. *a* show a kelp recruit without grazing marks, *b* and *c* show classic signs of sea urchin grazing with the irregular serrated bite marks, whereas *d* and *e* show clear crescent-shaped bite marks indicative of fish herbivory.
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KELP RECRUIT SURVIVAL

At each of two reefs within each region, 20 random kelp recruits were tagged by attaching a cable tie around the stipe of each individual (de Bettignies et al. 2013). Tagged individuals were collected after 1 month to assess survival.

RATES OF HERBIVORY

Rates of herbivory were quantified in a kelp tethering experiment (Wernberg et al. 2006, Vanderklift & Wernberg 2008), allowing equal exposure of kelps to fishes and urchins. At each of three reefs within each location, 8 kelp recruits (10-20 cm of total length, 2.2-6.8 g wet weight) were attached to chains (6 per reef) by clothes pegs. At each reef, 3 chains (= 24 recruits) were deployed inside crevices, and 3 chains were deployed on 'open reef'. Replicated chains were interspersed within *ca*. 500 m^2 on each reef. The wet weight (ww; to the nearest 0.1 g, after shaking off excess of water and blotting with paper) of each kelp recruit was measured at the beginning of the experiment and after 48 hours of deployment. Because of the short deployment and calm sea conditions during experimentation, algal loss due to autogenic changes was assumed to be minimal, and the change in weight considered as representative of the amount of tissue consumed by herbivores (Levenbach 2009). All kelp recruits were collected by hand at Mindelo (41.1° N), a shallow-water reef location that easily supplied hundreds of recruits. In all cases, tethered kelps were deployed <24 hours after collection and were always maintained in aerated sea-water to minimize stress and damage. Using recruits of the same origin removed any potential influence of location-specific differences in biochemical composition (nutritive value or grazing deterrents).

MACRO-HERBIVORE ABUNDANCE

At each of the five reefs within each region, all adult and sub-adult fishes were counted along five, randomly oriented, 25 x 4 m belt transects (Tuya et al. 2012). The number of *Paracentrotus lividus*, the most common sea-urchin in continental Portugal (Jacinto et al. 2013), was counted along five 25 x 1 m transects. Fishes were categorized according to their trophic affinities (Henriques et al. 2013; www.fishbase.org; Appendix 2). Herbivorous fishes were considered as those species able to consume algae, thus also including omnivorous species (Sala & Boudouresque 1997, Ruitton 2000, Horta et al. 2004).

STATISTICAL ANALYSES

Differences in the abundance of kelp recruits were tested by three-way ANOVA, including the factors: Region (2 levels: PEN *vs*. VIL, fixed), Reef (5 levels, random and nested within regions) and Habitat (2 levels: 'open reef' *vs*. 'crevices', fixed factor and orthogonal to the previous factors). Regions were considered fixed in our analyses, as these represented cool and warm ocean climates, respectively. Differences in the amount of kelp tissue consumed by macro-herbivores was analyzed by four-way ANOVA, including: Region (2 levels) and Habitat (2 levels) as fixed factors, while Reef (3 levels, nested within regions) and Chain (3 levels, nested within regions, habitats and reefs) were treated as random factors. The abundance of herbivorous fishes and sea-urchins was analyzed by three-way ANOVAs, including: Region (2 levels, fixed), Year (2 levels, random and nested within regions) and Reef (5 levels, random and nested within regions and years). Before each ANOVA, the assumption of homogeneity of variances was checked through a Cochran's C test; only the abundance of herbivorous fishes had to be Ln(x+1) transformed to achieve homogeneous variances ($P>0.05$). When differences were found, *a posteriori* comparisons were carried out using pairwise SNK tests (Underwood and Chapman, 1997). A Chi-squared (χ^2) was used to test for differences in the amount of grazing marks between regions and reef habitats. Kelp recruit survival was reported as percentage of lost individuals.

RESULTS

ABUNDANCE AND SURVIVAL OF KELP RECRUITS

Kelp recruit abundance was 3.9 times greater at VIL than PEN. Recruits at VIL were predominantly found in open reef habitats (Fig. 2). In contrast, the few kelp recruits at PEN were mainly found inside crevices (Fig. 2, Region × Habitat < 0.05, Table 1). There was zero survivorship of kelp recruits at PEN, where all tagged kelps disappeared, while the opposite was observed at VIL, where all tagged individuals were retrieved after 1 month (100% of kelp survival).

INTENSITY OF HERBIVORY ON KELP RECRUITS

The number of grazing marks on kelp recruits differed between regions and habitats (Fig. 3, χ^2 region= 193.7, χ^2 habitat= 96.3, df = 2, $P < 0.001$) with a larger number of heavy grazing marks at PEN and in open reef areas compared to VIL and crevices, respectively. Consumption of kelp recruit biomass was strongly influenced by the region and the type of habitat (significant interaction Habitat × Region in Table 1, Fig. 4). At PEN, the mean biomass consumption was between 2.0-3.2 g ww per recruit in open reef habitats relative to 0.5-1.6 g ww per recruit reef in crevices. In contrast, there was a mean consumption of 0.03-0.04 g ww per recruit in VIL that was irrespective of the habitat (SNK pairwise comparisons: all reefs at VIL: 'open' = 'crevice'; all reefs at PEN: 'open' > 'crevice', $P < 0.05$). Overall, these results suggest that herbivores strongly reduced kelp recruit biomass at PEN, including contrasting differences in consumption between open reef areas and crevices, but not at VIL.

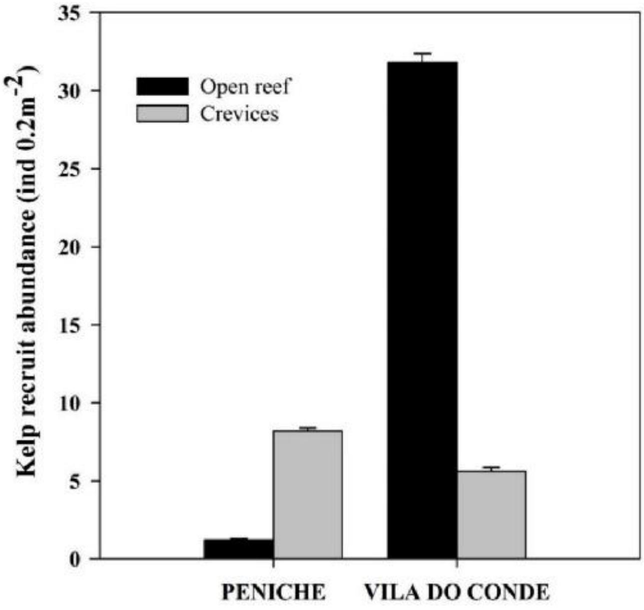


Figure 2.
Abundance of kelp recruits in each habitat at Peniche and Vila do Conde (mean +SE, n = 10).

Table 1. Summary of ANOVAs on the abundance of herbivorous fishes, sea-urchins and kelp recruits and grazing intensity on kelp recruits, at Peniche (PEN) and Vila do Conde (VIL) according to regions, years, reefs, habitats and chains. SNK tests resolved significant differences for fixed factors.

	abundance of herbivorous fishes				abundance of sea-urchins			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Transformation	Ln(x+1)				none			
Cochran’s	C = 0.1462 (non-significant)				C = 0.3152 (P < 0.01)			
Region	1	117.08	0	**<0.001**	1	12254.49	0	**<0.001**
Year	1	17.51	62.83	**<0.001**	1	756.25	5.74	**0.043**
Reef (Region)	8	1.65	5.94	**0.010**	8	1015.37	7.70	**0.004**
Region × Year	1	32.26	115.74	**<0.001**	1	650.25	4.93	**0.049**
Year × Reef (Region)	8	0.27	0.33	0.950	8	131.85	2.30	**0.028**
Residual	80	0.23			80	57.37		
SNK tests	Region × Year PEN > VIL: Both years				Region PEN > VIL: Both years			
	grazing on kelp recruits				abundance of kelp recruits			
Source of variation	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Transformation	None				None			
Cochran’s	C = 0.1281 (P < 0.01)				C = 0.3324 (P < 0.05)			
Region	1	270.6547	60.97	**0.001**	1	98.00	108.59	**<0.001**
Reef (Region)	4	4.4392	5.42	**0.003**	8	0.90	0.81	0.596
Habitat	1	52.5510	9.98	**0.034**	1	46.08	62.06	**<0.001**
Region × Habitat	24	0.8194	0.90	**0.032**	1	137.78	185.56	**<0.001**
Habitat × Reef (Region)	1	53.0792	10.08	**0.033**	8	0.74	0.66	0.722
Chain (Region×Reef×Habitat)	4	5.2653	6.43	**0.001**				
Residual	252	0.9095			180	1.11		
SNK tests	Habitat × Reef (Region) All reefs at VIL: open = crevice All reefs at PEN: open > crevice				Region × Habitat VIL: open > crevice PEN: crevice > open			

ABUNDANCE OF MACRO-GRAZERS

Although abundances varied significantly among surveys (Fig 5, Year < 0.05, Table 1, for both sea urchins and herbivorous fishes), the abundance of sea urchins was, overall, *ca*. 140 times greater at PEN than at VIL (significant Region < 0.05, Table 1, Fig. 5A). From the 19 fish species recorded, only 5 were considered herbivores (Table 2). The abundance of herbivorous fishes was, overall, *ca*. 45 times greater at PEN than at VIL (significant Region < 0.001, Table 1, Fig. 5B).

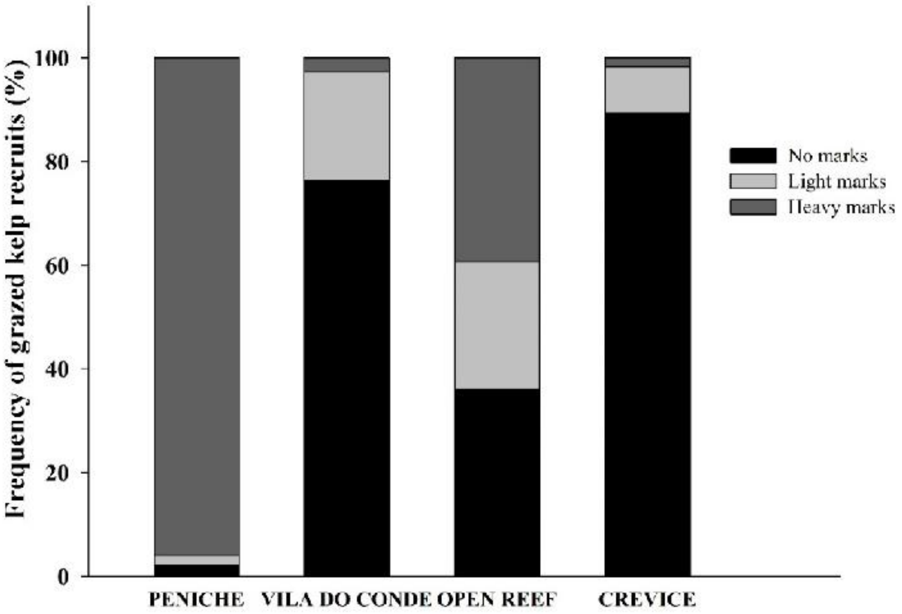


Figure 3.
Total number of grazing marks on kelp recruits (n=234) at Peniche, Vila do Conde, and in Open reef and crevices.

DISCUSSION

This study documented a clear difference in the intensity of herbivory on kelp recruits between sites in central and northern Portugal, two regions with different ocean climates. The differences in intensity of herbivory corresponded to concurrent differences in the abundance of kelp recruits and large-sized herbivores (sea-urchins and fishes). Differences in kelp consumption between open reef and crevices further demonstrated how varying herbivory intensity and local topographical features can contribute to spatial heterogeneity in the distribution of kelp recruits.

In central Portugal sampled sites, kelp recruits were mostly limited to crevices, in contrast to northern Portugal, where kelp recruits also occurred in large abundance in open reef habitats. These differences between regions suggest that strong herbivory pressure in open reef habitats likely prevents the growth of kelp recruits in central Portugal, where kelps recruits only survive when hidden inside crevices. Although crevices are not an ideal micro-habitat for kelp recruitment, including reduced light availability (Bartsch et al. 2008), the need for protection against consumers likely drive/limit their presence to these spatial refuges with localized reduced herbivory, i.e. where large schools of herbivorous fishes do not typically forage. In this sense topographical refuges provide a physical impedance to mortality from predation (see Dudley & D’Antonio 1991; Milchunas & Noy-Meir 2002; Fox & Bellwood 2007; Vergés, Alcoverro & Ballesteros 2009; Vergés *et al.* 2011; Bennett *et al.* 2010). In this study, the distribution of kelp recruits within reefs showed a strong reliance on high-complexity structural elements (crevices) when grazing was prominent. The capacity of topographic complexity to promote kelp recruitment where herbivores are abundant becomes particularly important in the context of the Portuguese coast as the southern limit of distribution of kelp species, such as *Laminaria ochroleuca or Laminaria hyperborea*.

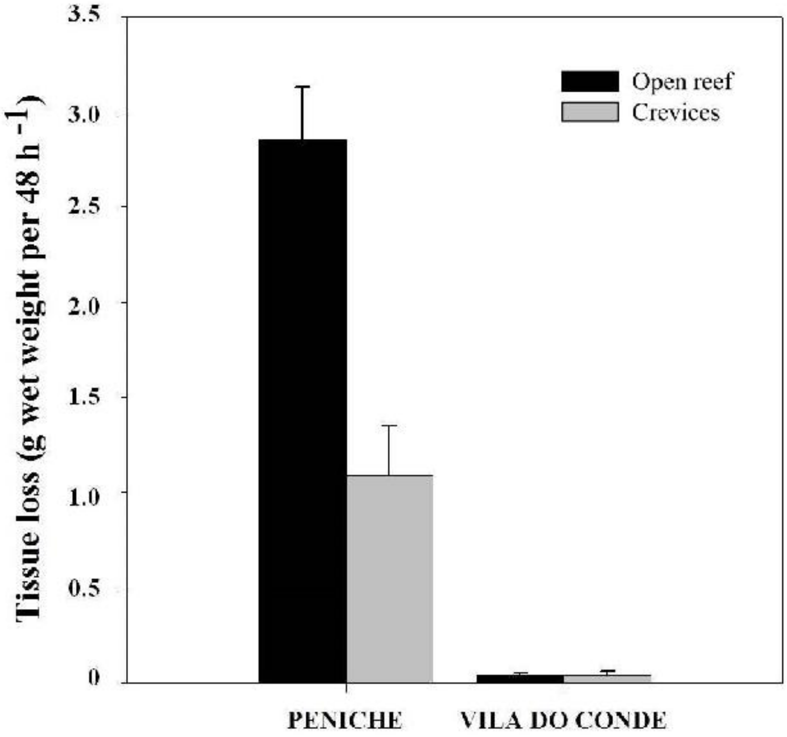
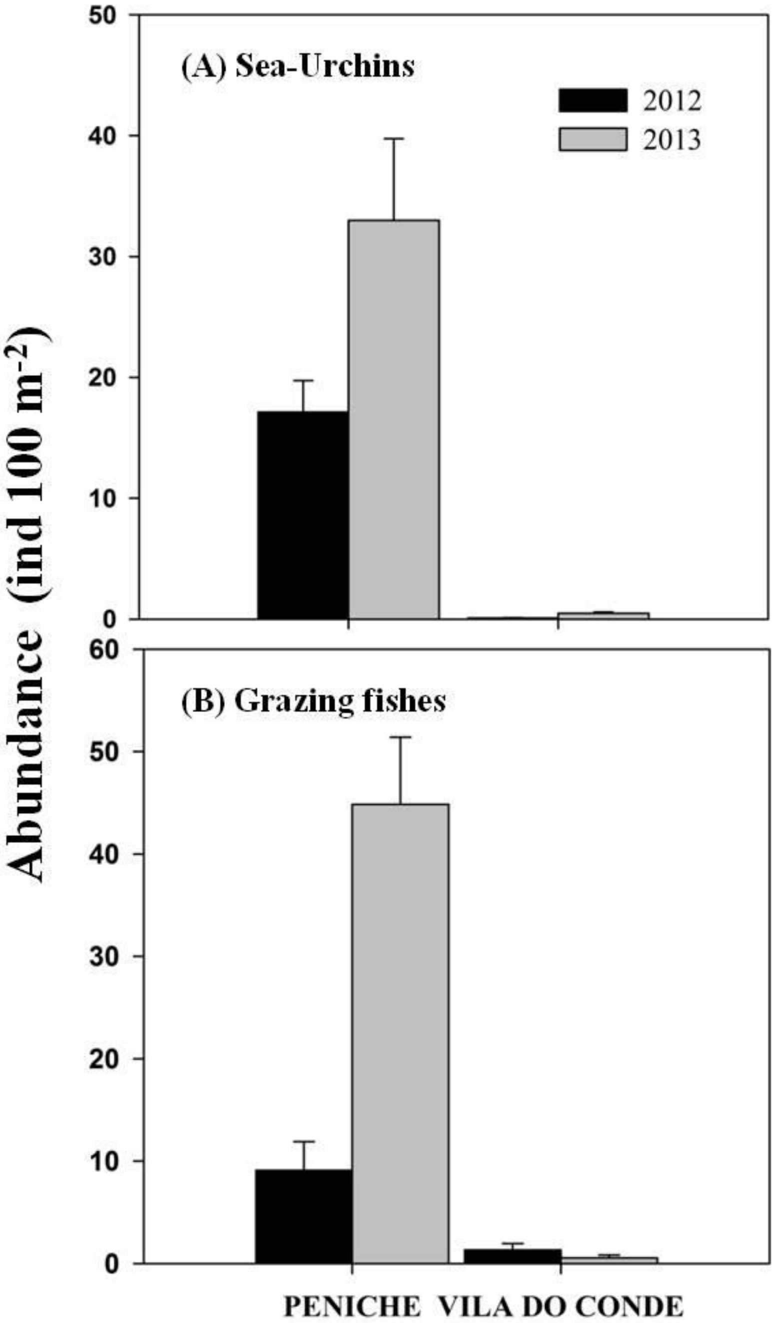


Figure 4.
Consumption of kelp recruits (tissue loss) in each habitat at Peniche and Vila do Conde (mean +SE, n = 48).

Marginal populations, in many cases, are characterized by low densities of adult individuals (Hampe & Petit 2005) and this that might lead to a limitation in the supply of propagules (e.g., Bennett & Wernberg 2014) and so compromise the recruitment. In addition, as demonstrated in this study, recruitment success can be further and drastically compromised by intense herbivory at the range margins. Given that recruits were common in crevices, kelp persistence in central Portugal seems to rely more on recruitment success, and so avoidance of consumption by herbivores, than in the lack of spores from adult individuals. In this study we did not address any potential physiological limitations on kelp distribution. However, short-term physiological experiments suggest kelp recruits of both *L. ochroleuca* and *S. polyschides* are capable of physiologically adjusting their metabolic processes (photosynthesis and respiration) to prevailing sea-water temperatures in the study region (Biskup et al. 2014). Still, we cannot rule out a possible physiological influence of varying ocean climate (abiotic conditions, e.g., Wernberg *et al.* 2010) between regions and the different observed patterns of kelp recruit abundance reflect the cumulative effects of biotic and abiotic influences.

Figure 4.
Abundance of (A) sea-urchins and (B) grazing fishes at Peniche and Vila do Conde in 2012 and 2013 (mean +SE, n = 5).



Despite the relatively small gradient in latitude (*ca*. 2º) an abrupt macroecological change in the diversity and structure of shallow-water subtidal assemblages was recently reported between central and northern Portugal (Tuya et al. 2012). This is, to some extent, supported by the findings of this study, though not specifically aimed at addressing such issues. The abundance of herbivorous fishes followed a sharp latitudinal cline, as those described for the western Atlantic (Floeter 2004), New Zealand, Australia and the Caribbean (Meekan & Choat 1997), where the abundance of herbivorous fishes decreased towards latitudes with cooler conditions. At sites in central Portugal, herbivorous fishes were numerically dominated by the genus *Diplodus*, particularly *D. vulgaris* and *D. sargus*, which are recognized as having a generalist feeding behavior (Sala & Ballesteros 1997, Horta et al. 2004). The diet of these species is typically omnivorous, including invertebrates and typically between 30% and 56% of frequency of occurrence of algae in the consumed food (Sala & Zabala 1996, Ruitton 2000, Horta et al. 2004). *Sarpa salpa* is the only obligate herbivorous fish in continental Portugal. This species is an ecologically important macrograzer in the Mediterranean (Vergés et al. 2008, 2009, Gera et al. 2013), particularly when occurring in schools of several hundreds of individuals (Verlaque 1990). While common in central Portugal, this species is normally found in very low abundances in northern Portugal (this study, Rodrigues et al. 2011, Henriques et al. 2013). Hence, although we do not have unambiguous evidence, it is likely that this species is responsible for a large part of the consumption of kelp recruits in central Portugal.

The sea-urchin *Paracentrotus lividus* displayed the same pattern of abundance as herbivorous fishes, being noticeably more abundant in central than in northern Portugal. In addition, a variable behavior of this echinoid may also contribute to minored grazing on northern Portugal. This is connected with the high densities of the predatory starfish *Marthasterias glacialis* found on rocky reefs in northern Portugal (Tuya & Duarte 2011). Although mussels are the preferred prey of this species (Tuya & Duarte 2011), its presence might induce indirect effects on sea-urchins. Predators that are continuously present in a particular habitat may provide persistent cues to alter the behavior of potential prey, including a reduction in their foraging area (Schmitz et al. 2004). In any case, sea-urchins seem to have a minor overall influence on kelp recruits regardless of the region, due to the low number of sea-urchin bite marks compared to fishes. This is an interesting finding due to the majority of the studies regarding herbivory on kelps are associated with urchin grazing, not with fish grazing. Although we cannot ascertain for sure that bite marks on kelp recruits were exclusively from fishes, the used methods allowed equal exposure of kelps to both fishes and urchins and the vast majority of kelp recruits showed bite marks consistent with fish herbivory (see example in Fig 1).

Table 2. Trophic guilds (inv-invertebrate feeders, ma-macrocarnivores, om-omnivores, her-herbivorous; according to Henriques et al. 2013 and www.fishbase.org) and abundances (ind 100 m^{-2}, mean, SD, n = 25) of fish species at each region and year.

Species	Trophic guild	**PENICHE**				**VILA DO CONDE**			
		2012		**2013**		**2012**		**2013**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Boops boops	om	2.18	5.14	0.12	0.60	0	0	0.24	0.59
Coris julis	inv	0.12	0.44	0.64	2.80	0	0	0.40	1.60
Centrolabrus exoletus	inv	0.16	0.80	0	0	0	0	0	0
Ctenolabrus rupestris	inv	0.08	0.28	0.08	0.28	0.72	2.13	0.56	0.64
Dicentrarchus labrax	ma	0	0	0	0	0.28	0.84	0.08	0.40
Diplodus sargus	om	0.24	0.83	12.60	19.58	0.40	1.41	0	0
Diplodus vulgaris	om	8.80	14.04	27.68	13.75	0.92	2.98	0.28	1.80
Gobiusculus flavescens	inv	0.32	0.69	0	0	0	0	0	0
Labrus bergylta	inv	1.92	2.10	1.84	1.07	0.40	0.58	1.68	1.86
Labrus mixtus	inv	0	0	0	0	0.08	0.28	0	0
Labrus spp.	inv	0	0	0	0	0.72	0.68	0	1.02
Labrus viridis	inv	0	0	0	0	0.04	0.20	0	0
Oblada melanura	om	0.04	0.20	0	0	0	0	0	0
Parablennius pilicornis	inv	0.32	1.03	0	0	0	0	0.24	0.59
Pollachius pollachius	inv	1.04	3.13	0.48	1.26	2.56	8.00	0	10.72
Sarpa salpa	her	0	0	1.60	5.54	0	0	0	0
Serranus cabrilla	ma	0	0	0.08	0.28	0	0	0	0
Symphodus spp.	inv	1.88	1.72	3.28	2.51	0.40	0.65	0.24	0.66
Trisopterus luscus	inv	0.12	0.44	0.36	0.57	4.76	10.87	0.56	1.04

The regional differences in herbivory on kelp recruits demonstrated in this study matches the inverse pattern of abundance of adult kelps across continental Portugal (Tuya et al. 2012; Assis et al. 2013). Previously, the presence and fitness of kelps across southern European waters had been exclusively linked to bottom-up and climatic influences (Lüning 1990, Bartsch et al. 2008, Fernández 2011, Voerman et al. 2013). Our results highlight that herbivory might also play a role by affecting the survivorship of kelp recruits towards their southern distribution limit in continental Portugal. Moreover, increasing sea-water temperatures are expected to increase the arrival and spread of herbivores and will increase consumer pressure on recipient macroalgal assemblages at temperate latitudes (Vergés et al. 2014). In this context, reports on the presence of new herbivorous species across southern Europe are becoming more frequent with records of species of tropical affinities located northwards of their usual distribution range increasing across the Iberian Peninsula during the last decade (Bañón & Mucientes 2009, Rodrigues 2012), including herbivorous fishes (Canas et al. 2005, Abecasis et al. 2009).

While no long-term quantitative data exist, local communities in central Portugal describe kelp beds as abundant in the past at this region (Assis et al. 2009). At present, we cannot say for sure if the apparent loss of kelp beds in the region is permanent. However, our results suggest that re-establishment is unlikely, or at best will be very slow, as long as herbivore pressure remains large in open reef habitats.

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CHAPTER IV

THE ‘GOLDEN KELP’ *LAMINARIA OCHROLEUCA* UNDER GLOBAL CHANGE: INTEGRATING MULTIPLE ECO-PHYSIOLOGICAL RESPONSES WITH SPECIES DISTRIBUTION MODELS

Figure Chapter IV
Overview of an outdoor mesocosm experiment where seawater temperature and nutrient availability are manipulated to assess the physiological responses on the kelp *Laminaria ochroleuca*. Photograph by J.N. Franco

João N. Franco[1,2], Fernando Tuya[3], Iacopo Bertocci[1,4], Laura Rodríguez[5], Brezo Martinez[5], Isabel Sousa-Pinto[1,2], Francisco Arenas[1]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal

[2] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4150-181 Porto, Portugal

[3] IU-ECOAQUA, Grupo en Biodivesidad y Conservación, Universidad de Las Palmas de Gran Canaria, 35017, Las Palmas, Canary Islands, Spain

[4] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy

[5] Rey Juan Carlos University, Calle Tulipán sn., 28933 Móstoles, Madrid, Spain

ABSTRACT

The loss of marine foundation species, in particular kelps at temperate latitudes, has been linked to climatic drivers and co-occurring human perturbations. Ocean temperature and nutrients typically co-vary over local and regional scales and play a crucial role on kelp dynamics. Examining their independent and interactive effects on kelp physiological performance is essential to understand and predict patterns of kelp distribution, particularly under scenarios of global change. Crossed combinations of ocean temperatures and availability of nutrients were experimentally tested on juveniles of the 'golden kelp', *Laminaria ochroleuca*, from the northwestern Iberian Peninsula. Eco-physiological responses included: survival, growth and total N content. Results were embedded into a Species Distribution Model (SDM), which relates presence records and climatic and non-climatic data to forecast distribution patterns of *L. ochroleuca* under different climate change scenarios. Temperatures above 24.6 ºC were lethal irrespective of nutrients. Optimal growth of juvenile sporophytes occurred between 12 °C and 18 ºC and no nutrient limitation. The SDM, where ocean temperature was the main predictor of kelp distribution in line with temperature thresholds given by eco-physiological responses, suggests a future expansion towards northern latitudes and a retreat from the southern limit/boundary of the current distribution. Range-shifting of the golden kelp can have severe ecological impacts at regional and local scales. The expansion or retraction of the species along the European coast seems to be modulated mainly by temperature, but nutrient availability would be key to maintain optimal physiological performance. Our work highlights that the combination of empirical and modelling approaches is accessible to researchers and crucial to building more robust predictions of ecological and biogeographic responses of habitat-forming species to forecasted environmental change.

KEYWORDS

brown seaweed, climate change, Laminariales, macrophytes, modelling, multiple perturbations, nutrients, Portugal, southern Europe, temperature

INTRODUCTION

There is widespread evidence of responses of flora and fauna inhabiting aquatic and terrestrial environments to climate change (Rosenzweig *et al.* 2008; Burrows *et al.* 2011; Poloczanska *et al.* 2013; Pinsky *et al.* 2013; O'Connor *et al.* 2015). In particular, shifts in biogeographic patterns have been reported worldwide for terrestrial, e.g. birds and butterflies (Parmesan 2007), as well as marine species, e.g. algae and fishes (Perry *et al.* 2005; Lima *et al.* 2007; Wernberg *et al.* 2010, 2015). Indeed, it is well known that species distribution patterns are directly controlled by climate (Pearson & Dawson 2003 and references therein), which has been considered responsible for either expansions at some species' cool range edges, or contractions at the warm range edges (Chen *et al.* 2011; Sunday, Bates & Dulvy 2012; Martínez *et al.* 2012; Cahill *et al.* 2014). The latter responses may ultimately lead to local extinctions, especially in the case of sessile organisms (Parmesan 2006; Wiens 2016).

In the marine realm, temperature drives basic biological processes (Bozinovic & Pörtner 2015), which critically modulate the survival, growth, reproduction and recruitment of macroalgae and, hence, their distribution patterns (Lüning 1990; Izquierdo, Pérez-Ruzafa & Gallardo 2002; Lima *et al.* 2007; Gómez *et al.* 2009; Harley *et al.* 2012). Examples of effects of temperature changes on patterns of distribution of seaweeds, due to either sporadic heat waves (Wernberg *et al.* 2013, 2016) or persistent increments in temperature (Lima *et al.* 2007; Rosenzweig *et al.* 2008), have been documented across many regions. Concurrently with temperature, the availability of nutrients is a necessary condition for the metabolism of macroalgae (Müller *et al.* 2009; Gordillo 2012). Ocean warming will probably enhance the stratification of the upper mixed layer, leading to changes in the nutrient availability for primary producers (Behrenfeld *et al.* 2006). Also, changes in oceanographic processes can directly and indirectly affect nutrient dynamics; for example, in some coastal areas, the productivity of macroalgae depends on the seasonality and/or intensity and frequency of upwelling events (Graham *et al.* 2007; Philippart *et al.* 2007; Black *et al.* 2011; Lobban & Harrison 2012). Climate change is apparently intensifying upwelling-favourable winds in most eastern boundary systems, but not along the west coast of the Iberian Peninsula, where the intensity of the upwelling regime is weakening (Sydeman *et al.* 2014) in association with an increase of sea-surface temperature (SST) (Lemos & Pires 2004). The relaxation of the spring to late summer upwelling regime along the Iberian Peninsula seems, therefore, to simultaneously affect both nutrient supply and ocean temperatures, with potential consequences for macroalgal eco-physiology. For example, nutrients directly affect recruitment and survival of juvenile sporophytes of the ‘giant kelp’ *Macrocystis pyrifera*, particularly during El Niño events, characterized by warm and nutrient-poor waters (Hernández-Carmona *et al.* 2011).

While increasing attention has been recently devoted to the effects of environmental or/and anthropogenic factors on aquatic ecosystems

(Halpern *et al.* 2008; Crain, Kroeker & Halpern 2008; Harley *et al.* 2012), these are often examined separately and considerable knowledge gaps remain concerning the effects of multiple stressors on species' functional responses (Wernberg, Smale & Thomsen 2012). Indeed, most marine climate change studies included only a single predictor – often temperature – of species' distributions, and thus did not explicitly take into account other potential drivers of change (but see Wahl *et al.* 2011; Bertocci *et al.* 2014; Gaitan-Espitia *et al.* 2014). Understanding the effects of multiple drivers is particularly challenging when their combined role cannot be predicted from single-driver studies, i.e. there are non-additive interactions (Folt & Chen 1999). This mechanistic knowledge, in conjunction with Species Distribution Models (SDMs), can predict shifts in species' distribution patterns under varying climate change scenarios (Martínez et al. 2015 and references therein). By correlating the occurrence of certain species with climatic and other physical factors, SDMs are a useful tool to predict habitat preferences or distributional changes (Araújo & Guisan 2006). The integration of physiological knowledge with SDMs into a convergent framework results in more robust predictions (Buckley *et al.* 2011; Wittmann *et al.* 2016), for example through taking advantage of knowledge on physiological lethal and sub-optimal values to predict areas of absence. However such an approach has been seldom taken (but see Martínez et al. 2015).

In the last decades, a growing body of literature has addressed changes in the structure and dynamics of several kelp species, including reductions in the stability of populations and regional distributional shifts (Wernberg *et al.* 2010; Smale *et al.* 2015; Filbee-Dexter, Feehan & Scheibling 2016). Even though kelps can adjust their physiological performance to environmental variation (Biskup *et al.* 2014), identifying thresholds of physiological acclimation is essential to understanding the impacts of climate change on the distribution of these organisms.

Here, we tested whether ecological responses of a kelp species to physical drivers were consistent with its current distribution range, and anticipated the future range extensions and retractions of the same species according to a SDM. Specifically, we assessed physiological thermal thresholds under varying nutrient availability to accurately determine the species’ functional responses (e.g. growth and survival). This information was then combined with SDMs to build more accurate predictions of the future distribution of the species. We used, as a model organism, the ‘golden kelp’, *Laminaria ochroleuca*, a seaweed with relevant ecological functions in the northern hemisphere (Arroyo *et al.* 2004; Rodil *et al.* 2015). This is a southern-Lusitanic, warm-water species, that is distributed from Morocco to southern UK, and also forms deep-water populations in the Azores, the Gorringe seamount (southwest of Portugal) and some Mediterranean locations (Birkett 1998; Assis *et al.* 2009; Flores-Moya 2012; Ramos *et al.* 2016). This kelp has declined in southern Europe (Fernandez 2011; Tuya *et al.* 2012; Assis *et al.* 2013; Voerman, Llera & Rico 2013), while it increased in southern UK (Smale *et al.* 2015). These shifts have been typically linked with temperature changes (Fernandez 2011; Smale *et al.* 2013; Pinho *et al.* 2015), even though herbivory may also play a critical role on the persistence of kelps from the Iberian Peninsula (Franco *et al.* 2015). Due to the warm water affinity of this species, we hypothesized a positive relationship between eco-physiological performance (growth, survival, and total nitrogen content) and experimental warming, until temperature exceeds the optimal levels. The positive effect of moderate increases in temperature was expected to be further enhanced by increased availability of nutrients. Conversely, decreased availability of nutrients was hypothesized to exacerbate, at least in part, the negative effects of temperature approaching the tolerance limit of *L. ochroleuca*.

MATERIALS AND METHODS

ALGAL COLLECTION

Juvenile sporophytes of *L. ochroleuca* (10-18 cm in total length, 6.1 ± 3.5 g, mean ± SD) were collected in January 2013 from the shallow subtidal habitat (~5 m depth) at three locations (São Bartolomeu, Amorosa and Viana do Castelo; 41º 34.39” N, 41º 38.47” N and 41º 41.93” N, respectively) from northern Portugal. The holdfasts of all individuals were cleaned of sediment and epiphytes in the field, immediately stored in a cool box and transported to the laboratory, where they were kept acclimatized for 7 days in a cooled (14 ºC) and aerated seawater tank (500 L) until the start of the experiment.

EXPERIMENTAL SETUP

Eight experimental levels of increasing temperatures were established: 12, 15, 18, 20, 22, 24, 26, and 28 ºC. The first four levels encompassed the annual average SST recorded from the southern Portuguese coast to the French Brittany coast in the last decades (Gómez-Gesteira *et al.* 2008). The remaining levels were chosen to represent high temperature events which *L. ochroleuca* sporophytes may experience at its southern distribution limit (22 and 24 ºC), and its probable upper thermal survival limit (26 and 28 ºC) (Flores-Moya 2012). During the course of the experiment, *L. ochroleuca* sporophytes were kept in outdoor tanks (four independent tanks per temperature, 60 L each) within temperature-controlled seawater baths to prevent the effects of ambient temperature oscillations. Water temperature was controlled in each tank by using simultaneously chillers and heaters, which are regulated by digital controllers and individual temperature probes (Aqua Medic ® AT Control System controllers, GmbH, Bissendorf, Germany). Temperature and salinity values were monitored daily and evaporation was compensated for by adding non-mineralized fresh water when needed. The average temperatures throughout the entire experiment were, respectively for each treatment: 12.3 ± 0.17, 15.0 ± 0.17, 18.0 ± 0.14, 20.0 ± 0.20, 22.0 ± 0.23, 24.0 ± 0.25, 25.9 ± 0.17, 28.3 ± 0.25 ºC (mean ± SD, n= 1536 measurements). Each temperature was crossed with two levels of availability of nutrients (high *vs.* low), providing a total of 32 experimental tanks, i.e. two tanks per combination of treatments). The enriched treatment (+Nut) was established by adding, every three days, inorganic N ($NaNO_3$) and P (NaH_3PO_4) to a final concentration ≥ 35 µM N and 5 µM P, respectively. Such values were about three times higher than the highest values typically recorded in the region (Doval, López & Madriñán 2016), and represented non-nutrient limitation similar to those recorded during upwelling events in coastal regions, i.e. >30 µM (Denny & Gaines 2007). The low level of nutrients (–Nut) corresponded to the lowest typical values recorded in the region (Doval, López & Madriñán 2016), and represented a nutrient-limited scenario similar to those found in summer in coastal systems with no land-source inputs. This treatment was established by using seawater specifically prepared before the experiment. This was achieved by maintaining 5000 L of natural seawater in an aerated tank with macroalgae (*L. ochroleuca* individuals different than those used in the experiment), naturally consuming nutrients. This aerated and open-to-air system prevented oxygen fluctuations and allowed to have a saturated system in terms of dissolved carbon avoiding pH fluctuations.

The concentration of macro-nutrients (nitrate and phosphate) was monitored every 4 days during one month through the collection of water samples that were immediately analyzed using a colorimetric auto-analyzer (Skalar® SAN Plus Segmented Flow Analyser), using

Skalar methods M461-318 (EPA 353.2) and M503-555R (Standard Method IP-450), respectively, and validating the analytical procedures with reference to samples containing known concentrations of each nutrient. When nitrate and phosphate concentrations dropped below 3.5 µM, algae were removed and the seawater was filtered (5 µ) and used to fill the experimental tanks allocated to the '-Nut' treatment. A total of 320 juvenile *L. ochroleuca* individuals were used, i.e. 10 individuals per tank. Each tank was continually supplied with air through the bottom to ensure water movement. Every 9 days, seawater and nutrients were renewed in each tank according to the corresponding treatment. Each tank was cleaned and *L. ochroleuca* fronds were gently scrubbed to remove overgrowing bacteria and epiphytes. This operation was carried out to minimize potential confounding effects due to shading and/or competition for nutrients by epiphytes. To check nutrient levels, water samples were taken during the third week of the experiment, when it was assumed that the algae had enough time to adapt to experimental conditions. Three replicated samples were taken from each treatment immediately after the weekly water change (initial) and 3 and 6 days later (final) for the +Nut and the -Nut treatment, respectively (see supplementary material, Table S1). The experiment ran for 36 days (from the 1st February until the 8th March 2013), under a natural photoperiod 10:14 light – dark cycle. The irradiance (PAR) was continuously monitored using a HOBO Micro Station with PAR light Smart Sensor (Onset Computer Corporation). To avoid exposure to direct sunlight, all tanks were covered with a neutral fiber glass mesh reducing by 30% incident PAR (see supplementary material, Fig. S1).

GROWTH AND MORTALITY

Before the start of the experiment, all *L. ochroleuca* sporophytes were individually tagged with a cable tie and a numbered by means of a plastic ring gently attached to the kelp stipe. During the experimental period, the fresh weight (FW) of each individual was measured at five times, i.e. 0, 9, 18, 27 and 36 days since the start of the experiment. Individuals were blotted dried with paper to remove excess water, weighted, and then returned to their respective tanks. Growth (g FW day^{-1}) was calculated as: Growth = $(W_t - W_o)/t$, where W_o was the initial fresh weight, W_t the fresh weight at each time, and t the number of days. Kelp individuals showing decay or discoloration over more than half of the lamina were considered dead, and so survival was estimated as the proportion over the total number of individuals per tank (Gao *et al.* 2012).

TOTAL NITROGEN CONTENT

The Nitrogen (N) content of kelp sporophytes was measured at the end of the experiment. Six individuals were selected at random from each treatment and dried in a convection oven at 60 °C for 48 h. After the measurement of dry weight, samples were crushed to a fine powder and the total N content determined through an Organic Elemental Analyzer – Flash 2000 and expressed as % of dry weight.

ANALYSIS OF DATA

Two-way analysis of variance (ANOVA) was used to test for effects of temperature and nutrients on the growth and the total N content of *L. ochroleuca* at the end of the experiment. Each ANOVA included the fixed factors ‘Temperature’ (six levels) and ‘Nutrients’ (two levels), with two replicates provided by the average of the ten *L. ochroleuca* individuals per tank. Before each ANOVA, the assumption of homogeneity of variances was checked through Cochran’s *C* test and data were transformed if necessary (Underwood 1997). Student-Newman-Keuls (SNK) *post-hoc* tests were used to compare significant means. We used logistic regression to explore the relationship between survival and levels of temperature and nutrients.

Data encompassing all individuals followed a binomial distribution (dead/alive). Since all observations were registered within a short range of the predicted variable (monotone likelihood), a logistic regression using a penalized likelihood method (Hilbe 2015) was carried out. All analyses were implemented through the R software (3.2.5), using the package logistf.

SPECIES DISTRIBUTION MODELS

Laminaria ochroleuca presence data were gathered from the Global Biodiversity Information Facility (GBIF) (http://data.gbif.org) and the Ocean Biogeographic Information System (OBIS) (http://iobis.org/es). Additional records were obtained through literature reviews (Pérez-Ruzafa *et al.* 2003; Assis *et al.* 2009; Díez *et al.* 2012), personal observations and communications. A total of 511 presence records of *L. ochroleuca* were finally compiled and geo-referenced onto a map using ArcGIS 10.1 (Fig. 1).

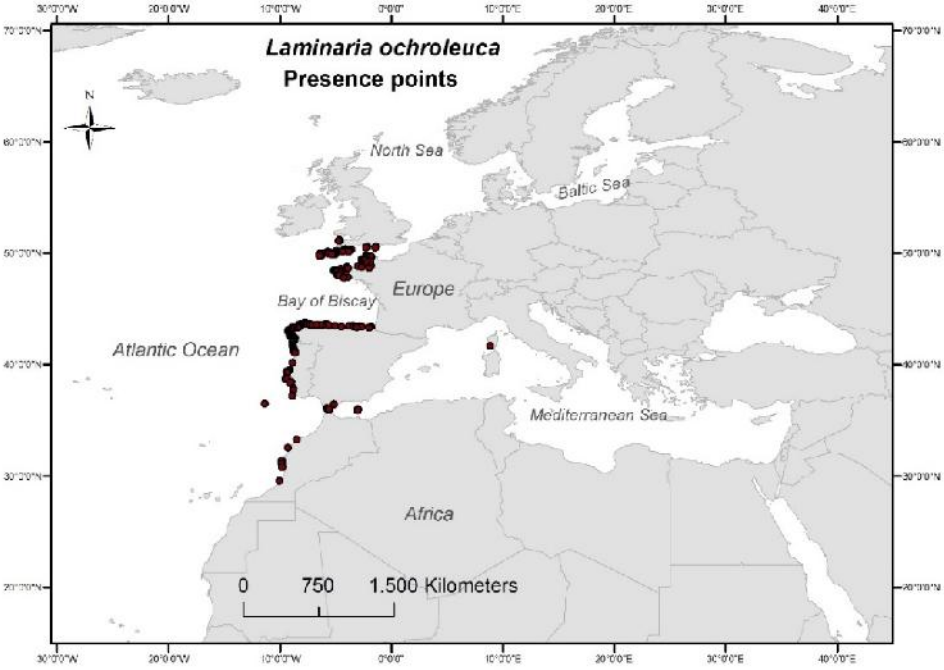


Figure 1.
Records of the kelp, L ochroleuca, across its distributional range (n=511).

The distribution area included the coasts of Europe and North of Africa restricted by a buffer of 20 Km from the coastline and a bathymetric mask of 40 m depth, encompassing the common depth range of this macroalgae. Environmental data were downloaded as raster layers from the global marine data set Bio-Oracle (Tyberghein *et al.* 2012), rescaled to 0.05 decimal degrees and restricted to the area of study. We initially evaluated the correlation between the available variables, which included the maximal, minimal, mean or range values of the sea surface temperature, calcite, chlorophyll *a*, diffuse attenuation (turbidity of the water column), dissolved oxygen, pH, salinity, phosphate, nitrate, silicate, cloud cover and PAR (see supplementary material, Table S2), by means of a Principal Component Analysis (PCA) (Lepš & Šmilauer 2005) and a Pearson's correlation matrix through the software CANOCOTM 4.5 (ter Braak J. F. & Šmilauer 2002). Among the groups of autocorrelated variables (r^2>0.80), we selected as predictors those with the highest potential importance for the biogeography of the target species (Lüning 1990). For example, maximum and minimum values were preferred relative to mean values due to their physiological influence (Martínez *et al.* 2015). The variables selected as potential predictors were: minimum of the monthly averages of sea surface temperature, maximum of the monthly averages of sea surface temperature, chlorophyll *a* concentration, nitrate, cloud cover and salinity, (see supplementary material, Table S2). We then applied Maximum Entropy Modelling (MaxEnt) to construct the SDM, a method that selects the statistical model which maximize the Entropy of the species probability distribution (Phillips, Anderson & Schapire 2006). Additionally, we built a generalized linear model (GLM) using the R package BIOMOD, a regression-like method that relates presence records and random pseudo absences with environmental layers (Thuiller *et al.* 2009). The contribution of each predictor variable in the model was analyzed by the MaxEnt permutation importance and percent contribution coefficients, as well as with the variable importance function of BIOMOD. By combining MaxEnt and the GLM, a final reduced model including the most important variables was finally computed. The performance of the models was evaluated using the predicted area under the curve (AUC) tool, provided by the Receiver Operating Characteristic (ROC) curve from MaxEnt (DeLeo & Campbell 1990). The ROC curve relates the sensitivity or true positives (fraction of presence records that are correctly classified as presences) against the proportion of false positives (1-specifity) (Allouche, Tsoar & Kadmon 2006). The performance in BIOMOD was measured by the AUC and the TSS test (i.e., sensitivity + specificity – 1). Models with AUC values higher than 0.85 indicated a good discrimination power (Phillips *et al.* 2006). Internal data-splitting validation was applied to confirm the variable importance of the final predictors in the training data (70% of presence points) and the consistency of the above evaluation metrics (AUC and TSS). MaxEnt was used to determine the habitat suitability index for all the study areas with the environmental conditions registered from 2002 to 2009, as well as to obtain future distribution projections by using rasters of forecasted physical conditions. The layers extracted from Bio-Oracle contained the information from the UKMO-HadCM3 model, which represents the conditions defined by the A2 scenario described by the Intergovernmental Panel on Climate Change (IPCC). The A2 scenario, with temperature increases of 2.6 ºC and CO_2 emissions >800 ppm until 2100, was the most severe among those provided by Bio-Oracle, (Meehl *et al.* 2007).The projections were run 10 times and the final projection was built up through averaging over all of them. A binarization of the map was generated by using the equal training sensitivity and specificity logistic threshold, as provided by MaxEnt (Liu *et al.* 2005), to discriminate the potential areas considered as suitable for the presence of the species and the areas where the species would be absent.

RESULTS

SURVIVAL, GROWTH AND TOTAL NITROGEN CONTENT

At the end of the experiment, *L. ochroleuca* survival was significantly affected by temperature (P < 0.001, Fig. 2, Table 1), but not by nutrients (P = 0.999, Table 1). No mortality of *L. ochroleuca* was found between 12 and 22 ºC. The threshold for the 50% survival (LC$_{50}$), was 24.6 ºC (Fig. 2), irrespectively of the nutrient treatment.

Table 1. Results of ANOVA (a) and logistic regression (b) testing, respectively, for the effects of temperature and nutrients on the growth and total N content and for the survival of juvenile sporophytes of *L. ochroleuca*. +Nut and –Nut indicate the high and low experimental nutrient concentrations, respectively. T12 to T24 indicates temperature (ºC). Ns = non-significant (at p>0.05). Significant results are highlighted in bold..

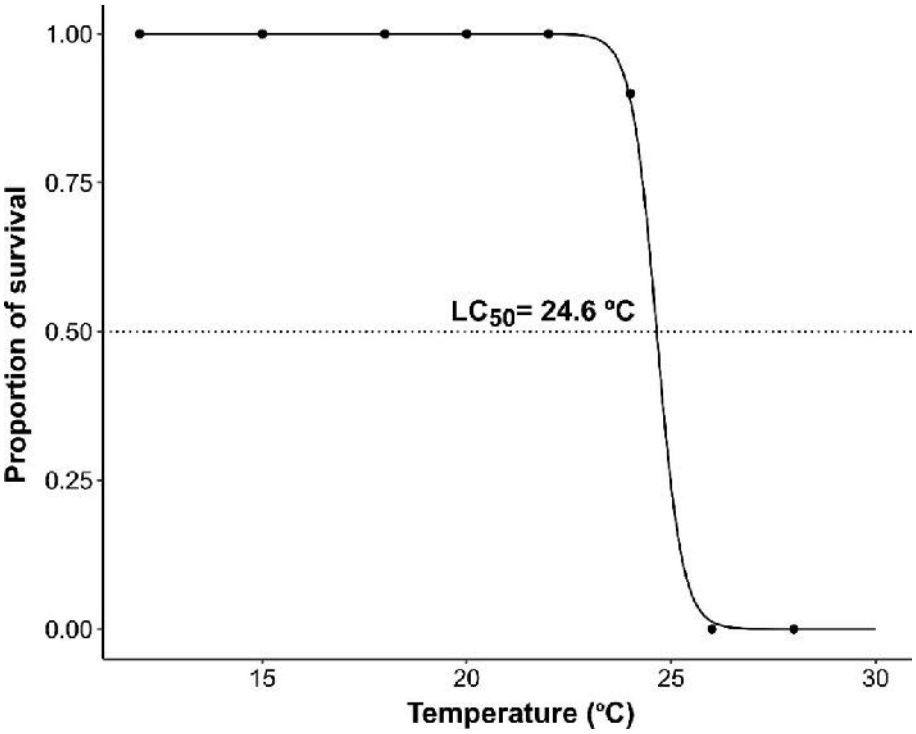
(a)				**(b)**	**ANOVA results**			
		Growth				**Nitrogen**		
Source of variation	**df**	**MS**	***F***	**P**	**df**	**MS**	***F***	**P**
Transformation		Ln(x+1)				none		
Cochran's		C = 0.436 (Ns)				C = 0.436 (Ns)		
Temperature	5	0.1985	6.56	**0.003**	5	0.0154	2.24	0.117
Nutrients	1	1.6985	56.08	**0.001**	1	0.97	141.48	**0.001**
Temperature X Nutrients	5	0.0568	1.88	0.172	5	0.0016	0.23	0.941
Residual	12	0.0303			12	0.0069		
SNK tests		Temp & Nut All Temp: +Nut > -Nut +Nut: T15=T12=T18>T20=T22=T24 -Nut: T15=T18=T12=T20=T22=T24				Temp All Temp: +Nut > -Nut		

(c)		**(d)**	**Regression results** [a]		
Model term	**Coefficient**	**Standard Error**	**95% C.I.** **Lower**	 **Upper**	**p**
(Intercept)	79.964	18.266	53.25	138.90	**<0.0001**
temperature	-3.244	0.754	-5.69	-2.15	**<0.0001**
nutrient	1.719 E-11	27.185	-65.98	65.98	0.999
temperature × nutrient	-7.09 E-13	1.115	-2.72	2.72	0.999

[a] likelihood ratio test=322.838 on 3 df, p=0, n=320
nutrient and temperature × nutrient were not included in the final model

The growth of juvenile sporophytes (Fig. 3; Fig. S2 Table 1) was larger under high nutrient concentration, particularly when temperatures were optimal ('+ Nut': mean growth = 4.54-5.57 g FW day^{-1} at 12, 15 and 18 ºC) and sub-optimal ('+ Nut': mean growth = 1.84-2.72 g FW day^{-1} at 20, 22 and 24 ºC), compared to low nutrient concentration at optimal temperatures ('- Nut': mean growth = 1.63-1.96 at 12, 15 and 18 ºC) and sub-optimal temperatures ('- Nut': mean growth = 1.31-1.42 g FW day^{-1} at 20, 22 and 24 ºC). The total N content was lower in the '-Nut' than in the '+Nut' treatment irrespective of temperature (Table 1, Fig. 4).

Figure 2.
Survivorship of *L. ochroleuca* sporophytes, at the end of the experimental period, according to varying temperatures
(y = 1 / 1 + e – (79.964535 + 3.244712 * χ, P<0.001).
The horizontal dotted line represents the 50% survivorship threshold.



SPECIES DISTRIBUTION MODELS

The final SDM was constructed including the predictors that were ranked as the three variables of highest importance in at least two of the three algorithms, namely: minimum of the monthly average of sea surface temperature (sstmin), the maximum of the monthly average of sea surface temperature (sstmax) and salinity, with a contribution of 57.5, 24.9 and 16.8%, respectively (Table 2). All the metrics applied to evaluate this final, reduced, model (AUC-MaxEnt, AUC and TSS from BIOMOD) were >0.92, indicating a good discrimination power of the models (Table 2). The MaxEnt current predictions showed the highest habitat suitability of *L. ochroleuca* on the northwestern coast of Spain, followed by the central and northern coasts of Portugal, along with the rest of the north of Spain and the westernmost coast of France and southern UK (Figs. 5A, 5C), representing conveniently the distribution of the species (Sheppard et al. 1978, tom Dieck (Bartsch) & de Oliveira 1993, Pérez-Ruzafa et al. 2003, this study, Fig. 1). The MaxEnt future projections using the A2 scenario suggested a partial reduction of the suitable habitat at the southern coasts of Portugal and the north of the Iberian Peninsula along with an increase of the suitable habitat northwards (Figs. 5B, 5D).

Table 2. (A) Percent contribution, permutation importance (MaxEnt) and variable importance (GLM BIOMOD) of selected models. Variables in bold were selected for the final model. (B) Results of metrics used to evaluate the models. Values are the same independent of the percentage of points used for training the model (30-70-100%).

(A)		**MaxEnt**	**MaxEnt**	**GLM BIOMOD**
Variable	Acronym	Percent contribution	Permutation importance	Variable importance
Minimum sea surface temperature	**sstmin**	**57.5**	**72.7**	**0.405**
Maximum sea surface temperature	**sstmax**	**24.9**	**26.5**	**0.376**
Salinity	**salinity**	**16.8**	0.0	**0.358**
Chlorophyll A Maximum concentration	chlomax	3.9	0.8	0.078
Nitrate	nitrate	0.7	0.0	0.059
Maximum Cloud cover	cloudmax	0.3	0.1	0.0

(B) Valuation test	**MaxEnt**	**GLM BIOMOD**
average test AUC for the 10 replicate runs	0.96	
AUC		0.99
TSS		0.92

The minimum sea surface temperature (sstmin), where presence records were found, ranged from 8 °C to 16.3 °C. Maximum sea surface temperature (sstmax) fluctuated between 15.1 °C and 24.6 °C. Regarding salinity, presence records varied between 34.4 psu and 38.2 psu suggesting that the total absence of records of *L. ochroleuca* in Baltic region is due to such low salinity (< 15 psu) environment (Meier, Kjellström & Graham 2006) (Fig. S3).

These results suggest that the algorithm used by MaxEnt approximated to the real values revealing a strong agreement between both used approaches and supporting temperature as the main predictor for this species distribution.

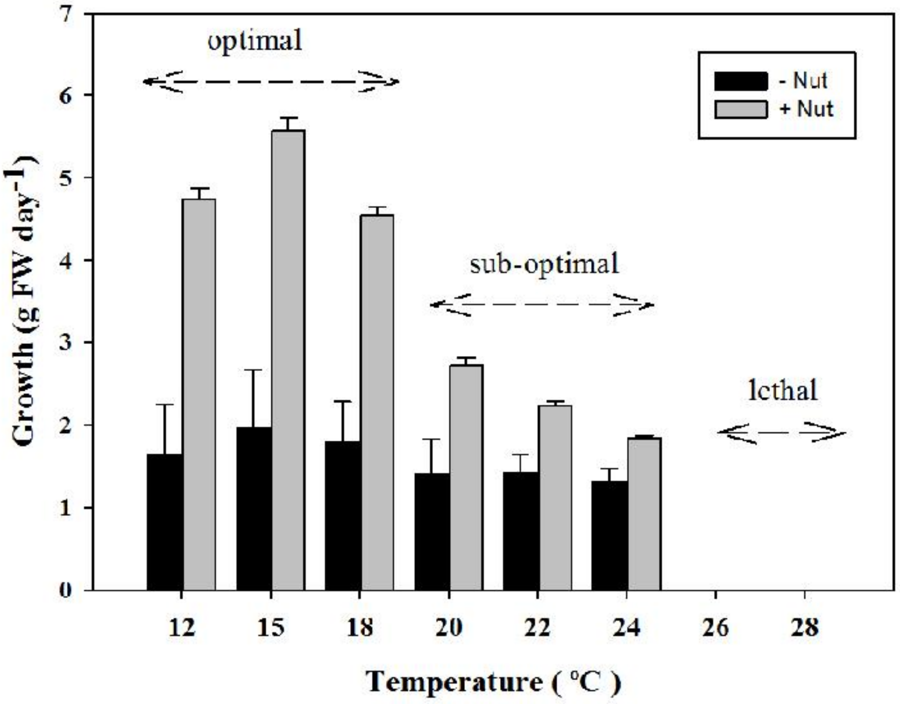


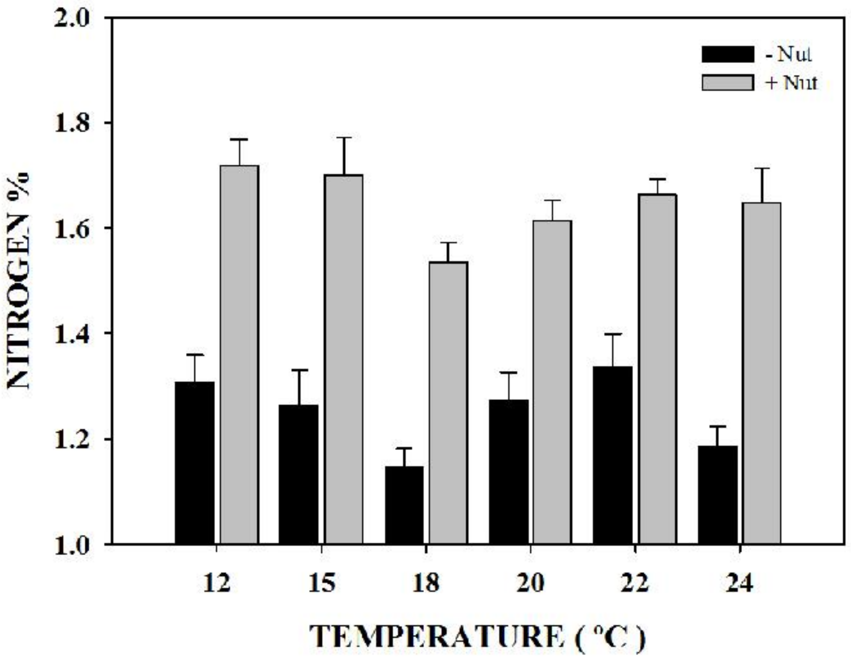
Figure 3.
Growth (mean + SE, n=20) of *L. ochroleuca* at the end of the experiment in each combination of temperature and nutrient availability.

DISCUSSION

In this study, we combined experiments manipulating environmental drivers with correlative modeling approaches, linking species presences to environmental conditions, to predict distribution shifts of the golden kelp, *Laminaria ochroleuca*. We found congruence between the two approaches, suggesting a range contraction at the Southern distribution range (Morocco and the Iberian Peninsula) and a potential expansion northward (e.g. the U.K.). The contraction of *L. ochroleuca* from southern Portugal (Assis *et al.* 2009; Tuya *et al.* 2012) and the rise at southern England (Smale *et al.* 2015) confirms these results. Temperature was the main driver contributing to forecast distribution trends, while salinity showed a comparatively lower contribution.

Our strategy is valuable for predicting macroecological patterns and by combining empirical physiological responses with correlative SDM models, we could 'double-check' and so enhance the accurateness of predictions of distribution shifts under climate change (Buckley *et al.* 2011; Martínez *et al.* 2015).

Figure 4.
Total nitrogen content (mean + SE, n=6) in *L. ochroleuca* sporophytes at the end of the experiment, in each combination of temperature and nutrient availability.



Our eco-physiological experiment revealed that juvenile kelp mortality reached a 50% (LC_{50} thermal threshold, e.g. Jones, Lima & Wethey 2010) when temperature was above 24.6 ºC, irrespective of nutrients. These results match the mean maximal SST the species experiences at its southern distributional limit in Morocco, suggesting a southern lethal distributional boundary in agreement with previous studies (Izquierdo *et al.* 2002; Flores-Moya 2012). The temperatures at which juvenile sporophytes achieved maximal growth in our physiological experiment (12 and 15º C) are within the lower range of mean maximum SST (~14º C) and the upper range of minimum SST (~16º C). Importantly, these values are within the mean annual water temperature where the species is frequent, such as along the Galician coast (42º N, 8º W and 44º N, 8º W, Torres 2003; Piñeiro-Corbeira, Barreiro & Cremades 2016). Physiological responses to temperatures were concurrently captured by the SDM, which included the mean maximal SST as the main climatic driver, followed by the regional variation in low salinity, which explains the absence of this species from the Baltic Sea. The importance of salinity in the SDM can be related with accentuated differences in this variable between areas in and outside the Baltic sea, which is characterized by mean low salinity < 15 psu (Meier *et al.* 2006); *L. ochroleuca* do not survive under these circumstances (John 1969). Moreover, the SDM suggests the importance of winter temperature (i.e., mean minimal SST) limiting the northern distribution of this species. Although low temperatures were not simulated in our manipulative experiment, this is likely because of the absence of *L. ochroleuca* at locations with mean minimal SST lower than 8º C. Previous experimental work indicated that, at temperatures below 10º C, reproduction *L. ochroleuca* gametophytes is compromised and the growth of young sporophytes is not competitive (Izquierdo *et al.* 2002). In our study, we focused on juvenile individuals. Even though thermal tolerances may differ among different life stages (Lüning 1984), the consequences of reaching a lethal thermal limit for juveniles are expected to exert an overall negative impact, potentially compromising subsequent ontogenic transitions and the maintenance of viable populations in southern Europe. In addition, the experimental part of our study was undertaken under natural light conditions and the potential modulation of responses to varying temperatures and nutrient regimes by different light scenarios (Brown, Edwards & Kim 2014) remains a matter to be tested.

Temperature is a key climatic variable explaining the biogeography of the golden kelp, as it is for many kelps worldwide. At low latitudes, these foundation macroalgae have shown declining trends linked to temperature increases, e.g. southern Europe (Fernandez 2011; Voerman *et al.* 2013) and western Australia (Wernberg *et al.* 2016). The predicted range contraction of *L. ochroleuca* at its southern distributional range is likely to be further exacerbated by the reduction in the intensity of upwelling that has occurred along the Iberian Peninsula in the last decades (Lemos & Pires 2004; Llope *et al.* 2007; Sydeman *et al.* 2014). Several kelp species are widely distributed along the Atlantic coast of Europe, showing a continuous distribution from the Arctic to Brittany, but southwards they are only present in areas of intense upwelling (Lüning 1990). Moreover, concomitant biological factors, difficult to include into large-scale SDMs, may also play a role in the predicted retraction. For instance, Franco et al. (2015) reported that kelps from warmer, southern, locations along the Portuguese coast are exposed to more intense grazing pressure than kelps from colder, northern, locations. Given the lack of detailed information on such possible influences, caution is required in predicting the expansion of *L. ochroleuca* northwards of its current distribution.

This is especially true since a common assumption of ecological niche theory is that species distributions are static in space and time, meaning that the occurrence of a species is in equilibrium with its environment (Guisan & Thuiller 2005).

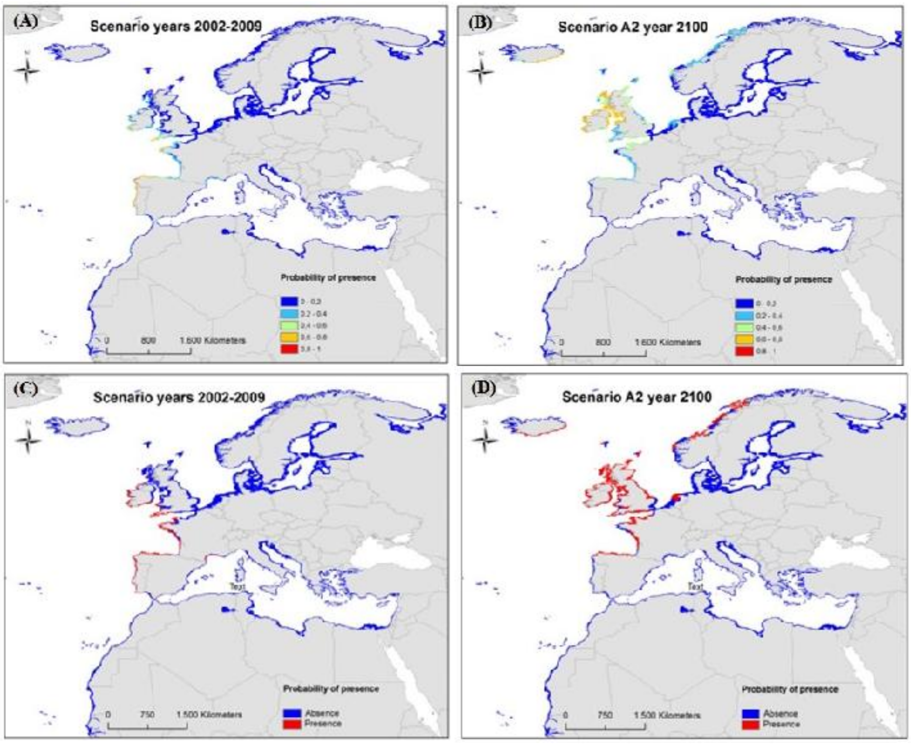


Figure 5. MaxEnt projections, including (A) current habitat suitability (from year 2002 to 2009), (B) predicted habitat suitability in 2100 (A2 IPCC scenario), (C) current presence/absence distribution, and (D) predicted presence/absence distribution in the 2100 scenario.

During range expansion, instead, a pioneer species might not be in such a condition due to several factors, including biological interactions and dispersal limitation (Pearson *et al.* 2006; Araújo & Luoto 2007). Therefore, it is hard to known how much of a species’ suitable habitat, exclusively determined by the species’ requirements and/or tolerances, is represented by its current or future habitat (Guisan & Thuiller 2005; Pacifici *et al.* 2015). Nevertheless, expected contracted distributional edges, where environmental conditions are close to species physiological tolerance thresholds, support the trends forecasted by SDMs (e.g. this study, Martínez *et al.* 2015).

Our results suggest a non-additive effect of temperature and nutrients on kelp growth, while no nutrient-related effects on survival were found. Kelp growth was considered ‘optimal’ at 15 ºC and sub-optimal between 20-24 ºC with growth being significantly reduced by

48% and 76% under high and low nutrient conditions, respectively. Responses of species to abiotic variables are complex and may vary geographically depending on environmental conditions. Our experiment identified the relative importance of nutrients for kelp performance at optimal and suboptimal temperature conditions that were otherwise not captured by the SDMs. Specifically, at optimal temperatures (12-18 ºC), the benefit of nutrient availability was fully taken by the kelp. Outside the optimal thermal range, on the contrary, *L. ochroleuca* seems able to accumulate reserve nutrients, but not to use them to support growth. Variable nutrient uptake, assimilation, and storage capabilities were reported for perennial kelps which, for example, exhibit seasonally-varying periods of growth to overcome subsequent periods of low resource availability or sub-optimal environmental conditions (Sheppard *et al.* 1978; Bartsch *et al.* 2009; Gordillo 2012). Interestingly, the reduction of kelp physiological performance (growth) at low nutrient availability was not negative *per se*; kelp growth rate under such circumstance was still positive (> 1 g FW day $^{-1}$). However, if extended periods of low nutrient availability occur, particularly under high temperatures resulting in reduced growth, the fitness of individuals would likely be compromised. Such mechanisms may critically contribute to reducing the resilience of kelp populations (Wernberg *et al.* 2010). In this context, the non-inclusion of N as a predictor in the SDM was not crucial in setting the distributional limits of the species. On the other hand, the manipulative experiment indicated that nutrients are important supporting kelp growth, especially at optimal thermal conditions. This apparent inconsistency is explained as SDMs are well suited to capture latitudinal changes in patterns of distribution, while eco-physiological experiments are expected to capture responses of individuals to factors that operate mainly at local to regional scales. Indeed, scaling is a major issue for any kind of SDM (Guisan & Thuiller 2005) and, if regional models of higher resolution (reduced geographic area) are performed, the contribution of non-climatic physical variables, such as nutrients, might be more determinant (Guisan & Thuiller 2005; Kearney & Porter 2009).

Distributional range shifts of habitat formers may be predicted with greater confidence by combining mechanistic and correlative approaches, which are, however, rarely combined together (but see Martínez *et al.* 2015). Here, we have performed eco-physiological experiments under realistic and ecologically relevant conditions to be transferred, and embedded, into SDMs by using mean monthly SST, as reference to thermal conditions. Sea surface temperature data by satellite have the ability to detect general patterns of ecological importance and can be used as a reference to real environmental conditions (Smale & Wernberg 2009). This is particularly the case for ocean temperatures, not subjected to large oscillations relative to other variables, such as aerial temperatures, for which mean values would be much less precise (Kearney & Porter 2009; Martínez *et al.* 2015). We acknowledge that our strategy, i.e. mechanistic SDMs, are sometimes difficult to construct, due to the requirement of collecting specific data on life-history traits of a given organism, and because of performing extensive field and laboratory validation. However, in some cases, available literature may offer appropriate information for the construction of better predictions to combine knowledge on physiological thresholds to environmental conditions and correlative SDMs. This was the case in our study, which took advantage of the northern distributional temperature limit of *L. ochroleuca*, e.g. Bartsch (1993). In a broader perspective, such an option should be applicable in climate change scenarios, where range contractions due to physical stress are expected to be mostly driven by physiological responses of species reaching their tolerance limits. The multidisciplinary approach established in this study represents a valid tool to assess range shifts of ecologically important species induced by climate change, especially when consistent outcomes from different procedures strengthen the robustness of their derived predictions.

SUPPLEMENTARY MATERIAL

Table S1. Mean (± SE, n = 3) nutrient concentrations before (initial) and after (final) weekly water changes at 3 and 6 days for high (+Nut) and low (-Nut) treatments, respectively.

		NO_3 (μ mol L^{-1})		PO_4 (μ mol L^{-1})	
Temperature (°C)	**Nutrient concentration**	**initial**	**final**	**initial**	**final**
12	**High**	39.96 ± 0.98	12.69 ± 0.30	5.18 ± 0.40	2.33 ± 0.18
	Low	3.49 ± 0.11	0.35 ± 0.01	0.44 ± 0.02	0.09 ± 0.00
15	**High**	39.74 ± 0.68	11.92 ± 0.20	5.17 ± 0.27	2.07 ± 0.11
	Low	3.56 ± 0.13	0.25 ± 0.01	0.45 ± 0.02	0.05 ± 0.00
18	**High**	39.88 ± 0.14	12.76 ± 0.04	4.92 ± 0.14	2.16 ± 0.06
	Low	3.41 ± 0.12	0.27 ± 0.11	0.45 ± 0.02	0.08 ± 0.01
20	**High**	39.60 ± 0.61	17.82 ± 0.27	5.28 ± 0.08	2.64 ± 0.04
	Low	3.54 ± 0.12	0.53 ± 0.02	0.44 ± 0.03	0.10 ± 0.01
22	**High**	39.70 ± 0.88	21.83 ± 0.48	4.76 ± 0.11	2.62 ± 0.06
	Low	3.62 ± 0.11	0.62 ± 0.02	0.47 ± 0.01	0.12 ± 0.00
24	**High**	41.06 ± 0.82	24.64 ± 0.49	5.33 ± 0.37	3.46 ± 0.24
	Low	3.66 ± 0.24	0.73 ± 0.05	0.45 ± 0.02	0.13 ± 0.01

Figure S1.
PAR (hourly means from daily measurements n=36) during the experimental period.

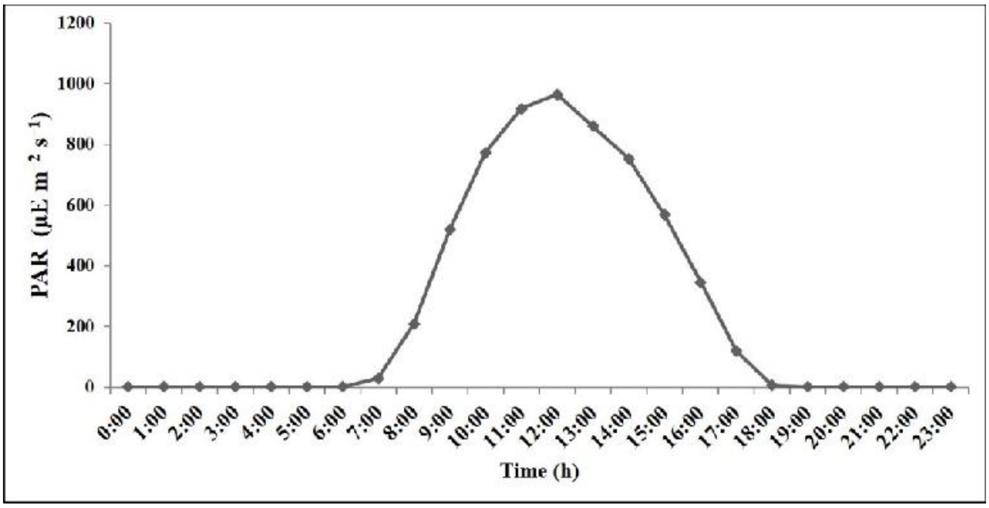
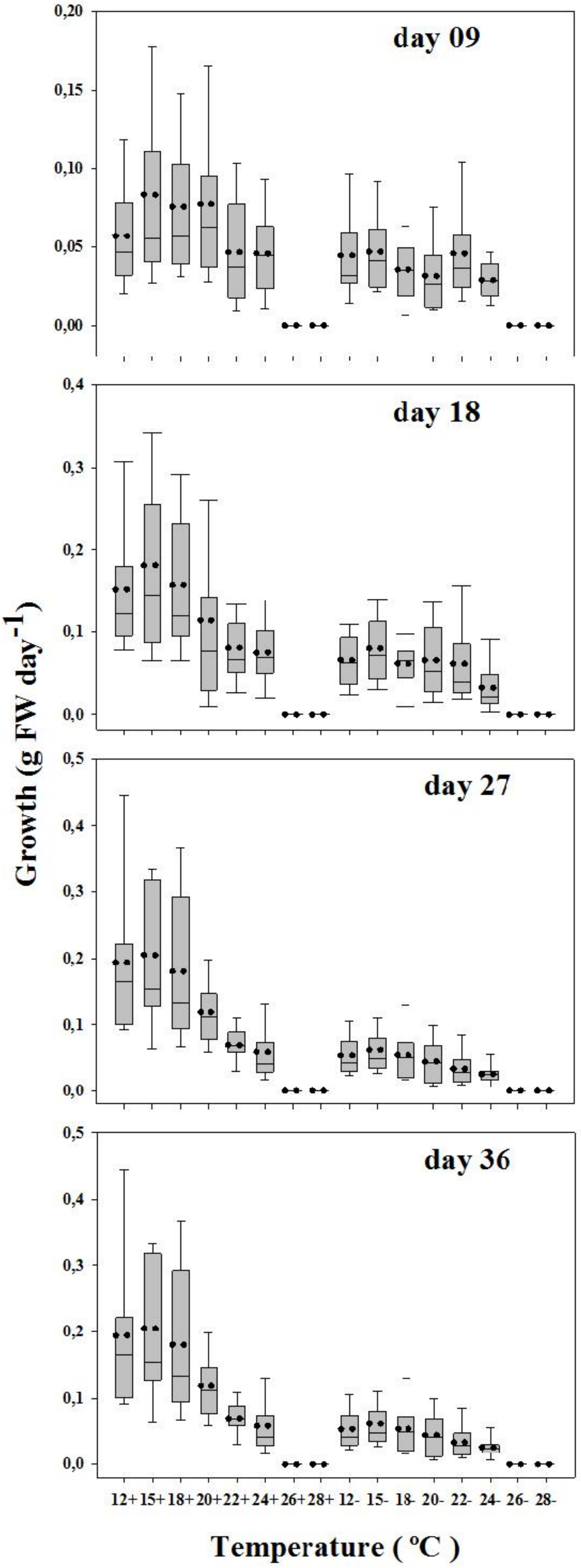


Table S2. Environmental variables extracted from Bio-Oracle (Table adapted from Tybergheim et al. 2012). Variables in bold were selected by the full model.

Acronym	Variable	Units	Origin	Source	Original Resolution
chlomax	Chlorophyll *a* Max. concentration	mg/m^2	Temporal maximum from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)
chlomean	Chlorophyll *a* Mean concentration	mg/m^2	Temporal mean from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)
chlomin	Chlorophyll *a* Min. concentration	mg/m^2	Temporal minimum from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)
chlorange	Chlorophyll A Range	mg/m^2	Temporal range from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2Km)
cloudmax	Cloud cover Max	%	Temporal maximum from monthly images (2005-2010)	Terra-MODIS	5arcmin (9.2 Km)
cloudmean	Cloud cover Mean	%	Temporal mean from monthly images (2005-2010)	Terra-MODIS	5arcmin (9.2 Km)
cloudmin	Cloud cover Min	%	Temporal minimum from monthly images (2005-2010)	Terra-MODIS	5arcmin (9.2 Km)
damax	Diffuse Attenuation Max (turbidity of the water column)	1/m	Temporal maximum from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)
damean	Diffuse Attenuation Mean	1/m	Temporal mean from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2Km)
damin	Diffuse Attenuation Min	1/m	Temporal minimum from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)
nitrate	Nitrate	µmol/l	DIVA interpolation of in-situ measurements	WOD 2009	5arcmin (9.2 Km)
parmax	photosynthetically available radiation Max	Einstein/ m^2/day	Temporal maximum from monthly climatologies (1997-2009)	SeaWiFS	5arcmin (9.2Km)
parmean	photosynthetically available radiation Mean	Einstein/ m^2/day	Temporal mean from monthly climatologies (1997-2009)	SeaWiFS	5arcmin (9.2 Km)
phosphate	Phosphate	µmol/l	DIVA interpolation of in-situ measurements	WOD 2009	5arcmin (9.2 Km)
salinity	Salinity	PSS	DIVA interpolation of in-situ measurements	WOD 2009	5arcmin (9.2 Km)
sstmax	sea surface temperature Max	°C	Temporal minimum from monthly climatologies (2002-2009)	MODIS	5arcmin (9.2 Km)
sstmean	sea surface temperature Mean	°C	Temporal mean from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)
sstmin	sea surface temperature Min	°C	Temporal minimum from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)
sstrange	sea surface temperature Range	°C	Temporal range from monthly climatologies (2002-2009)	Aqua-MODIS	5arcmin (9.2 Km)

Figure S2.
Box and whisker plots showing the growth of kelp sporophytes, according to experimental temperatures and nutrients availability, at 9, 18, 27 and 36 days from the start of the experiment. Whiskers are minimum and maximum and black dotted line is the mean of the growth at each treatment combination (n=20). (+) and (-) are high and low nutrient treatments, respectively



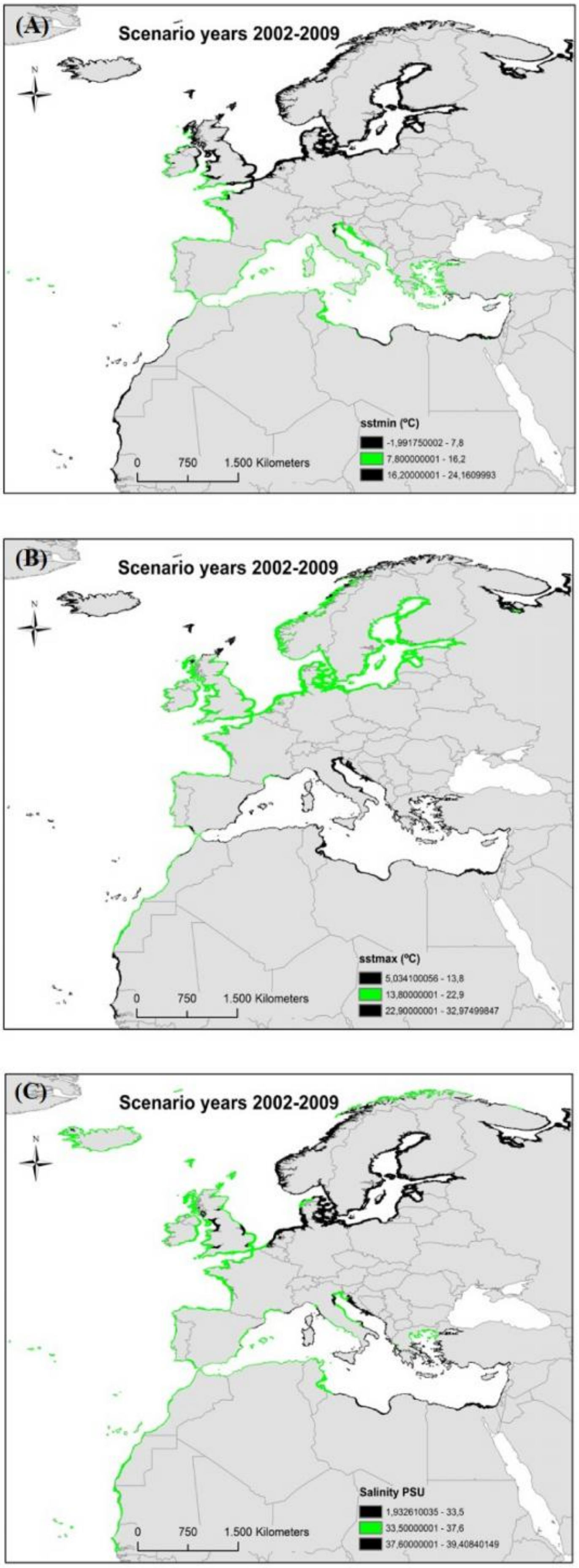


Figure S3. Maps representing the areas where the values of the variables: (A) sstmin, (B) sstmax and (C) salinity fall inside the presence threshold determined by MaxEnt.

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CONCLUSIONS AND FUTURE REMARKS

Figure Conclusions. Underwater landscape of Viana do Castelo region where the sea urchin *Echinus esculentus* and *Laminaria hyperborea* are present. Photograph by J.N. Franco

CONCLUSIONS

Through extensive observational surveys, the present study on kelp ecosystems in the Atlantic Iberian Peninsula increased the current knowledge on spatial and temporal patterns of distribution, abundance and diversity of kelp populations and their major consumers in southern Europe. In parallel, a set of manipulative experiments contributed to the understanding of how abiotic and biological processes, acting separately or in complex interactions, can shape their ecological and physiological responses and their expected shifts in distribution under climate change scenarios. The main findings and implications of the evidences gathered during this PhD project are summarized below.

1. Despite kelp beds of the Iberian Peninsula are naturally variable over temporal (interannual) and spatial scales (among reefs located kilometres apart), there is an overall decreasing trend, as reported by previous studies.
2. The persistent occurrence of kelps is restricted to northern Portugal and Galicia. This suggests that the north-western part of the Iberian Peninsula represents a ‘refuge’, where multi-specific kelp forests are still present.
3. Herbivory is a key regulatory process of kelp performance, at local and regional scales, and across different life stages of kelp individuals, particularly in the warmer regions of western Iberia.
4. Herbivore impacts are relatively more important in the ‘warm’ region, i.e. the central Iberian coast, where juvenile kelp individuals may be heavily consumed, especially when growing on flat reefs particularly exposed to herbivorous fishes.
5. Juvenile kelps are less consumed if they can take advantage of the protection provided by crevices, hence reef topography plays

an important role as buffer against grazing pressure where kelp consumers are abundant.

6. At present, neither the compensatory growth of adult kelps nor the physical protection given by reef topography seems very effective in counterbalancing kelp consumption. Therefore, the re-establishment of depauperated kelp populations is unlikely, or at best will be very slow, as long as herbivore pressure remains high or is even increasing, such as along central and southern coasts of western Iberia, where tropical and subtropical-affinity fish species are becoming more frequent.
7. Seawater temperature plays an important influence on kelp performance. Increases in seawater temperature, due to either sporadic heat waves or persistent increments, are predicted to drive a displacement of the distributional range shift of particular kelp species, such as *L. ochroleuca*.
8. Overall, Iberian kelp forests are modulated by both biotic (“top-down”) and abiotic (“bottom-up”) processes. Under climate projections for the 21st century, synergistic effects of both types of processes are expected to compromise Iberian kelp communities.

FUTURE REMARKS

Emphasis in future studies should be given to understand abiotic and biotic interactions, and their dynamics, as drivers of kelp performance, abundance and distribution. This study clearly indicated that multiple processes do not operate alone in coastal environments, naturally raising new ecologically relevant questions, including: is really kelp decline in Iberia only or mainly driven by temperature, nutrients and herbivory? What other factors are contributing? Are kelp responses to stressors general and pervasive, or do they vary between species not yet assessed? How deeply and on which scales is the resilience of these ecosystems affected? Is it possible for these kelp ecosystems to recover in regions where they were abundant in the past, possibly also through appropriate and feasible restoration measures? Will the current trend of kelp decline continue through time, or is it a snapshot resulting from the lack of long term data suitable to identify complex cycles of increases and decreases in abundances?

Addressing such questions has crucial implications at the ecological, social and economic level, although is made further difficult by expected non-linear responses of kelp to multiple pressures. However, present findings and results of other studies performed in other kelp-dominated regions may contribute to better direct future studies in Iberia. For example, there is evidence that varying wave energy scenarios, competition with introduced species, or increase of substrate sedimentation associated with dominant turf-forming algae can negatively impact kelp-dominated ecosystems. Understanding the actual responses of species to changes in multiple stressors requires a detailed understanding of what drives physiological vulnerability, recognizing that changes that are stressful to one species may benefit another species, even in the same system. Such complex interactions can be explored and disentangled only through a holistic approach involving the collection of data suitable to capture variation in the underlying environment across spatial and temporal extents, preferably at fine spatial resolution, combined with manipulative experiments. Subsequently, the obtained information will also provide the basis of refined and more general predictive models of shifts in kelp distribution able to include the largest possible range of relevant variables, including the nature and strength of species interactions, dispersal abilities and geographic variation in genotypic and phenotypic traits.