UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologia

Ecology of west Iberian kelps: *Laminaria ochroleuca* and *Saccorhiza polyschides* living near their distributional limits

Tânia Ribeiro Pereira

Doutoramento em Ecologia
Especialidade de Ecologia das Populações

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Tânia Ribeiro Pereira

Tese orientada por:
Prof. Doutora Ester A. Serrão
Prof. Doutora Myriam Valero
Prof. Doutor Gareth A. Pearson

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DECLARAÇÃO DE AUTORIA DE TRABALHO.

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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RESUMO

Esta tese aborda a ecologia das espécies de kelp, pouco estudadas, do Oeste da Península Ibérica.

O capítulo I centrou-se na dinâmica populacional. Com excepção de *Laminaria hyperborea*, as florestas de kelp pouco profundas demonstraram ser auto-sustentáveis, embora fortemente impactadas por condições ambientais extremas. É de esperar que, com o aquecimento global, estas populações de áreas menos profundas se tornem mais jovens e dependentes da dispersão de esporos subtidais.

No capítulo II, a resiliência das populações pouco profundas de *L. ochroleuca* foi estudada. Num dos locais, a cobertura de algas foi completamente removida durante uma tempestade. Os resultados indicam que destruições extensas dificultam a recuperação da população dado que desprovêem a população de indivíduos maiores, produtores de esporos, e da protecção do dossel.

O histórico de vida de *S. polyschides* varia com a latitude. A Norte os esporófitos estão presentes e recrutam o ano inteiro, enquanto a Sul são sazonais. Embora esta diferença tenha sido atribuída a limitações de temperatura, no capítulo III é demonstrado que tal não explica estas observações, dando origem à nova hipótese que tal diferença é causada pela limitação de nutrientes a Sul.

O capítulo IV focalizou o desenvolvimento microscópico de *L. ochroleuca* e *S. polyschides*. Embora o limite de temperatura das duas espécies seja semelhante, *S. polyschides* demonstrou menor mortalidade e um desenvolvimento mais rápido, apesar de crescer vegetativamente, em oposição aos gametófitos femininos unicelulares de *L. ochroleuca.*

Visto que tanto *L. ochroleuca* como *S. polyschides* são encontradas a baixa profundidade, no capítulo V o stress a que estão sujeitas devido ao rápido aquecimento foi estudado expondo as duas espécies, dos limites geográficos de *L. ochroleuca*, a choques térmicos sucessivos, simulando a baixa-mar. *L. ochroleuca* do Sul foi a mais resistente, enquanto entre *S. polyschides* dos dois locais não foram observadas diferenças.

PALAVRAS-CHAVE

Laminaria ochroleuca; *Laminaria hyperborea*; *Saccorhiza polyschides*; dinâmica populacional; temperatura; afloramento costeiro

ABSTRACT

This thesis focused on the understudied western Iberian kelps.

Chapter I focused on population dynamics. With exception of *L. hyperborea*, shallow kelp forests seemed to be self sustainable, although highly impacted by extreme environmental conditions. With further climate change, it might be expected that the shallower population will become younger and dependent on spore dispersal from the subtidal.

In Chapter II the resilience of shallow *L. ochroleuca* populations was studied. In one of the sites, canopy was completely removed during a storm. Results indicate that extensive removal hampers the population's ability to recover as it stripes the area from individuals big enough to produce spores. In addition, the lack of protection offered by the canopy hampers recruitment.

S. polyschides has different life histories across latitudes. At the poleward range, individuals are able to overwinter and recruit year-round, while low-latitude macroscopic sporophytes are seasonal. While this difference has commonly been attributed to temperature limitations, chapter III shows that temperature does not offer an explanation for the different life histories, raising the novel hypothesis that such difference was caused by nutrient limitations at low-latitudes.

Chapter IV focused on the microscopic development of *L. ochroleuca* and *S. polyschides*. While they showed similar temperature limitations, *S. polyschides* showed overall lower mortality and quicker development, despite showing more vegetative growth by the gametophytes when compared to unicellular *L. ochroleuca* female gametophytes.

As both *L. ochroleuca* and *S. polyschides* can be found in shallow areas, their thermal stress responses were compared by exposing recruits of the two species, from the geographical edges of distribution of *L. ochroleuca,* to repeated heat shock, simulating low tides. Recruits of *L. ochroleuca* from the low latitude areas were the most resistant, while no differences were observed between *S. polyschides* from the two latitudes.

KEYWORDS

Laminaria ochroleuca; Laminaria hyperborea; Saccorhiza polyschides; Population dynamics; Temperature; Upwelling

INDEX

PUBLICATIONS

CHAPTER 3

Pereira, T.R., Engelen, A.H., Pearson, G.A., Valero, M. & Serrão E.A. (*in press*). Contrasting timing of life stages across latitudes – a case of a marine forests forming species. *Marine Ecology Progress Series*

CHAPTER 4

Pereira, T.R., Engelen, A.H., Pearson, G.A., Serrão, E.A. & Valero, M. (2011). Temperature effects on the microscopic haploid stage development of *Laminaria ochroleuca* and *Saccorhiza polyschides*, kelps with contrasting life histories. *Cahiers de Biologie Marine*. 52: 395-403

CHAPTER 5

Pereira, T.R., Engelen, A.H., Pearson, G.A., Valero, M. & Serrão, E.A. (2015). Response of kelps from different latitudes to consecutive heat shock. *Journal of Experimental Marine Biology and Ecology*. 463: 57-62

INTRODUCTION

INTRODUCTION

WHAT IS A KELP?

The term kelp was firstly used to designate the ashes of brown seaweeds. It was later given an ecological meaning, designating large brown algae capable of forming marine forests. Although it is sometimes used as a synonym for the order Laminariales, kelp is not a taxonomical term, as it includes species from various orders: Laminariales (as, for instance, *Laminaria, Saccharina and Macrocystis*), Tilopteridales (as is the case of *Saccorhiza*), Desmarestiales (such as *Desmarestia*) and Fucales (like *Durvillaea* and the Norwegian kelp *Ascophyllum*).

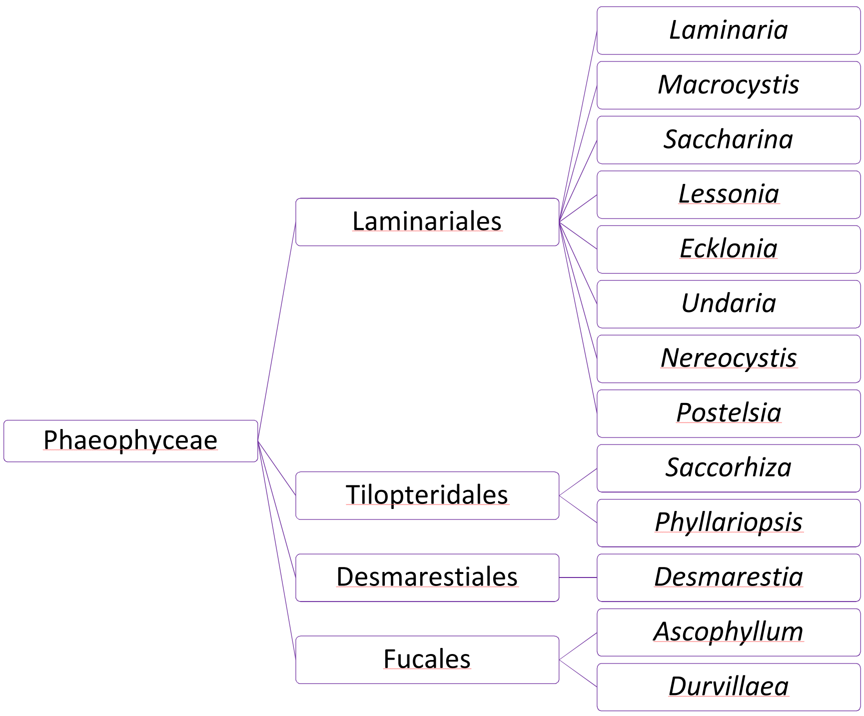

Fig. 1: As the term kelp designates large brown algae (class Phaeophyceae) capable of forming marine forests, kelp species can be a part of different orders.

The typical kelp individual is attached to the substrate by a holdfast, which is composed of root like structures called hapterae (Fig. 2). Their only purpose is fixation, without any role in assimilating nutrients. In some species, such as *Saccorhiza sp,* these may be substituted by a bulb. The stalk like part is called the stipe and may be round or flattened, depending on the species. The stipe supports the blade, the wider and flatter part of the individual (Fig. 2). At the base of the blade a strip of meristematic tissue is responsible for both the stipe and the blade's growth. In some species, the reproductive tissue is formed in darker areas called sori in the distal part of the blade, while in other species it may develop as specialized overgrowths of the stipe, the sporophylls (Fig. 2). When mature, the reproductive tissue disintegrates releasing the spores, which develop into male and female gametophyte and, after fertilization, give raise to a new sporophyte.

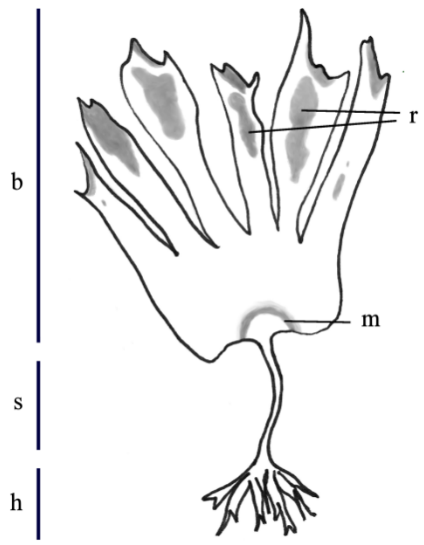

Fig. 2: Typical kelp sporophyte. b, blade; s, stipe; h, holdfast; r, reproductive tissue; m, meristem.

IMPORTANCE

ECOLOGICAL ROLE

Kelp importance, both ecological and economical has been recognized for centuries (Darwin 1913, Gittins 1966, 1982). Their forests constitute the habitat for a wide variety of algae and animal species making it one of the most biodiverse and productive systems on earth (Mann 1973). They are habitat structuring species and, by increasing spatial structure, they also increase trophic complexity (Angel & Ojeda 2001). Their canopy, by reducing light, creates suitable conditions for shade-adapted species and, as they serve as shelter and nursery for a wide variety of animals, kelp forests are associated with higher abundance and diversity of fish, among which economically important species (Bodkin 1988, Steneck et al. 2003, Graham 2004).

Kelp forests are a highly productive and steady carbon and nitrogen source year-round. They may account for more than 65% of total Particulated Organic Mater (POM) (Bustamante & Branch 1996), and form a reliable food source for several marine animals, both grazers and filter-feeders (Duggins et al. 1989, Fredriksen 2003). In addition, in rocky areas, kelp rafts and dissolved material can be carried to neighboring areas increasing secondary production in communities ranging from a few meters to kilometers away (Krumhansl & Scheibling 2012). Kelp rafts form an important protective structure and their detritus is the main nutrient source in sandy beaches, which otherwise would rely on the limited microalgae and their seasonal growth (Dugan et al. 2011, Orr et al. 2014).

Kelp forests are also very competent storing atmospheric carbon (Wilmers et al. 2012) and their canopies, by damping waves reduce water flow, influence sedimentation and reduce coastal erosion (Jackson & Winant 1983, Løvås & Tørum 2001).

ECONOMICAL RELEVANCE

Kelps have historically been used by coastal communities as a source of feed and income in times of famine and war. The first uses of kelps as a food source can be traced as far back as the 5th century. The Chinese "Classic of poetry" indicates that during the 5th century, kelp, among other seaweeds, was recognized as a delicacy, and the Greeks used the seaweeds washed ashore to feed animals in times of scarcity as early as the 1st century BC (Newton 1951). During the Tang Dynasty (6th to 9th century), kelp was used to treat iodine deficiency, goiter and thyroid enlargement in inland China (Wang & Xie 1999). In the 7th century kelps were an important part of the Japanese cuisine and became a part of the Irish diet during the 18th century potato famine. During this century, many Scottish people, displaced during the highland clearances, had no other means of survival other than gathering kelp to obtain potash for the soap and gunpowder industries (Gittins 1966). In some Neolithic sites in the Orkney Islands, Scotland, sheep ingested fresh seaweed during winter, rather than dry fodder (Balasse et al. 2006). In the 19th century, due to the overpopulation of these Islands, a 1.5 to 2m high dyke was built around North Ronaldsay to keep sheep off the central part of the island. Being relegated to the coast line, the only available forage was seaweeds. Nowadays they still feed almost exclusively on seaweeds, mainly *Laminaria digitata* and *Laminaria hyperborea* (Hansen et al. 2003). In the coastal villages of Brittany, Northern France, Northern Portugal and Galicia, Northern Spain, seaweeds washed ashore have also been used for decades as a fertilizer in agriculture (Braud 1974). In the Northern Portuguese coast seaweed collection was such an important activity it was regulated by forals and seaweed collection rights in some areas were given to monasteries (Cabral 2005). In some areas of northwestern Iberia seaweeds are still collected and used as fertilizer nowadays, although on a smaller scale (Fig. 3)(Cabral 2005, Araújo et al. 2006).

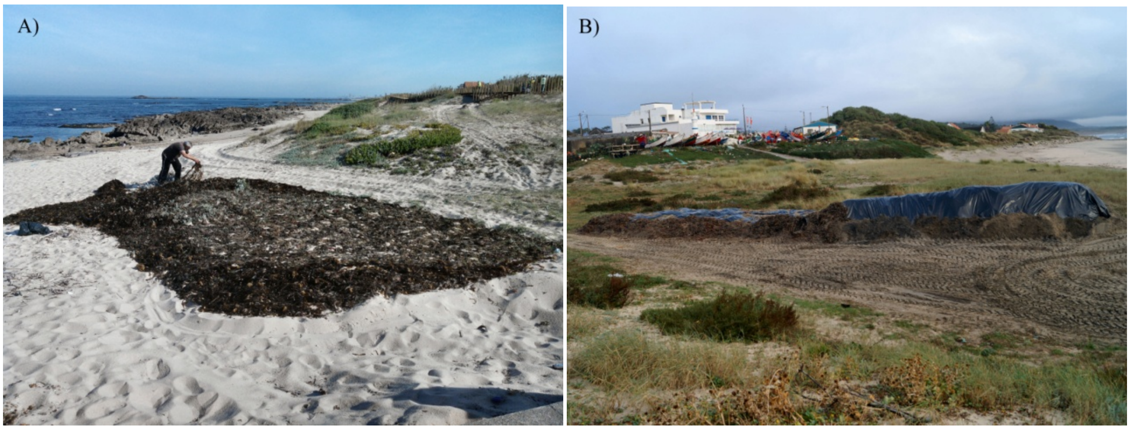

Fig. 3: Drying macroalgae in Northern Portugal, mainly kelp, to use as fertilizer in agriculture. A) Mindelo, May 2014. B) Castelo de Neiva, October 2014

While kelps have for centuries been part of the human diet in Asia, their use is nowadays widespread, and the different species are commercialized as types of kombu and wakame (Sánchez-Machado et al. 2004, Rodríguez-Bernaldo de Quirós et al. 2010). Presently, kelp extracts, among which alginates, have an important role in as food additives (EU food additives E400 to E405) and appetite suppressant (Gallardo et al. 1990). It is widely used in cosmetics and health care, as coating in pharmaceutics, analgesics, anti-inflammatory, anti-fungal, impression making material in prosthetics and in the treatment of ulcers, burns and skin grafts (Attwood 1989, Vázquez-Freire et al. 1994, Hellio et al. 2000, Paul & Sharma 2004, Bonneville et al. 2007, Cheng et al.

2009). Its ability to encapsulate living cells is driving an endless variety of studies, from treatment of diseases such as diabetes (Soon-Shiong et al. 1993), to control fermentation processes in alcoholic beverage industry, production of novel beverages (Kourkoutas et al. 2004) and synthetic seed production (Naik & Chand 2006).

Studies with ruminants indicate that kelp might be a good nutrient supplement, and its applications in aquaculture vary from feed supplement for fish to biofiltration of effluents (Neori et al. 2004, Castro et al. 2009). Furthermore, their ability to remove toxic pollutants confers a great potential for wastewater treatment (Lodeiro et al. 2005). They are also considered a suitable biomass source for the production of biofuels, because they're abundant, grow quickly, no land space is needed for their production and there is no competition between food and fuel, as opposed to corn and sugar cane (Aresta et al. 2005, Adams et al. 2008, Vivekanand et al. 2011).

CURRENT STATE

Given the importance and potential of these marine forests, it is mandatory to gather information on their ecology and resilience to the quick changes their environment is undergoing.

CLIMATE CHANGE

Climate change of the last few decades has been causing changes in several habitats all over the world (Cai et al. 2014). The effects of El Niño Southern Oscillation (ENSO) and the North Atlantic Oscillation (NAO) have become important concerns with the increased frequency of extreme events, such as storms, droughts and floods (Drinkwater et al. 2003, Seager et al. 2010, Cai et al. 2014). Such episodes impact both aquatic and terrestrial ecosystems, affecting a large myriad of species, both directly and through cascading effects (Harrison 2000, Holmgren et al. 2001, Wang 2005, Doney et al. 2012, Marengo et al. 2013, Cai et al. 2014). This impact is especially important when foundation species, such as kelps, are impacted.

Extreme variations in environmental conditions may cause devastating effects on kelp forests. Such is the case of highly energetic storms that may destroy entire forests by breaking the stipes. Adding to the removal of the protective canopy, storms have the potential to decrease the population's ability to recover both by burying and scouring spores and gametophytes, decreasing recruitment, and affecting the survival of new sporophytes (Dayton & Tegner 1984, Hoffmann & Santelices 1991, Tegner et al. 1997, Vásquez et al. 2006). Since these are foundation species, their removal may cause cascading effects, harming species that depend on them (Ebeling et al. 1985, Byrnes et al. 2011).

POLLUTION, OVERFISHING AND ALIEN SPECIES

In addition to storms, pollution and overexploitation of these habitats can further affect kelp populations (Sangil et al. 2012). Several areas have become barren grounds and are unable to recover due to grazer pressure, in particular by sea urchins (Leinaas & Christie 1996, Norderhaug & Christie 2009). This can be potentiated by overfishing of the herbivore's predators (Watling & Norse 1998, Coleman et al. 2008, Guarnieri et al. 2014). These hindrances to recover may also be worsened by the introduction of alien species with competitor advantage (Stæhr et al. 2000, Casas et al. 2004), such as *Sargassum muticum* and *Undaria pinnatifida*. *U. pinnatifida* was introduced in Brittany, France, in 1983 for commercial exploitation and has since extended its distribution as far as the North Sea and the Northern Portuguese coast (Fletcher & Farrell 1998, Pérez-Ruzafa et al. 2002, Araújo et al. 2009). It is now found in both hemispheres, in the eastern and western coasts of both the Atlantic and Pacific Oceans (Hay & Luckens 1987, Pérez-Ruzafa et al. 2002, Silva et al. 2002, Casas et al. 2004, Araújo et al. 2009). In contrast, the introduction of *S. muticum* was most likely due to shipment of Japanese oyster. Ever since, it has invaded both the Eastern Pacific and Western Atlantic coasts (Ambrose and Nelson 1982, Stæhr et al. 2000, Harries et al. 2007, Olabarria et al. 2009). It competes with other macroalgae species for both substrate and light and may cause variations in species abundance or even prevent their recruitment (Ambrose & Nelson 1982, Stæhr et al. 2000, Sanchez et al. 2005, Harries et al. 2007). Although this species has been associated with an increase in biodiversity and primary production, its competitive ability may be potentiated by the lesser palatability of *S. muticum* when compared to native species (Harries et al. 2007; Engelen et al, in press).

ENVIRONMENTAL CONDITIONS

SUBSTRATE

Kelps are usually found on rocky substrate. In the absence of waves and if the current is not strong enough to dislodge them, they may also grow attached to smaller rocks, like pebbles and on annelid tubes. In these conditions their life span becomes dependent on their size, as bigger individuals will be more easily washed away and broken or transported ashore or out of the photic zone (Birkett et al. 1998). As such, kelps are ephemeral when growing on unstable substrate. In bedrock, small-scale substrate rugosity is an important factor for kelp population persistence because it enhances spore aggregation (Muth 2012).

TEMPERATURE

Temperature is recognized as one of the main factors affecting kelp distribution and seasonality. Kelps are stenothermal and as such are able to survive and reproduce within a small range of temperatures. As a consequence, thermal conditions narrow their distribution not only geographically but also in the shore. Throughout the year kelps have different temperature requirements, as survival, vegetative growth and reproduction have different constraints (Bolton & Lüning 1982, Hoek 1982, Izquierdo et al. 2001). Temperature is also thought to be one of the factors regulating seasonality of the reproductive period in kelps. However its role is difficult to access in the field, since changes in temperature are usually accompanied by changes in other environmental factors such as light intensity, photoperiod and nutrient availability (Kain (Jones) 1989).

LIGHT INTENSITY, QUALITY AND PHOTOPERIOD

Light quantity and quality is one of the main factors affecting kelp depth distribution. Turbidity reduces the light penetration and changes its spectral composition. However, seaweeds can, to an extent, acclimate to different light compositions by varying the proportion of their pigments, (Fairhead & Cheshire 2004, Brey 2009).

Kelp's light requirements vary not only between species, but also between life stages (Lüning & Dring 1972). Spectral characteristics and levels of irradiance are used to control gametogenesis in several kelp species (Lüning 1980, Hoffmann & Santelices 1982). Blue light to triggers the gametogenesis of kelps, and the irradiance must exceed certain levels (Andersen 2005). Moreover, critical light demand is increased by higher temperatures, decreasing the depth limit of kelp (Staehr & Wernberg 2009).

The effect of day length varies with species. In experiments with *Saccharina latissima*, where the meristem was isolated from the rest of the frond, a short-day period was necessary for sporogenesis (Buchholz & Lüning 1999) and in *S. japonica*, sori onset was faster under a short-day regime (Mizuta et al. 1999a). In contrast, day-length had no significant impact in *L. digitata* (Buchholz & Lüning 1999, Pang & Lüning 2004).

HYDRODYNAMICS: WAVES & CURRENTS

While water motion is an important cause of mortality in kelps, the ability to survive in disturbed waters varies with species. As such, hydrodynamics, especially waves, affect the distribution of kelp species on the coast (Birkett et al. 1998). Storms may have a nefarious effect on these populations, especially when occurring during low tide, causing larger and stiffer individuals to be removed. As a result, kelps in shallow waters are usually younger than those in deeper areas (Birkett et al. 1998, de Bettignies et al. 2012). As such, wave action will influence a kelp bed's species composition, demography and individual's morphology (Birkett et al. 1998, de Bettignies et al. 2012). Currents, on the other hand, may have a positive effect on kelp communities as by moving the canopy, shading is reduced and nutrient availability increased (Hepburn et al. 2007, Pedersen et al. 2012).

As kelps are subtidal species, tidal range and heights don't usually have an effect on the community. However, in locations where the low tide of very low spring tides occurs around midday, kelps might suffer from desiccation, which might lead to death or to bleaching of portions of the blade.

Nutrients

Kelps are recognized as efficient absorbers of nutrients (Probyn & McQuaid 1985, Broch et al. 2013). Uptake depends on the species, availability of light and the conditions the individuals are accustomed to (Lüning 1990). When nutrient are available in excess, their uptake is greater than their immediate need and the excess is stored as mannitol, laminarin and alginate (Black 1954, Skriptsova et al. 2004). These are stored irregularly through the individual and their concentration varies throughout the year with the individual needs (Honya et al. 1993, Skriptsova et al. 2004, Adams et al. 2011, Handå et al. 2013). These polysaccharides, accumulated during times of abundance can be used immediately or when nutrient availability becomes diminished (Iwao et al. 2008, Sánchez-Barredo et al. 2011).

Salinity

Although the optimum salinity for kelps varies between 30 and 35 psu, kelps can survive salinities of between 16 and 50 psu (Lüning 1990, Schoch & Chenelot 2004). Kelp resistance to salinity varies with species and stage and impacts life processes differently (Buschmann et al. 2004, Schoch & Chenelot 2004, Fredersdorf et al. 2009).

Life cycle

While there are some exception (such as species in the order Fucales), the typical kelp life cycle consists of a microscopic haploid gametophyte phase, alternating with macroscopic diploid sporophytes (Fig. 4). Due to the difficulty of following the microscopic stages in the field, kelps have been classified as annual or perennial based on the macroscopic sporophyte life span only. In annual species, sporophytes life is ephemeral and usually limited to a portion of the year. In contrast, perennial species have macroscopic sporophytes whose life expectancy may vary between 2 and 25 years, depending on the species and local conditions (Birkett et al. 1998, Steneck et al. 2003). Spores are produces in reproductive tissue, called sori, formed in the blade of adult sporophytes. They germinate and develop into dioecious male and female haploid gametophytes, producing antheridia and oogonia, respectively. After fertilization and syngamy, the diploid zygote forms a new sporophyte (Dayton 1985). These microscopic forms are commonly referred to as “bank of microscopic forms” (Chapman 1987). After the decay of the annual sporophytes, survival of the microscopic stages is crucial for population persistence. In perennial species, on the other hand, microscopic stages are only this crucial in cases of massive destruction. When such events take place, microscopic stages can quickly recruit and reoccupy the newly available space (Ladah & Zertuche-González 2007, Barradas et al. 2011, Carney 2011).

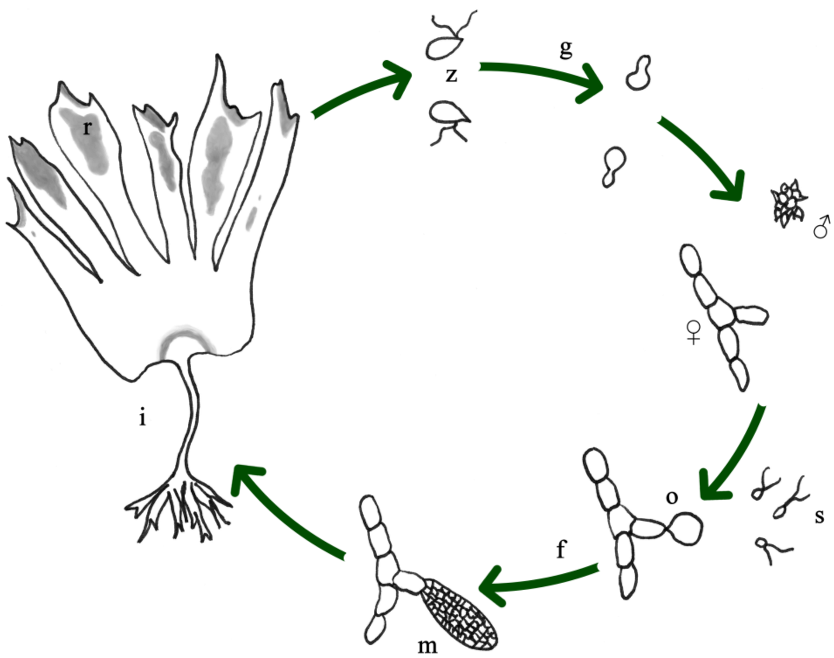

Fig. 4: Typical kelp life cycle. i, macroscopic sporophyte; r, sorus; z, zoospores; g, germination; ♂ male gametophyte; ♀ female gametophyte; s, spermatozoids; o, oogonia; f, fertilization; m, microscopic sporophyte.

GROWTH AND REPRODUCTIVE SEASON

The life cycle of macroalgae is frequently regulated by environmental factors, such as temperature, light and nutrient availability. As such, seasonal environmental variations can induce growth and reproduction in most species (Reed et al. 1996). When it comes to seasonality, subtidal species can be divided in two groups: those that respond to an environmental trigger, anticipating favorable season, and those that respond to favorable conditions. Most kelp species have been described as examples of the first, growing during winter, when light availability and temperatures are lower and nutrient availability is higher. Many kelp species have been described to accumulate nitrogen and phosphate during winter, when nutrients are more common (Gagne & Mann 1987, Broch & Slagstad 2012). Their growth coincides with this abundance period and decreases when the reserves are depleted (Nielsen et al. 2014). When summer arrives, their blades have reached their peak length, the growth is halted and energy is allocated to reproduction (Lüning 1979, Nielsen et al. 2014). Yet, it has also been reported that transplantation from low nutrient concentrations to an artificially enriched environment may result in fast formation of sori (Mizuta et al. 1999b).

This differentiation between growth and reproductive seasons has been observed for species in both the *Laminaria* and the *Saccharina* genus, which led to formulate the hypothesis of a sporogenesis inhibitor being released by the active meristem. In *L. digitata*, tissue discs isolated from the distal part of the frond formed sorus after 7-12 weeks, while discs from the blade base did not show any sori development (Buchholz & Lüning 1999). This insinuates that meristem activity generates inhibitors of sporangium formation which move to the distal parts of the blade, keeping the frond clear of reproductive tissue during the season of rapid growth. This theory was supported by yet another experiment where cutting holes near the base of the frond, blocking the pathway of the hypothesized sporulation inhibitor (Lüning et al. 2000), resulted in sori production in the distal margin of the hole after 5-10 weeks. The production of sori in discs, despite the season, was also reported for *S. japonica* (Mizuta et al. 1999b) and *S. latissima* (Buchholz & Lüning 1999). The sporulation inhibitors are thought to move mainly in longitudinal pathways converging laterally. This is supported by experiments where transverse cuts were made in *S. latissima* (Pang & Lüning 2004). When these transverse cuts were too short, the blade stood clear of sorus, while in larger cuts sori developed in the distal part of the blade with a flame-like shape, indicating a lateral movement of the inhibitors from the margin to the middle. In blades with multiple cuts, the sori produced in the distal part of the cuts were progressively smaller in distal direction, suggesting a gradient of physiological competence for sorus formation, higher at the base and lower at the distal end of the blade (Pang & Lüning 2004). Discs may develop sori independent of individuals' thickness or fresh weight, indicating that the effect of the meristematic sporogenesis inhibitor is independent of age (Mizuta et al. 1999b).

Life phases of macroalgae are frequently associated with environmental conditions, and while in controlled conditions it is possible to assess the effect of each, in the field they may vary considerably and their effect on kelp life may be coupled. As an example, kelps are more sensitive to high temperatures when these are associated with strong irradiance (Staehr & Wernberg 2009), and more resistant when nitrogen is not limiting (Gerard 1997).

SPORE DISPERSAL

Spores of typical kelp are usually smaller than 100µm in diameter and motile, although very slow, for variable time after release from the reproductive tissue (Clayton 1992, Norton 1992, Gaylord et al. 2012). Their permanence in the water column depends on the species, the height from which they are released and the environmental conditions (Norton 1992, Reed et al. 1997, Gaylord et al. 2004, Stevens et al. 2008). Their propagules probably are released in pulses, increasing density and dispersal ability (Bobadilla & Santelices 2005). Spores may settle immediately or remain viable in the water column for days, even after losing their mobility and germinating (Reed et al. 1992). As these retain the ability to attach to a substrate, dispersal may be attained by stages other than spores and enhanced by wave action and strong currents which might resuspend them (Reed et al. 1988, 1992, Cie & Edwards 2011).

Most studies that aimed to determine how far species can disperse were done based on how far from the spore source new recruits appear. Not only does this depend on the initial spore density, it neglects their survival ability and propagule longevity, indicating only the furthest they are able disperse in enough density to develop into a visible sporophyte. While, the majority of spores settle within a few meter of the source, a small portion may disperse as far as kilometers which makes them very relevant for population connectivity (Gaylord et al. 2002). Also, the topography of the site is extremely important in determining the spore dispersal ability as even small roughness elements may drastically reduce dispersal distances (Gaylord et al. 2002). Still, some species have different strategies to achieve long distance dispersal. *Macrocystis pyrifera* form rafts that can float for long periods and, as these maintain their reproductive ability, zoospores may disperse over large distances (Macaya et al. 2005). *Saccorhiza polyschides* has been reported to produce two sets of spores: one that settles in the proximity of the parent sporophyte and another one for wider dispersal (Fernandez et al. 1988). Long distance dispersal from fixed stands may also be attained via the dispersal of fragments or whole individuals (Forrest et al. 2000). It was also described that zoospores can be transported by fishing activities and on the hull of boats. This is thought to be one of the main responsible mechanisms for the wide spread of *U. pinnatifida* (Hay 1990).

On the other hand, in areas with strong hydrodynamics, spores may be diluted in the currents. As such, some kelp species have developed mechanism to reduce dispersal, which increases the chances for local recruitment and may be fundamental in exposed areas. Such is the case of *Nereocystis leutkeana*, whose sori tissue is released synchronously from the blade, in one piece, and half its spores are released shortly after sinking (Amsler & Neushul 1990). In other species, such as *Postelsia palmaeformis*, spores are released encased in mucilage, sinking in clumps. Maximum spore dispersal for this species is estimated at 3 m (Dayton 1973, Paine 1979). Although *U. pinnatifida* has successfully dispersed to most of the Atlantic Ocean, long-range dispersal of this species is mostly due to boats (Farrell & Fletcher 2006), as experiments indicate that its spores are able to disperse no further than 10m (Forrest et al. 2000).

Fertilization

As fertilization occurs after spore dispersal, synchrony of spores release is essential to ensure viable densities (Norton 1992). Female eggs are stationary and usually remain connected to the gametophyte, while male antherozoids are motile. Antherozoids are however devoid of an eye spot and are not phototactic (Brawley &

Johnson 1992). It has been reported that the release of the egg is accompanied by the release of pheromones, including lamiroxene, which induces the release of the spermatozoids and directs them to the oogonium. Antherozoids also respond to a decrease in the hormone concentration, directing them to the pheromone origin (Maier & Muller 1986, Maier et al. 1988, 2001).

Eggs and spermatozoid formation may be triggered by changes in temperature, light intensity and long-day or most commonly by short-day photoperiods (Lüning & Neushul 1978, Brawley & Johnson 1992). In some species of kelps gametogenesis occurs in blue light while red light induces gametophytes to grow vegetatively (Lüning & Dring 1972, 1975). However, lower temperatures may decrease light intensity demands for gametogenesis (Lüning & Dring 1972, Lüning 1980).

Environmental conditions are also responsible for triggering the release of eggs (Müller et al. 2008). *Laminaria* species, for instance, release their eggs within a few minutes of the dark cycle (Henry 1987, Lüning 2007). As the pheromones are released by egg release, this synchrony is important as it increases the pheromone concentration and induces the spermatozoid release in more male gametophytes, increasing the synchrony of egg and spermatozoid release and, thus the chances of fertilization. As these pheromones are easily diluted by currents, it is essential that gametophytes are dense enough to communicate by such chemical signals. (Reed 1990) determined that a minimum of 1 spore / mm^2 is needed for successful recruitment.

Although spermatozoids from one species are only attracted to one pheromone, most species produce a combination of them. This has been speculated to have an allelopathic role as the female gametophyte is going to induce the release of spermatozoids from other species, disrupting gamete release synchrony of other species (Bartsch et al. 2008). Such an example is the interaction between *Ectocarpus* and *Cutleria*. *Ectocarpus* male gametes respond to the pheromone ectocarpene, which is secreted in small amounts by *Cutleria* (Brawley & Johnson 1992). Reed (1990) also speculated that in areas where Macrocystis and Pterygophora were mixed, Pterygophora caused the premature release of Macrocystis male gametes, reducing its ability to recruit.

Kelp gametophyte sex determination is believed to be mainly genetic (Evans 1963). However, environmental conditions may influence the ratio between males and females, particularly in stressful conditions (Funano 1983, Lee & Brinkhuis 1988, Nelson 2005, Oppliger et al. 2011). Also, environmental conditions determine if females are uni or multicellular and if the later bears one or more eggs (Hoffmann & Santelices 1982, Hoffmann et al. 1984, Izquierdo et al. 2001, Muñoz et al. 2004).

“BANK OF MICROSCOPIC FORMS”

The microscopic stages of the kelp life cycle play a fundamental role in the persistence of populations. In perennial species, their role is, usually, to just make up for the losses. In cases of massive destruction however, they become responsible for recovering the population. In annual species the macroscopic sporophyte is present only during a period of the year, when the next recruiting season arrives, the species rely on microscopic stages to recover the population. While in culture these microscopic forms can be kept in arrested development, their longevity in the field is unknown. (Chapman 1987) termed these forms as “bank of microscopic forms” and has often been considered a seed bank analogue. However, as these forms have no protective structures and can be easily grazed, scoured and buried, it is unlikely that they survive in arrested development for more than a year (Hoffmann & Santelices 1991, Schiel & Foster 2006). While field surveys show that recruitment occurs within a few months after the perennial sporophytes were removed, there is no evidence of them remaining in a dormant state for long periods, as plant seeds (Ladah et al. 1999, Barradas et al. 2011).

ATLANTIC KELP SPECIES

LAMINARIA OCHROLEUCA

L. ochroleuca is a Lusitanian species, found very deep in Azores, in the warm and temperate waters from Morocco to the Northwestern Spanish coast and from Brittany (France) to the English and Bristol channels, as well as some areas in the

Mediterranean (Braud 1974, Drew 1974, Hoek 1982, Birkett et al. 1998, Tittley & Neto 2000, Bartsch et al. 2008). It usually occupies rocky substrates from 0 to 30m deep, although in clear water, such as in the Strait of Messina, it can survive as deep as 50m (Drew 1974, Birkett et al. 1998). Together with *S. polyschides, L. ochroleuca* is the main forest forming kelp species in the temperate Northeastern Atlantic. Individuals of *L. ochroleuca* are perennial. Their growth is seasonal and usually takes place from winter to spring. During the reproductive season, in the summer and fall, blade growth is suppressed and the reproductive tissue is produced. When the reproductive tissue is mature, the distal part of the blade, where the sporangia are, disintegrates releasing the spores (Fig. 5a) (Mann 1973, Lüning 1990, Buchholz & Lüning 1999). This seasonal pattern of growth and reproduction is, however, mostly based on the observations of other *Laminaria* species. Furthermore, information about the state of *L. ochroleuca* low-latitude populations is almost exclusively based on the empirical knowledge of fishing communities along the Portuguese coast. As this is the main perennial kelp species populating the Portuguese coast, many species depend on its maintenance and resilience. Understanding the processes which allows them to persevere is, therefore, essential (Chapter 1). Still, resilience may sometimes be masked by a high survival rate and only in cases of destruction is the population's ability to recover observed (Chapter 2). Also, highly energetic storm have the potential to destroy extensive areas of kelp forest by breaking their stipes. As with climate change these events are expected to become more frequent, it's important to understand the extent of their impact, the populations' ability to recover and the frequency at which the storm's destructive effects may become permanent (Chapter 1 and 2).

L. ochroleuca has been reported to have an upper temperature limit for sporophyte' survival and reproduction of around 25°C (tom Dieck & de Oliveira 1993). Its minimum survival temperature was not found in earlier reports but has been hypothesized that its geographical distribution is limited at high-latitude by the 10 °C winter isotherm (Braud 1974, Hoek 1982). Although able to develop sori at temperatures up to 23°C, its gametophytes are expected to survive and reproduce only at temperatures between 5 and 20 °C (Lüning 1980, Birkett et al. 1998). However, very few studies have been performed to assess the temperature limits for this species and the information is mostly based on their geographical distribution and its similarity with *L. hyperborea* (Chapter 4).

Populations of *L. ochroleuca* are often found in areas influenced by tides, where environmental conditions may change quick and drastically, with individuals being exposed to extreme conditions for short periods of time. In addition, the few experiments made with this species don't account for possible ecotype differentiation that might occur along their geographical distribution (Bolton & Lüning 1982, Gerard & Du Bois 1988, Peters & Breeman 1993) (Chapter 5).

LAMINARIA HYPERBOREA

L. hyperborea is typical of the rocky shores of the Northern Europe's cold waters. It is present around Iceland and Norway, extending south to Denmark, but absent from Arctic influenced water and the Baltic Sea. It is present further south around the United Kingdom, the Coast of Brittany and in the Northwestern Coast of Spain, extending Westward till Cape Mondego in mid-Portugal (Birkett et al. 1998). Studies show that this species low-latitude limit has been shifting north during the last few decades (Ardré 1970, Pérez-Ruzafa et al. 2003). Although at the northernmost coast of Portugal this species has been observed to populate deep intertidal pools, little is known about the stability of these populations or their ability to persist (Chapter 1).

L. hyperborea is a cold water and shade-adapted species and in some areas can be found as deep as 47m. Although able to survive between 0 and 20°C, its reproduction becomes impaired at temperatures above 15°C (Lüning 1980).

L. hyperborea sporophyte is perennial, with a maximum life expectancy of more than 10 years, which varies with latitude and environmental conditions (Sjøtun et al. 1993). This species does not have a continuous growth, growing at different rates throughout the year. Its meristem remains inactive all through summer and, when temperature and day length conditions, around November, are right, the meristem resumes its activity giving rise to a swelling at the top of the stipe. This continues growing until it forms a disc. The meristematic activity than shifts from the base of the stipe to the center of the disc, causing it to expand (Lüning 1986). As such, once a year, this species grows a new blade, keeping the old one at the distal extremity (Fig. 5b).

SACCORHIZA POLYSCHIDES

The macroscopic sporophyte of *S. polyschides* is annual, growth starts in spring and maximum size is reached in the summer. In early autumn, sporophytes typically reach reproductive maturity. Unlike *L. ochroleuca*, *S. polyschides* develops sporophylls at the base of the stipe, besides the sori in the blade. With this, this species forms two sets of spores, for local and longer distance dispersal (Fig. 5c) (Fernandez et al. 1988).

S. polyschides has the same southern distribution limit as *L. ochroleuca.* Still, it can be found further north along the South and West coasts of England, Wales, Scotland and Ireland, reaching the west coast of Norway (Norton 1977, Birkett et al. 1998). Like *L. ochroleuca*, *S. polyschides* can usually occupy rocky substrate up to 35 m deep (Norton 1970). In the southern portion of its geographical range, only microscopic stages overwinter, and macroscopic sporophytes recruitment in the following spring depends completely on their development and survival (Mann 1973, Hoek 1982). Similarly to *L. ochroleuca*, and despite being the main annual forest forming species in the Northeastern Atlantic (Smale et al. 2013), little information exists about its seasonal low-latitude populations (Chapter 1). In contrast, in central and high-latitude population, macroscopic sporophytes can also be observed in the field during winter and recruitment takes place year-round (Norton & Burrows 1969, Jensen et al. 1985). While it is commonly accepted that this difference in population history reside in the dependence of low-latitude populations on the typical summer, which lowers water temperature allowing the sporophyte persistence during summer, this hypothesis has not been tested until now (Chapter 3).

Upper temperature limit of *S. polyschides* has been reported to be 24ºC, very close to that of *L. ochroleuca* (Norton 1977). Similarly, no data on its lower survival temperature was found and the species distribution is thought to be limited by the 4 °C isotherm in Norway (Norton 1977, Hoek 1982). Just as for *L. ochroleuca*, despite its relevance, little information on how environmental conditions affect this species is available (Chapter 3 and 4).

While it has been reported that in the Strait of Messina *S. polyschides* is able to occupy the coast up to the surface while *L. ochroleuca* is present only below 30 m, in Northern Portugal the opposite is observed. While *L. ochroleuca* dominates intertidal pools, *S. polyschides,* despite occurring, is unable to reach maturity (Drew 1974, Hoek 1982). This indicates that these species might be delimited by different factors in the two sites and their ability to endure recurrent quick and drastic temperature changes felt in the high subtidal and intertidal pools may differ (Chapter 5).

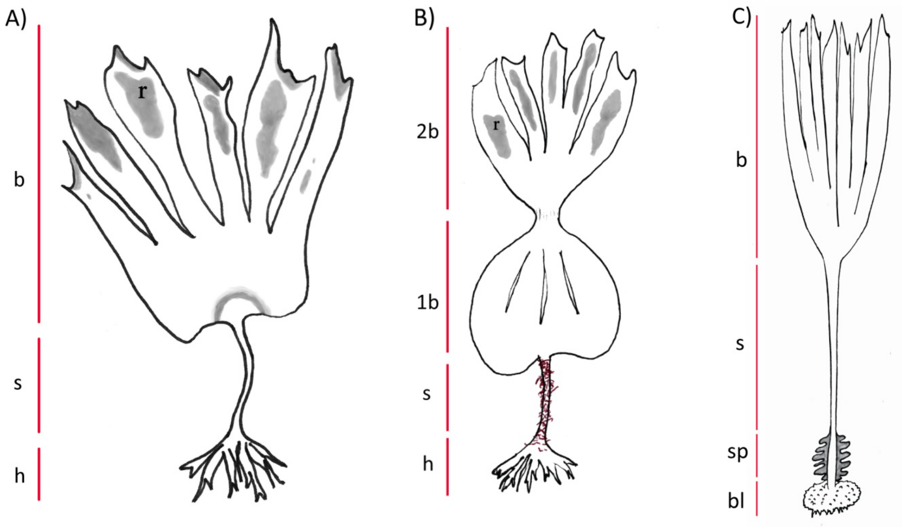

Fig. 5: Schemes of A) *Laminaria ochroleuca*; B) *Laminaria hyperborea*; C) *Saccorhiza polyschides* and their morphological differences. While *L. ochroleuca* and *S. polyschides* blades (b) grow somewhat continuously, in *L. hyperborea* a 1st year (1b) and 2nd year (2b) blade can be distinguished. The stipes (s) of both *Laminaria* species is round. The stipe surface of *L. hyperborea* is rough and frequently covered by red algae. *S. polyschides* has a flat stipe, being easily distinguished from the other two species. While the *Laminaria* species have their reproductive tissue in the distal part of their blades (r), *S. polyschides* develop specialized tissue at the base of the stipe, the sporophyll (sp). *Laminaria* species are fixed to the ground by haptera (h) while *S. polyschides* develops a bulb (bl).

CHAPTER 1

POPULATION DYNAMICS OF TEMPERATE KELP FORESTS NEAR THEIR LOW-LATITUDE LIMIT

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ABSTRACT

This study assesses the most important life processes for maintenance and growth of kelp forests at their shallower and lower latitude distribution limits. This gives information about the most sensitive phases of the life cycle, the possible causes for population extinction and, if necessary, where preservation effort should be concentrated.

Due to its seasonal life-history, persistence of *Saccorhiza polyschides* depended totally on survival of the microscopic forms. While few *Laminaria hyperborea* became reproductive during the census period, *Laminaria ochroleuca* spore production was considerable. Still, both species relied mainly on individual's survival. Half-way through the censuses, late winter storms caused extensive destruction along the coast. Shallower populations of *L. ochroleuca* fully recovered from the microscopic stages, showing resilience to major perturbations. However, it is predicted that recurrence of such highly energetic storms will increase with climate change. These populations will likely become gradually younger and dependent on spore dispersal from the subtidal. In contrast, local reproductive success of *L. hyperborea* and dispersal from the subtidal were already insufficient for repopulation. Records indicate a northward distribution shift of this species and our data is in agreement with these findings.

While sporophytes of *S. polyschides* are absent during winter, storms might bury the microscopic stages. Also, yearly climate variations may delay their recruitment, preventing them from reaching maturity. Although dispersed spores were enough to maintain the population, its density was lower.

This study indicates that with increased storm frequency, marginal kelp populations will become younger and scattered. Changes in depth distribution will likely occur prior to geographical ones.

Keywords: *Laminaria ochroleuca*; *Laminaria hyperborea*; *Saccorhiza polyschides*; population dynamics; matrix modeling

INTRODUCTION

Over the last decades, global warming has caused drastic changes in habitats all over the world, affecting both terrestrial and marine habitats and causing changes in the distribution of plants and animals. Climate-driven alterations are manifested not only in rising temperatures but also by increased frequency of droughts, floods, storms and changes in wind and current speed and direction (IPCC 2007). Terrestrial species, such as plants (Allen & Breshears 1998, Lenoir et al. 2008), insects (Parmesan et al. 1999, Chen et al. 2009) and birds (Brommer 2004, Hitch & Leberg 2007), among the best studied, have been shifting their distribution not only poleward but also to higher altitudes (Hickling et al. 2006, Chen et al. 2011). A similar trend is observable in the marine realm (Burrows et al. 2011) where distribution shifts, both geographically and in depth, have been reported for macroalgae (Bartsch et al. 2008, Gallon et al. 2014), plankton (Beaugrand & Reid 2003) and fish (Perry et al. 2005, Sabatés et al. 2006). With these changes, structurally complex marine habitats and biogenic temperate reefs, such as coral reefs, seagrass meadows and kelp forests are becoming increasingly rare, threatening the existence of entire ecosystems (Poloczanska et al. 2013).

Climate change related local extinctions have been detected for several kelp species worldwide and a decrease in resilience is expected (Müller et al. 2009, Merzouk & Johnson 2011, Raybaud et al. 2013, Voerman et al. 2013). Yet, this alteration is sometimes difficult to access since populations may remain intact in the absence of disturbance (Wernberg et al. 2010). Severe storms, usually associated with El Niño Southern Oscillation (ENSO) events, have been reported to destroy kelp populations on both hemispheres (Dayton & Tegner 1984, Ebeling et al. 1985, Ettinger-Epstein & Kingsford 2008). After the 1982-83 ENSO events, for instance, *Lessonia berteroana* (formerly *Lessonia nigrescens*) northern distribution limit in Chile shifted south and was able to recover only 60 km in 20 years (Martinez et al. 2003). Similarly, the North Atlantic Oscillation (NAO) is known to influence European climate and species abundance, distribution and assemblages (Drinkwater et al. 2003). Also, kelp forests worldwide have lost resilience due to human activities, disease and overgrazing, and are now absent from several areas they used to inhabit (Leinaas & Christie 1996, Cole & Syms 1999, Ling et al. 2009, Wernberg et al. 2010, Davoult et al. 2011, Bartsch et al. 2012, Díez et al. 2012, Voerman et al. 2013, Oppliger et al. 2014).

In Europe, kelps have undergone distributional changes: *Laminaria ochroleuca* seems to have extended its distribution northward (Spooner 1950, Smale et al. 2014) while disappearing from the north coast of Spain (Fernández 2011), while *Laminaria hyperborea* is retreating from Asturias and the Portuguese coast (Ardré 1970, Pérez-Ruzafa et al. 2003), and *Saccorhiza polyschides* expansion to the Basque coast can be related to climate change driven current alterations (Fernandez et al. 1988). The 2009/2010 winter was characterized by a record persistence of the NAO negative phase that, together with ENSO, caused a temperature drop all over Europe (Seager et al. 2010, Cattiaux et al. 2010). In February 2010, a strong late winter storm occurred in Europe, causing massive destruction in the Northern Portuguese coast, greatly affecting canopy forming macroalgae populations (Grumm 2010, personal observations). Studies indicate that the recurrence interval of such energetic storms is of about 10 years (Dias et al. 1994). However, climate change is predicted to cause an increase in its frequency (IPCC 2007, Young et al. 2011, Hemer et al. 2013).

Kelp forests are among the most diverse and productive systems in the world (Mann 1973, Steneck et al. 2003). Given their economical relevance, it is important to know their current state, resistance and resilience to disturbance, which may help predict the fate of their populations. In this study we used a demographic approach to assess population fitness and identify the most important life events for the maintenance of three kelp species: *L. ochroleuca*, *L. hyperborea* and *S. polyschides* on the northern Portuguese coast, the lowest latitude area where these species form forests (Tuya et al. 2012) and inhabit intertidal pools, being exposed to rapid variation in environmental conditions.

The current state and trend of kelp populations from the Portuguese coast is unknown as no long term monitoring efforts have been made during the last decades. However, local coastal communities have the empirical knowledge of the decline of kelp forest and the high abundance of *S. polyschides* in the northern part of the country. As such, we expected to observe a decline in *Laminaria sp*. populations as the small dimension of their individuals, indicatory of high death rate and low reproductive output, agreed with popular knowledge. *S. polyschides* populations, on the other hand, as a fast growing opportunistic species, was expected to be self sustainable, producing enough spores locally for their recruitment in the next spring.

METHODS

STUDY SITE

Tidal and wave regimes in the Portuguese coast are highly energetic. During summer, wave height generally varies between 1 and 3m with periods lower than 10s (Vitorino et al. 2002). Although these usually result in stiffer individuals with shorter stipes, some studies show that it might also have a positive effect on individual's performance by reducing self-shading and facilitating nutrient uptake (Hepburn et al. 2007, Pedersen et al. 2012). However, during winter storms, waves may reach 12m, with 13 to 18s periods with a 10 year recurrence interval (Dias et al. 1994, Boaventura et al. 2002).

SPECIES

The life cycle of kelps consists of an alternation between macroscopic diploid sporophytes and microscopic haploid gametophytes. In *Laminaria* species, sporophytes are perennial and their life is divided in periods of rapid vegetative growth and reproduction. The reproductive tissue is formed in the distal area of the blade, which later disintegrates releasing the spores. In contrast, *S. polyschides* is an annual species and its spores are formed in a reproductive frill at the base of the stipe (Norton & Burrows 1969). The first autumn storms usually break the stipes, near the blade, ending the macroscopic phase. Spores, gametophytes and microscopic sporophytes constitute a bank of microscopic forms that is believed to stay in a slow or arrested development state during the period that is most unfavorable for the macroscopic sporophytes, which are regenerated from the microscopic stages in spring (Chapman 1987, Hoffmann & Santelices 1991, Schiel & Foster 2006). As such, in cases of massive destruction, the recovery of kelp forests depends completely on survival and development of these forms.

The distribution of *L. hyperborea*, a West European cold-temperate species, extends from the northern Portuguese coast, to the Russian Barents Sea coast. In warm-temperate areas, it is replaced by *L. ochroleuca*, sharing a habitat from the Northern Portuguese to Brittany's coast. *L. ochroleuca* can still be found all along the west coast of Portugal, in Morocco and in the warmer waters of the Strait of Messina and the Azores (Hoek 1982, Birkett et al. 1998). *S. polyschides* shares its distribution with both these *Laminaria* species, from the Western coast of Norway, to the Moroccan coast, including the Irish, Western Scottish, and Welsh coasts. The Northern Portuguese coast is the lowest latitude area where these species are able to form forests and inhabit intertidal pools (Tuya et al. 2012).

To study these populations, censuses were made for two sites with *L. ochroleuca* from May 2008 to May 2010. Due to the impossibility of finding an area with only *L. hyperborea*, a pool with both *Laminaria* species was followed from August 2009 to August 2010. As such, the censuses included the winter storms of 2009/2010, which allowed observing its effect and assess population's resilience to storm disturbance events. Similar protocol was used to follow a population of *S. polyschides* from August 2010 to August 2011.

POPULATION MONITORING

Due to limitations in accessing intertidal and high subtidal areas only during spring tides, censuses of the three species were done in different periods. In May 2008 a 4 m^2 area with about 100 individuals of *L. ochroleuca* was chosen in both a 1.3 m deep intertidal pool and a subtidal area in São Bartolomeu do Mar (SB: 41°34'27.50"N; 8°48'1.70"W) and Viana do Castelo (VC: 41°41'44.80"N; 8°51'8.40"W), respectively. A census was carried out on a monthly basis, when sea conditions allowed, for two years. Since no intertidal pool was found with *L. hyperborea* alone, two intertidal pools 1 and 2 m deep with both *L. hyperborea* and *L. ochroleuca* in Moledo do Minho (MM: 41°50'7.20"N; 8°52'33.70"W and MM2: 41°50'5.80"N; 8°52'34.80"W, respectively) where chosen in August 2009 and followed till August 2010. However, in September

2010, MM2 was completely buried in sand, and although in the next census the sand had already been removed by wave action, no kelps recruited back. Another suitable pool, with enough *L. hyperborea*, was not available as most pools were small (0.3 m deep) and mainly occupied by *L. ochroleuca*. As such, only MM was considered in data analyses. A census of *S. polyschides* was initiated in a subtidal area in August 2010 in Areosa (Ar: 41°42'33.56"N; 8°51'48.81"W) and carried out for one year.

On a monthly basis, as long as the sea conditions allowed, stipe and blade length of all individuals in the predetermined area were measured and their reproductive state recorded. Individuals were distinguished by mapping their position and /or a tag around their stipe (for larger individuals). Maps were made by trillaterations, i.e., measuring the distance of each individual to two fixed points or previously mapped individuals, giving their relative position in the pool. Maps were made with Site Recorder, student edition (3H Consulting Ltd.). New recruits were triangulated and tagged when large enough.

To assess population resilience to the late winter storm, all individuals at the sites with *Laminaria* stands were measured and population structure was determined two years after the last census, in May 2012 for the *L. ochroleuca* stands and in August 2012 for the mixed *L. ochroleuca* and *L. hyperborea*. Although data on *S. polyschides* population does not allow the study of its dynamics, a census was also made for the new generation, in August 2012, to check how individual density and development varied for the same period in three consecutive years.

Matrix models

The relative contribution of each size class to the reproductive output was estimated by measuring stipe and blade length and sori area of 300 reproductive *L. ochroleuca* individuals between September and October 2008. Individuals were taken to the lab and sori area was measured using the image software Gimp (GNU Image Manipulation Program) and considered to be equal in both sides of the blade. The best predictor for an individual's reproductive effort was chosen by studying the correlation between sori area and stipe, blade and total size using linear regression (Reed et al. 1996, 1997). After concluding that reproductive effort increases exponentially with individual's size, total size was considered the best predictor of an individuals' reproductive output. Reproductive individuals of *L. hyperborea* were not abundant enough to make a similar assessment of spore production. However, studies indicate that spore production in *Laminaria* species is within the same order of magnitude (Chapman 1984, Joska & Bolton 2007). As we didn't aim at assessing the amount of spores produced by each stage class but only its relative contribution, this was assumed to be similar to that of *L. ochroleuca*. No reproductive effort assessment was performed for *S. polyschides*.

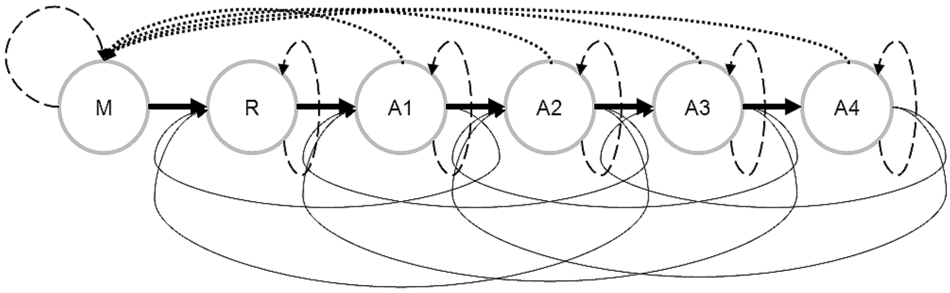
Individuals were divided in six stages: a microscopic stage (M), which includes both female spores, female gametophytes and microscopic sporophytes; and five macroscopic stages: recruits (R), individuals too small to be reproductive, and adult stages A1, A2, A3 and A4 based on total length (Table 1). The macroscopic sporophyte was divided in stages according to size as this is the best predictor of an individual's role in the population (Chapman 1993). These were chosen as to allow a description of yearly growth dynamics and degradation of the blade. Since the minimum size for an individual to become reproductive and maximum size within populations varied between *Laminaria* species and *Saccorhiza*, different size categories were used for each species (Table 1).

Table 1: Size stages, based on total length (cm) defined per species for individual-based descriptions of yearly growth and degradation dynamics.

	Laminaria spp	*S. polyschides*
M	Microscopic stages	
R	≤ 20	≤ 30
A1	≤ 40	≤ 100
A2	≤ 70	≤ 200
A3	≤ 100	≤ 300
A4	> 100	> 300

Projection matrices were constructed based on the life cycle shown in Figure 1 (top). Transition probabilities between stages and mortality were calculated based on their relative frequencies, within each stage, between censuses. Mortality was estimated based on the number of mapped/tagged individuals of each stage/size class that disappeared between censuses (Ang & De Wreede 1990).

The transition data was entered in a square matrix as described in Ang (1987), Fig. 1 (bottom). In this, values are relative probabilities of the transitions in Figure 1 (top): persistence in the same size class, growth to a larger class, erosion to a smaller class, and the number of offspring originated per individual in the stage.



$$\begin{bmatrix} P_{M} & F_{R} & F_{A1} & F_{A2} & F_{A3} & F_{A4} \\ G_{M,R} & P_{R} & E_{A1,R} & E_{A2,R} & E_{A3,R} & DE_{A4,R} \\ G_{M,A1} & G_{R,A1} & P_{A1} & E_{A2,A1} & E_{A3,A1} & E_{A4,A1} \\ G_{M,A2} & G_{R,A2} & G_{A1,A2} & P_{A2} & E_{A3,A2} & E_{A4,A2} \\ G_{M,A3} & G_{R,A3} & G_{A1,A3} & G_{A2,A3} & P_{A3} & E_{A4,A3} \\ G_{M,A4} & G_{R,A4} & G_{A1,A4} & G_{A2,A4} & G_{A3,A4} & P_{A4} \end{bmatrix}$$

Fig. 1: Diagram of the life cycle graph of *L. ochroleuca*, showing the possible transitions between stages defined in Table 1 (top) and structure of the monthly matrix (as described by Ang 1987) (bottom), where each value in the matrix is a relative probability of the transition depicted in the diagram: dotted arrows and F: reproductive success of a stage; thin arrows and E: erosion to a smaller class; thick arrows and G: growth to a bigger class; dashed arrows and P: persistence in the same size classes.

Based on the period needed for a spore to germinate and produce a sporophyte large enough to be observed in the field (Chapman 1986; Hoffmann & Santelices 1991, unpublishes data from lab cultures), reproductive success was calculated based on the amount of recruits that appeared during the following year divided by the number of fertile adults in the previous year and multiplied by the stage's relative reproductive contribution. Relative contribution was calculated based on the relative mean sori area of each stage. As no demographic data of microscopic stages can be gathered in the field, the best available approach was to consider a 100% survival. This stage is essential to the model because it allows considering the time lapse between production of a spore and transition to a macroscopic sporophyte. It doesn't aim at answering what takes place at the microscopic level.

Matrix population models assume that the area studied is closed in terms of spore transport, i.e., that the input and output of spores from the neighborhood are balanced (Engelen et al. 2005). This assumption was made based on previous studies that indicate that most spores settle in the vicinity of their origin (Norton 1992, Gaylord et al. 2002). Moreover, the small size of the individuals provides the spores with a lesser ability to remain in the water column (Gaylord et al. 2004). Also, roughness of the substrate reduces significantly spore dispersal ability (Gaylord et al. 2002). As such, the northern Portuguese coast, with its rocky outcrops almost parallel to the coast will likely present obstacles to spore dispersal. Still, it can be assumed that a small portion of the spores will still disperse to the neighbor areas. As the areas studied are not isolated patches, it was assumed that this will be true for the surrounding areas, evening out spore input and output. However, due to this assumption this method could not be used in *S. polyschides* population, since no reproduction occurred locally in 2010. In 2011 individuals most likely originated from spores dispersed from more subtidal areas. Therefore, only population structure, density and state of maturity were compared.

Annual composite matrices, describing the transitions in size structure during one year, obtained by multiplying the monthly matrices sequentially, were used to determine population growth rate (λ) (Caswell 2001). Random monthly matrices were constructed using a 500 replicate bootstrap, following Caswell (2001). These were in turn used to calculate 500 random year matrices from which the stochastic population growth rate (μ) was calculated. A proportional change in each of the month matrix elements was used to calculate the elasticity of population growth and the relevance of each transition during that month to the yearly population growth, according to Åberg (1992). Since elasticities sum to one, the transition values can be interpreted as the contribution of that matrix element to the population growth, identifying the most important phases of the species life history when summed over each phase. The relative importance of demographic processes, such as vegetative growth, survival and reproductive success were examined by summing the regions of the elasticity matrix referring to these different transitions (de Kroon et al. 1986).

Model simulation

Modeling the dynamics of a population allows predicting its future state. We therefore propose that the dynamics of the years when the populations were followed can be used to simulate the state of the population in 2012. By comparing it with the observations, it is possible to assess the population's ability to recover from destruction caused by the 2010 late winter storm. As such, two simulations for the two years were made. The first assumed that no storm took place in either 2011 or 2012, by using the dynamics from the 2008/2009 period to simulate both years. The second considered that 2011 was a storm year, followed by a calmer 2012.

However, dynamics after disturbance are different from those in a stable population. Therefore, we propose that if no destruction had occurred, the state of the population in 2012 could be predicted by using the dynamics from the 2008/2009 period. Thus, a third simulation was performed in which it was considered that no storm took place between the beginning of the census and 2012. We hypothesize that a similarity between this prediction and the observed state would indicate that the population recovered completely. Thus, such comparison allows assessing the population recovery state. Results of the three simulations were compared with the observed population structure of May 2012. Stage M individuals were not considered due to the impossibility of observing them in the field.

Results

Laminaria ochroleuca and *L. hyperborea*

Population structure

Although the relative abundance of the different stages in the population varies through time, structural differences can be observed between the population of the two sites and the two census years (Fig. 2). For the period before the storm (February 2010), VC is composed of larger individuals and few recruits, while in SB a relatively high number of recruits is always present, contrasting with the few A3 individuals and no A4. After the storm however, VC lost all the bigger individuals and in proportion, the number of recruits reached its highest. SB, on the other hand, was able to maintain a fair amount of adults and the proportion of recruits was even with the period before the storm. Population structure of the MM mixed stand varied between the two species (Fig. 3). In both, most individuals were in the recruit stage. However, *L. ochroleuca* had some stage A3 and A4 individuals, while *L. hyperborea* had just a few A3 individuals and lacked A4. Whilst before the storm the pool was mostly occupied by *L. hyperborea*, the opposite was observed after the storm as no new *L. hyperborea* appeared and recruits of *L. ochroleuca* promptly grew on the newly available substrate.

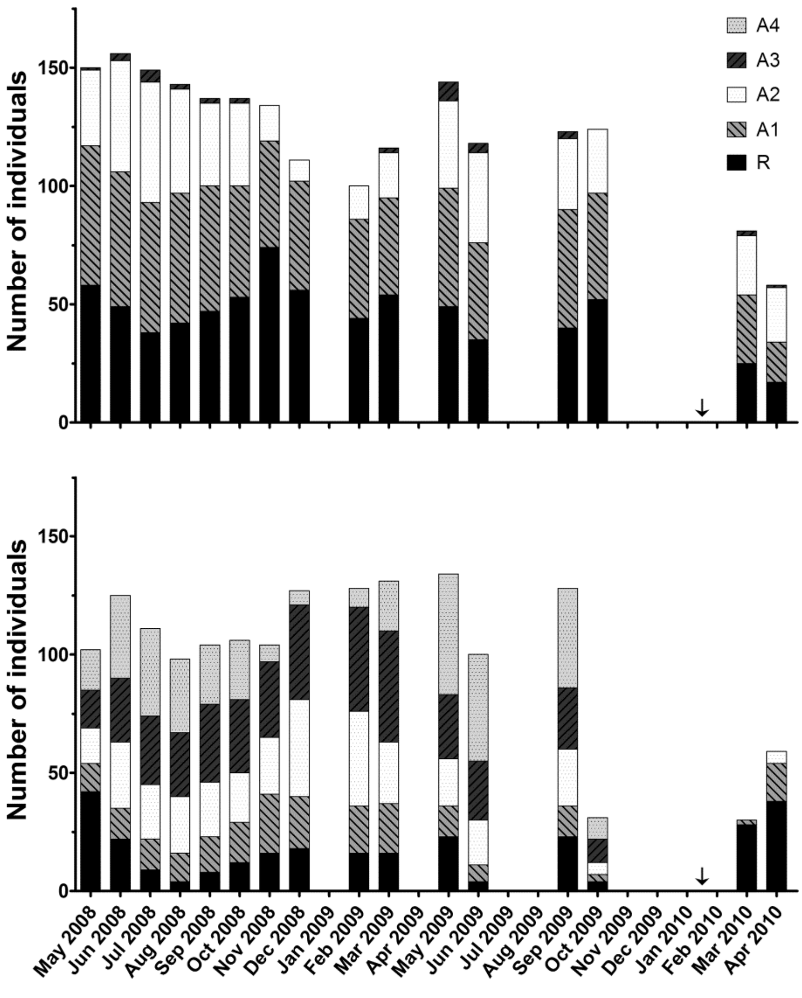


Figure 2: Population stage structure of *Laminaria ochroleuca* at S. Bartolomeu (top) and Viana do Castelo (bottom) from May 2008 to April 2010. Stage classes: R: recruits, A1: individuals from 20 to 40cm in length, A2: individuals from 40 to 70cm, A3: 70 to 100cm, A4: individuals longer than 100cm. Microscopic stages were not considered as they are not observable in the field. Arrows indicate the time of the late 2009/2010 winter storm.

Population growth rates varied with population and year. During 2008-2009, the *L. ochroleuca* population at VC had negative growth (λ = 0.8265) while at SB positive growth was recorded (λ = 1.0351). From 2009-2010, populations at both sites had negative population growth (λ = 0.3526 and λ = 0.4796, for VC and SB respectively). However, it is important to note that the storm took place 4 months before the end of the census, causing an 88% and 36% mortality in VC and SB, respectively.

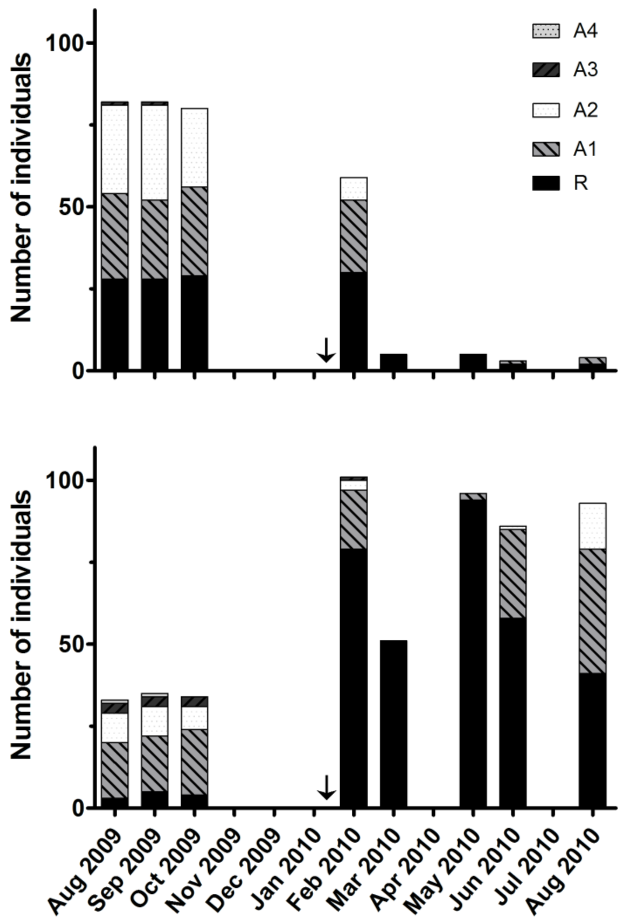


Figure 3: Population stage structure of *Laminaria hyperborea* (top) and *L. ochroleuca* (bottom) from August 2009 to August 2010. Stage classes: R: recruits, A1: individuals from 20 to 40cm in length, A2: individuals from 40 to 70cm, A3: 70 to 100cm, A4: individuals longer than 100cm. Microscopic stages were not considered as they are not observable in the field. Arrows indicate the time of the late 2009/2010 winter storm.

At the mixed site both species decreased throughout the year (λ = 0.0344 and λ = 0.5565, for *L. hyperborea* and *L. ochroleuca*, respectively). However, it is important to note that the natural destruction took place half way through the year, causing the death of 84% of *L. ochroleuca*, while mortality of *L. hyperborea* reached 91.5%.

ELASTICITY

Reproductive success had a negligible contribution to population growth. Although always below 0.1, its contribution was slightly elevated in the beginning of spring (May and June) when the new recruits appear, and at the beginning of autumn (October and November) when spores are typically released. Consequently, as the contribution of survival and vegetative growth varied with time, a negative relationship between the two processes can be observed. Survival contributed the most to population growth, with the exception of March 2009 in VC, and especially in autumn and winter months, whereas vegetative growth had a slightly more important role roughly between January and June, when individual blade growth exceeds erosion (Fig. 4). The contribution of vegetative growth, survival and reproductive success also varied between the two species at MM. Population growth of *L. hyperborea* was totally dependent on individual survival, except for October 2009, when it slightly relied (1%) on vegetative growth. Contributions to *L. ochroleuca* population growth, however, varied throughout the year. During March and June 2010, vegetative growth contributed the most to λ, while in February 2010 growth relied mostly on reproductive success. For all the other months, λ was mostly dependent on survival (Fig. 4).

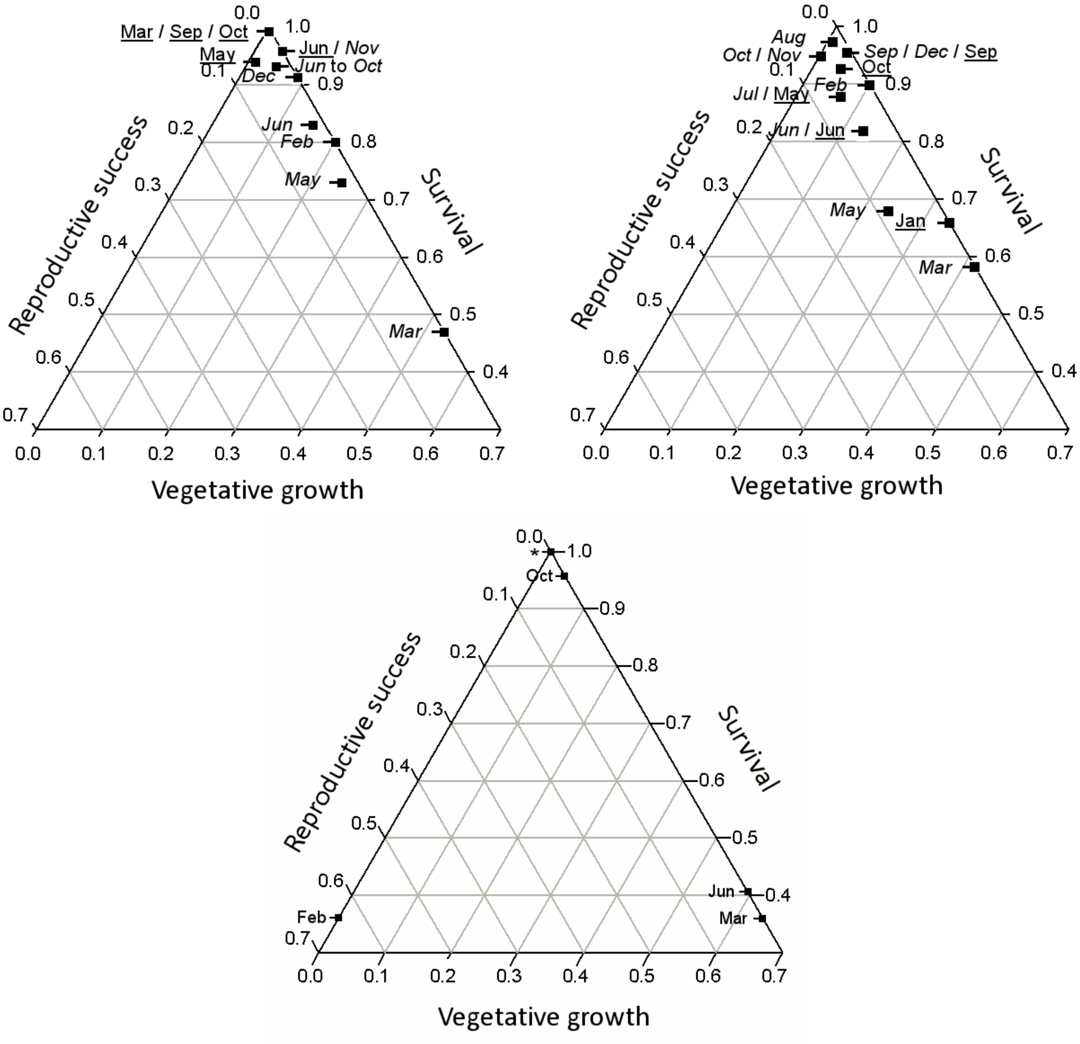


Figure 4: Triangular ordination of the relative contribution of vegetative growth, survival and reproductive success in each month to population growth rate (λ) for *L. ochroleuca* at Viana do Castelo (left) and S. Bartolomeu (right) in the periods of 2008-09 (non underlined) and 2009-10 (underlined), and mixed population of *L. hyperborea* and *L. ochroleuca* at Moledo do Minho (bottom). Points with a month label refer to *L. ochroleuca*. * refers to *L. ochroleuca* in August and September 2009 and March to June 2010 and *L. hyperborea* for each of the considered months.

Model simulation

Considering the 2008/2009 dynamics after the storm, the two year simulations predict the appearance of few individuals. Therefore, population structure at both sites in 2012 differed from both the two year simulations, either if both years were simulated as regular years, with no storms, or if 2011 was considered a high disturbance year and 2012 a regular one.

At both sites, the four year simulation depicted the observed structure the best. However, at SB there were significantly fewer individuals in stages B and C. At VC, on the other hand, the number of individuals in smaller stages (R and A) was significantly lower than predicted.

<u>*SACCORHIZA POLYSCHIDES*</u>

Population structure by the end of the macroscopic sporophyte season, at the same time of year, varied between the three years. In May 2010, there were barely any *S. polyschides* recruits in the field. Since recruitment usually occurs between February and April (personal observations), in 2010 individuals appeared in the field somewhat later than usual. By the end of the macroscopic season, in August 2010, the population was composed of smaller individuals, when compared to 2011 and no reproductive individuals were present (Fig. 5).

During 2011, the maximum population density was 7.5 individuals m^{-2}, less than half the density registered in August 2010 (20 individuals m^{-2}). In 2012 density was the highest, reaching 27 individuals m^{-2}, 135% that of 2010 and 360% of 2011.

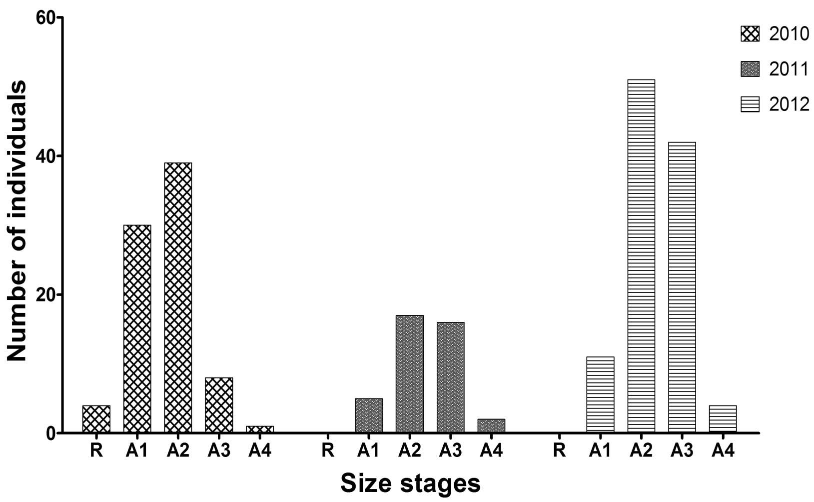


Figure 5: Population stage structure for the end of the macroscopic season (August) of 2010, 2011 and 2012. Stage classes: R: recruits with less than 30cm, A1: individuals from 30 to 100 cm in length, A2: individuals from 100 to 200 cm, A3: 200 to 300cm, A4: individuals longer than 300cm. Microscopic stages were not considered as they are not observable in the field.

Discussion

With climate change, variations in environmental conditions will become broader and species populating shallower areas will most likely be the first to suffer their effect and lose resilience (Guo et al. 2005, Hampe & Petit 2005, IPCC 2007, Wernberg et al. 2010, Harley et al. 2012). The young age of individuals of *L. ochroleuca* is a reflection of such adversities (Norton et al. 1982). While their reproductive effort is considerable, it took them longer than 2 years to fully recover after a massive destruction. As with global warming such highly energetic storms will become more frequent, individual's life expectancy is likely to decrease, impairing local spore production and rendering the population dependent on spore dispersal from deeper areas (IPCC 2007, Byrnes et al. 2011, Hemer et al. 2013).

Contrastingly, *L. hyperborea* was totally dependent on individual's survival as reproductive effort and recruitment were negligible. After the storm, this species was unable to recruit back, indicating that the deeper scattered population is unable to disperse enough spores to these areas (Reed et al. 1992, Gaylord et al. 2004) and in accordance with the reported tendency of the northward distribution shift of the last decades (Ardré 1970, Pérez-Ruzafa et al. 2003).

Yearly climate variations seem to influence *S. polyschides* life cycle. Recruitment seems to have decreased as a result of the winter storm (Ballantine 1984, Hoffmann & Santelices 1991, Burrows et al. 2011), and was delayed due to the late summer. This resulted in delayed development of sporophytes, which were unable to reach maturity before decline. With this, local yearly production of spores was precluded and, since spores are unlikely to survive more than a year (Lüning 1980, Hoffmann & Santelices 1991), the next generation most likely relied on spore dispersal from more subtidal areas.

L. ochroleuca

During 2008-09, the population at SB appears to be younger than that at VC, where longevity seems to be higher, leading to a low recruitment. For this period, λ of VC was negative, recruitment was low and elasticity shows that it relied mostly on survival rather than compensated for losses. However, VC population was mainly composed of individuals with considerable reproductive effort and, as most spores settle in the vicinity of their origin, it may be assumed that spore supply was not limiting (Norton 1992, Gaylord et al. 2006). Individuals at this site formed a dense canopy reducing the light that reaches the substrate and probably limiting early stage growth (tom Dieck 1993). Recruits were able to reoccupy the available space only when death locally reached a threshold, reducing the inhibition. Therefore, negative growth of the population is not unexpected and doesn't necessarily mean a loss of function or an inability to recover. Such population structure is usually found in areas where disturbance is lower (Dayton et al. 1984).

The SB population, in contrast, was mostly composed of relatively small individuals, which indicates that it may be younger and depend more on recruitment to compensate a high mortality. Although, the percentage of recruits in this population was higher than for VC, reproductive success had an extremely low contribution to population growth, indicating the high mortality they suffer. Nevertheless, the low number of survivors was enough to maintain a positive population growth.

During the second period of the census, in January 2010, a highly energetic storm caused the destruction of 88 and 36% of VC and SB population, respectively, probably as a result of smaller individuals being more resistant to strong hydrodynamics. As this took place four months before the last census, the negative population growth is mainly an indicator of the storm's effect. After the storm, recruitment in VC became higher than for SB, supporting our initial hypothesis that is was inhibited by canopy (tom Dieck 1993). Since all individuals big enough to reproduce were destroyed in VC, it became almost totally dependent on survival of microscopic stages and the contribution of reproductive effort to population growth is even lower than for the preceding period.

For both VC and SB, the two year simulations of population structure resulted in significantly fewer individuals than observed. This disagreement is caused by the differences in processes between the first year of census, when population was set, and the dynamics after the destruction, when available space drove to an increase in recruitment (Engelen et al. 2011).

Considering the first census, in May 2008, and simulating as if 4 regular years took place, should provide an estimate of what the population structure would be if no disturbance took place. SB and VC show opposing deviations between simulated and observed data. In SB, the simulation is in agreement with the smaller stages only, while the larger individuals are yet to be recovered, indicating that more than two years were required for complete recovery. In contrast, in VC the smaller stages disagree with the simulation results, as no R and very few A1 individuals were present in 2012. Here, the storm caused the destruction of almost all adults after the reproductive season, leaving microscopic stages available to recover the population. However, as it takes longer than a year for a recruit to become adult, it is to be expected that in the following reproductive season these new individuals would have been too small to reproduce. Dispersal range varies highly with local conditions and the rocky outcrops, parallel to the shore, most likely hamper dispersal from the subtidal (Reed et al. 1992, Gaylord et al. 2004). Also, the microscopic forms are unlikely to survive more than 1 year (Chapman 1987, Hoffmann & Santelices 1991, Tegner et al. 1997, Vásquez et al. 2006). Thereby, a shortage of spores in 2012 might explain the absence of stage R and A1 individuals. As such, it seems that although the macroscopic population has recovered, the bank of microscopic stages has not yet been replenished.

L. HYPERBOREA

Population structures of both *L. ochroleuca* and *L. hyperborea* growing in the same pool indicate that these were young when compared with VC and SB. Still, *L. hyperborea* had even smaller individuals than *L. ochroleuca*. This might be associated with its lower resistance to light and high temperature exposure (Lüning 1986, Han & Kain (Jones) 1996). Considering that most individuals were under the canopy of *L. ochroleuca*, it might be proposed that this protection is fundamental for the settlement of *L. hyperborea*. Adults were small, with low reproductive effort and in a non-reproductive state for most of the year. In September and February, the range of their typical reproductive season (Han & Kain (Jones) 1996), respectively only 10% and 50% of adults had sori, resulting in only 2 new recruits throughout the census period. Impairment of fertilization caused by temperatures above 15 °C may also be a contributing factor (Lüning 1980). Sporophytes of *L. ochroleuca,* on the other hand, are able to grow and form sori up to 23 °C and gametophytes survive and reproduce at temperatures up to 20 °C (Lüning 1980, Pereira & Engelen 2011).

λ values express recovery but also the storm impact, which caused a destruction of 84% of *L. ochroleuca*, and 91.5% of *L. hyperborea*. Observations in 2012, two years after the last census, show that the population had more individuals in all stages forming a denser canopy. With this, protection for *L. hyperborea* was likely recovered. However, this species was absent from the area, as the local population was not able to maintain itself and the scattered subtidal populations (personal observations, in 2009) don't seem to be able to disperse microscopic forms in enough density (Reed et al. 1992, Gaylord et al. 2002).

Reports indicate a Northward shift of the southern distribution limit of *L. hyperborea*. In 1969, it was present till Cape Mondego (40º10'49.23"N; 8º54'20.88"W) (Ardré 1970). In 2003, it shifted further North, to Praia da Aguçadoura, Porto (41º25'40.79"N; 8º46'57.98"W) (Pérez-Ruzafa et al. 2003). At the study site, 1km south from the Minho River estuary (41º50'06.79"N; 8º52'30.58"W), this species was not able to recover its populations in the intertidal pools, supporting the tendency of distribution shift which may not only be geographically but also in depth.

S. POLYSCHIDES

During the 2010 late winter storm, the *S. polyschides* population, exclusively composed of microscopic forms, most likely suffered scouring and burial by sediments (Hoffmann & Santelices 1991). Also, instead of recruiting between March and April, only a reduced number of scattered small sporophytes could be observed in May, indicating that this species life cycle can be dramatically affected by yearly climatic variation. As a consequence of this delay individuals died off before reaching reproductive maturity. In 2011, however, recruitment took place in April. Since spores are unlikely to survive for more than one year (Hoffmann & Santelices 1991, Schiel & Foster 2006), the spores that gave rise to the new population were most likely descended from the neighbor subtidal individuals. Still, the failure to reproduce in the area seems to have caused a shortage of spores resulting in a 50% lower population density in 2011, compared to 2010. Nevertheless, as recruitment took place earlier, the population was significantly more developed in 2011, with a higher percentage of mature reproductive individuals. Maybe as a result of a higher local spore production, population density in 2012 was significantly higher than in the two previous years.

Conclusion

Shallow kelp populations are highly impacted by anomalous environmental conditions (Guo et al. 2005, Hampe & Petit 2005, IPCC 2007, Wernberg et al. 2010, Harley et al. 2012). The constant spore production of the perennial *L. ochroleuca* population provides an advantage, allowing a complete recovery without depending on spore dispersal from adjacent areas. For *S. polyschides* however, in years with abnormal conditions, the development of the macroscopic sporophyte may be delayed, hampering their annual spore production and rendering the population dependent on spore dispersal from more subtidal areas. Our results suggest that a complete recovery of shallow *L. ochroleuca* forests takes longer than two years while *S. polyschides* is able to recover after one year. In contrast, *L. hyperborea* was unable to recover from destruction as local spore production and dispersal from deeper areas were insufficient to repopulate the destroyed area. With climate change, it is believed that the recurrence of such extreme events will become more frequent (Young et al. 2011, Byrnes et al. 2011, Hemer et al. 2013). This might, in the future, result in a decrease in density of shallow kelp forests and later shift of their upper distribution limit to deeper areas, before geographical distribution shifts may be perceived (Bartsch et al. 2008).

CHAPTER 2

LIVING ON THE EDGE: KELP FOREST RESILIENCE AT THE EDGE OF DISTRIBUTION.

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ABSTRACT

Kelp forests have been disappearing from several areas worldwide. However, natural disturbance effects do not occur homogeneously across the species range, and are expected to affect first the distributional edges where species occur in limiting conditions. Further, their loss of resilience is often only noticeable when populations suffer massive destructions. We aimed to assess the resilience of *Laminaria ochroleuca* to perturbations at the geographical and vertical edge of its distribution, and to look for evidence of possible competitive interactions affecting recruitment.

Following experimental canopy removal, within 6 months *L. ochroleuca* recruited and grew almost to the initial state. At the storm affected site, however, little recovery could be observed 6 months after the storm and no significant differences between the control and the canopy removal treatments, equally affected by the storm, was found. Thus, the extension of the destruction seems to have been more important than its consecutiveness. Major destructive events may deprive the population from individuals big enough to reproduce, which may lead to a lesser availability of spores as local absence of spore production is not compensated by dispersal from the surrounding area. Also, new individuals lack the protection usually provided by the canopy, which results in higher mortality. Although there was available substrate and recruitment was rapid, population density was lower after the storm, indicative of the high recruit mortality and possibly due to limited availability of spores.

Density and size of other canopy forming species, such as *Saccorhiza polyschides*, *Sargassum muticum*, *Cystoseira tamariscifolia* and *Cystoseira baccata* varied over time. However, this was due to seasonal variations and did not depend on, nor affected, the density or size of *L. ochroleuca*.

Keywords: *Laminaria ochroleuca*, algal recruitment, kelp recovery, edge populations

INTRODUCTION

Over the last decades, climate change effects have become ever more noticeable, with increasing extreme environmental conditions observed all over the world (Cai et al. 2014). Concerns about effects of El Niño Southern Oscillation (ENSO) have been raising, as storms, droughts and floods impact aquatic and terrestrial ecosystems worldwide, affecting many species, both directly and through cascading effects (Harrison 2000, Holmgren et al. 2001, Wang 2005, Doney et al. 2012, Marengo et al. 2013, Cai et al. 2014). Similarly, the North Atlantic Oscillation (NAO) has been influencing climate and the abundance of several species (Drinkwater et al. 2003, Seager et al. 2010). This has an especially important impact when affecting habitat foundations species such as trees, corals, seagrass and kelp, on which a wide variety of organisms depend.

Kelp forests are particularly susceptible to variations in environmental conditions, which can cause massive death and impede the onset of new individuals. Since most species rely on environmental cues for growth and reproduction, such oscillations may have dramatic effects on populations (Ottersen et al. 2001, Yang & Rudolf 2010, Voerman et al. 2013, Bartsch et al. 2013). Such is the case of storms, which are prone to breaking kelp stipes, may cause a decrease in recruitment by scouring and burying spores and gametophytes and, by removing the canopy that offers protection to new recruits, affects their survival and, consequently, the population's ability to recover (Dayton & Tegner 1984, Hoffmann & Santelices 1991, Tegner et al. 1997, Vásquez et al. 2006). Moreover, such destruction may have cascading effects, affecting species that find their habitat in kelp forests (Ebeling et al. 1985, Byrnes et al. 2011). In addition to storms, pollution and overexploitation of natural resources disturb food webs and ecosystems, with potential effects for kelp populations (Slik 2004, Sangil et al. 2012). Several areas have become barren grounds and are unable to recover due to anthropogenic pressure, such as overfishing the herbivore's predators, which results in their increased density (Watling & Norse 1998, Coleman et al. 2008, Guarnieri et al. 2014). This difficulty to recover from destruction may also be maximized by the introduction of alien species that have a competitor advantage (Stæhr et al. 2000, Casas et al. 2004). Atlantic kelp forests can be severely impacted by the introduction of invading species such as the Japanese *Sargassum muticum* and *Undaria pinnatifida*. Kelp competes with other large algae for both substrate and light, and invasive species may cause variations in abundance of native kelp or even prevent their recruitment (Ambrose & Nelson 1982, Stæhr et al. 2000, Sanchez et al. 2005, Harries et al. 2007). Grazing pressure is also a factor frequently impeding the recovery of kelp populations, especially by sea urchins (Leinaas & Christie 1996, Norderhaug & Christie 2009).

This study aims to assess the ability of kelp forests to recover from destruction when occurring near distributional edges. This includes their ability to compete for space and the identification of potential space competitors. Experimental kelp destruction was initiated in 2009 on the Northern Portuguese coast, near the southern limit of large continuous kelp forests of *L. ochroleuca*. There, forests extend from the subtidal to deep intertidal pools, where they are constantly subjected to strong currents, wave action and wide variations in environmental conditions. Although these populations are composed of relatively small individuals, they are able to reproduce and recruit locally, forming self-sustained populations (Unpublished data). Still, little is known about their ability to recover from canopy removal due to either harvest or natural destruction and how long it takes for populations to completely recover. Also, canopy shading can inhibit development of other macroalgae and, with this inhibition halted, other species might find the conditions to resume their growth and compete with kelps for space. With invasive species extending the area they populate, it's important to find out how capable kelps are of overgrowing and reoccupying the area, and what species may pose a threat to recovery.

METHODS

Dense kelp forests in the high subtidal and intertidal pools of Northern Portugal are mainly composed by *L. ochroleuca*, while the lower subtidal is mainly dominated by *S. polyschides.* In autumn 2008, a two year long destructive experiment was initiated in large lower intertidal pools with dense *L. ochroleuca* beds, at two sites less than 2km apart: Viana do Castelo (VC: 41º41'36.83"N, 8º51'08.00"W) and Areosa (Ar: 41º42'27.19"N, 8º51'43.81"W) (Fig.1). This was initiated by removing all canopy forming seaweeds from three 1m^2 quadrats at each site. All kelps were taken to the lab, weighted and their stipe and total length measured. To serve as control, in both sites, canopy forming algae in three additional 1m^2 quadrats were tagged and measured. In spring 2009, a second set of three destructive quadrats was added to VC. Around both the control and removal quadrats, a 50 cm buffer zone was set, were all canopy was removed to ensure that blades of neighbor individuals wouldn't reach the study areas. The census was repeated every six months, with exception of autumn 2009 due to the severe storms that ravaged the northern Portuguese coast. The winter of 2009/2010 was characterized by an unusual persistence of the NAO negative phase, which caused a temperature drop over North and Western Europe (Seager et al. 2010). In the Northern Portuguese coast, extremely energetic storms occurred, accompanied by unusually strong waves that caused extensive destruction along the coast. After this, several areas where clean from most macroalgae. Maybe due to coastal configuration, this destructive storm affected just one of the studied sites (VC), allowing the study of the effect of single and consecutive destructions to continue, and giving clues on what might happen when such energetic storms, or possibly harvest, become more frequent. In autumn 2010, 2 years after the beginning of the experiment, all kelps in both control and removal quadrats were removed and taken to the lab for measuring and weighting (Fig. 1).

	Autumn 2008	**Spring 2009**	**Autumn 2009**	**Feb 2010**	**Spring 2010**	**Autumn 2010**
Storm site	1st destruction	2nd destruction		Storm	Census	Final sampling
No-Storm Site	1st destruction	Census			Census	Final samping

Fig.1. Chronology of destructions and censuses in the two study sites.

Since the contrasting storm impact between Ar and VC hints for different conditions, data from the two sites was analyzed separately to study the effect of single and consecutive destructions, respectively. Stipe and total length, weight, density of kelps, number of new recruits and mortality data was analyzed with 2-way repeated measures ANOVA, considering time (repeated measures) and type of quadrat, on Sigma Plot (Systat Software, San Jose, CA).

Due to the difficulty in transforming the data on density of other canopy forming species to meet the normality and homoscedasticity assumptions required for an ANOVA, the data were analyzed with the PERMANOVA+ module of Primer 6 (Anderson et al. 2008). Both PERMANOVA, PERMDISP and pairwise analyses were made with 9999 permutations.

After checking for significant differences between sites, prior to the 2009/2010 winter storm, data from the storm impacted site (VC) was used to study the resilience of *L. ochroleuca* to multiple destructions. To check how long it would take for populations to completely recover from either multiple or single destructions, data from both sites, considering time from last destruction, was used.

RESULTS

SINGLE REMOVAL

In the no-storm site (Ar), where canopy was removed only once from each quadrat, in autumn, stipe length varied significantly through time and with type of quadrat (2-way RMANOVA, time*quadrat type, $p = 0.007$; Fig. 2). Prior to kelp removal, no significant difference could be observed between control and removal quadrats. After six months, stipes of individuals in the removal quadrats were significantly smaller than in the control, although not significantly different from the initial mean. In contrast, from a year and a half on, stipe length in removal quadrats was significantly higher from not only the initial value but also the control. No significant difference could be observed, through time, for the control quadrats. Total length varied significantly with time but not with type of quadrat (2-way RMANOVA, time, $p < 0.001$, Fig. 2), and was significantly lower at the beginning and end of the experiment than after 6 and 18 months.

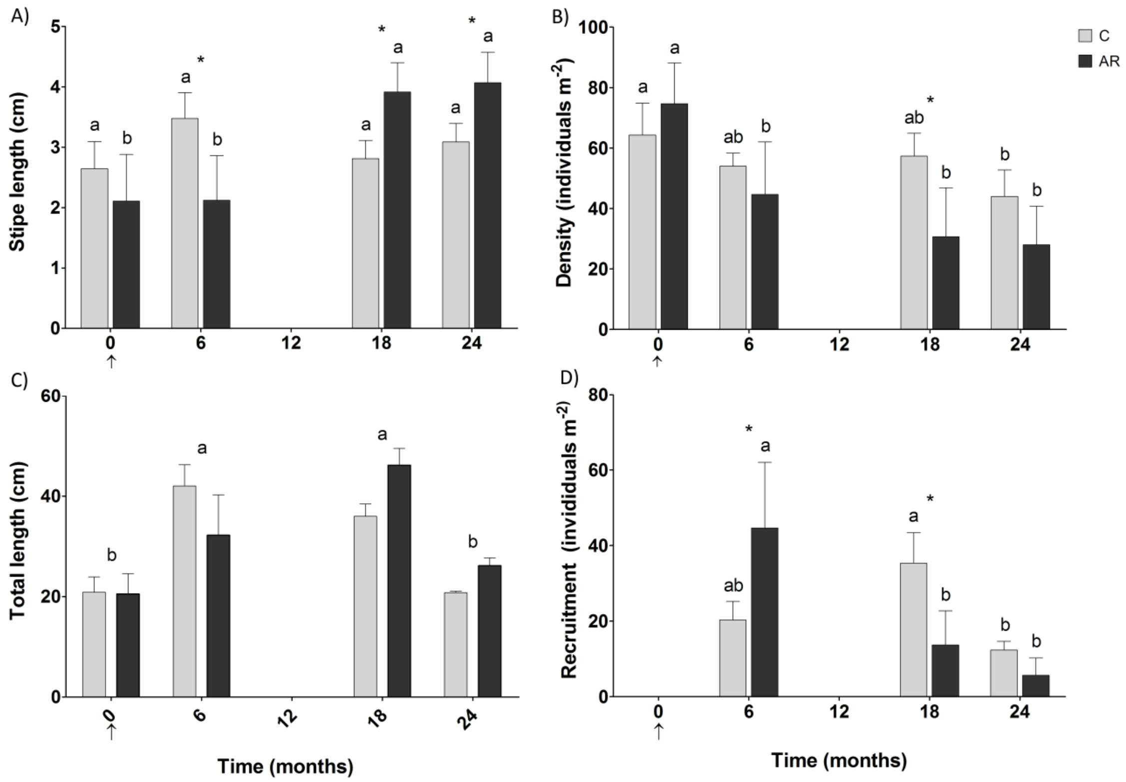

Fig. 2: A) Mean stipe length, B) Density, C) Total length and D) Recruitment of *L. ochroleuca* through time in the no-storm site. Time 0 refers to the beginning of the experiment and the arrows indicate the points where removal took place. C: control quadrats; AR: autumn canopy removal quadrats. . Different letters indicate significantly different temporal variation within each type of quadrat. * indicates a significant difference between control and experimental removal quadrats for the same time period.

Density of *L. ochroleuca* varied significantly with time and type of quadrat (2-way RMANOVA time * quadrat type, p = 0.005, Fig. 2). Both types of quadrats show a tendency for density to decrease with time, but significant difference between control and removal was observed at 18 months. Although PERMANOVA results indicate that the density of other canopy forming algae varied with time and type of quadrat (PERMANOVA, time * quadrat type, p = 0.0051; PERMDISP, quadrat type, p = 0.2819; time, p = 0.0004), pairwise comparisons detected no significant difference between census times. The number of canopy forming individuals other than *L. ochroleuca* was higher in spring, while almost absent in the autumn censuses (Fig. 3). Also, these variations showed no pattern relatable with *L. ochroleuca* density.

Although not significantly different, mortality for the 2009/2010 winter storm period was lower than for the rest of the monitoring period, indicating that the Ar population was not significantly impacted. During the two years, no significant difference was observed between control and removal quadrats (2-way RMANOVA, quadrat, $p = 0.105$), but mortality was significantly higher for the period after the storm between 18 and 24 months (2-way RMANOVA, time, $p = 0.035$; Fig. 2).

The number of new individuals varied with both time and type of quadrat (2-way RMANOVA, time * quadrat type, $p < 0.001$). A maximum of new individuals was observed after the canopy removal. At 18 months, however, significantly more new individuals were found at the control quadrats than in the removal ones (Fig. 2). The number of new individuals followed neither a seasonal variation nor did it vary according to individual density or *L. ochroleuca* mortality.

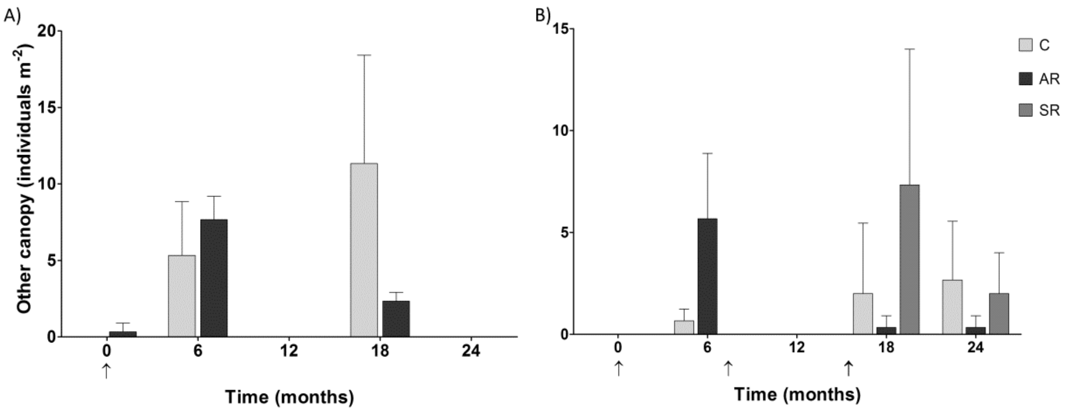

Fig. 3: Abundance of canopy forming species other than *Laminaria ochroleuca* varied throughout the year in both the A) no-storm site and the B) storm site. The pattern was, however, unrelated to density, size or recruitment of *L. ochroleuca*. C: control quadrats; AR: autumn canopy removal quadrats. SR: spring canopy removal quadrats

MULTIPLE DESTRUCTION

In the storm-site, both stipe and total length varied significantly with time and type of quadrat (2way RMANOVA, time * quadrat type, $p = 0.013$, Fig. 4). Stipes were significantly longer in the first 6 months of the experiment and in the control quadrats during that period. After the second experimental destruction no significant difference in stipe length was found among the different types of quadrats. Total length measurements followed a similar pattern but pairwise comparisons detected no significant difference between types of quadrats for any given period. Individuals were significantly shorter after the storm, with the exception of the spring removed quadrats at 24 months, for which there was no significant difference from their initial state (Fig. 4)

Density of *L. ochroleuca* varied significantly over time between types of quadrats (2-way RMANOVA, time * quadrat type, $p < 0.001$, Fig. 4). Significant difference between quadrats could be found at 6 months only, when the spring destroyed quadrats had significantly higher density, than prior to removal. Density was lower after either the experimental destruction or the storm. Mortality varied significantly with time and type of quadrat (2-way RMANOVA, time * quadrat type, $p = 0.018$). Pairwise comparisons show that this was significantly lower for the control quadrat between 0 and 6 months and for the spring removed quadrats between 18 and 24 months (Fig. 4). For the rest of the points, mortality was almost total. As such, after the storm, almost all individuals in the quadrats were new. The density of other canopy forming species, such as *Saccorhiza polyschides*, *Cystoseira baccata*, *Cystoseira tamariscifolia* and *S. muticum*, varied significantly with time and between quadrat treatment (PERMANOVA, time * quadrat type, $p = 0.0232$, Fig. 3; PERMDISP, quadrat type, $p = 0.574$, time, $p = 0.2213$). Nevertheless, pairwise comparisons did not show significant difference between quadrat types at each time period. Density of such species showed no seasonality or variation with density of *L. ochroleuca*.

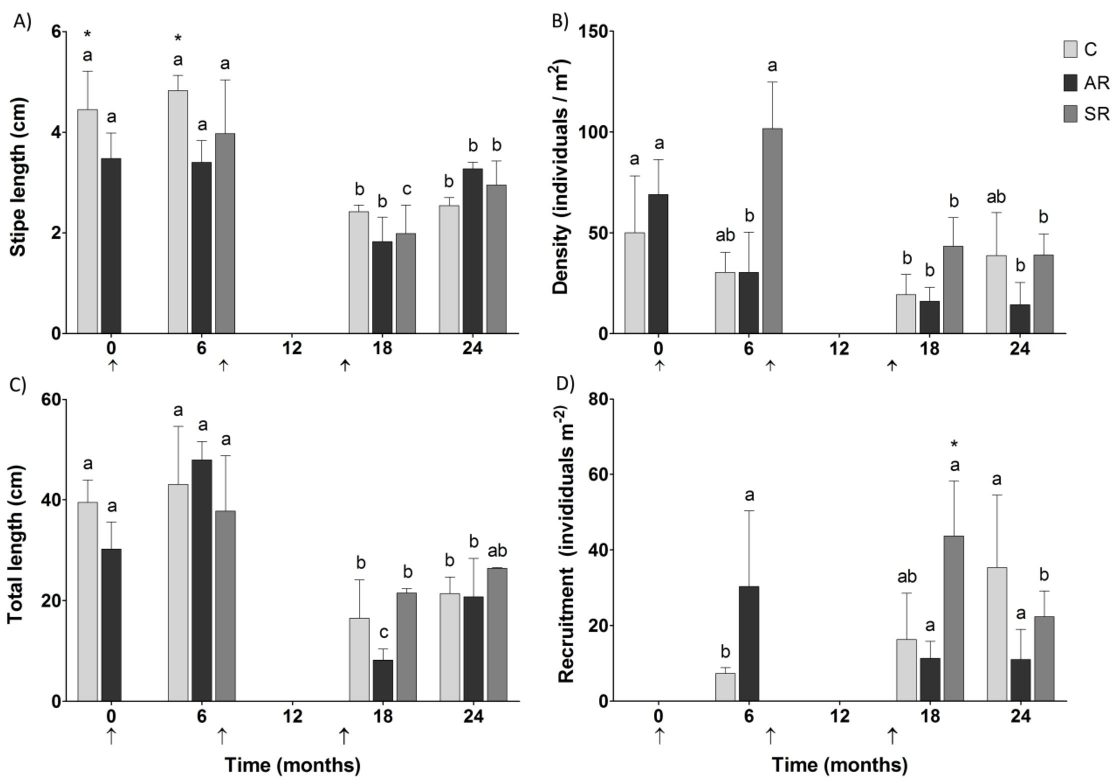

Fig. 4: A) Mean stipe length, B) Density, C) total length and D) Recruitment of *L. ochroleuca* through time in the storm site, where consecutive destruction took place. Time 0 refers to the beginning of the experiment and the arrows indicate the points where removal took place. The bold arrow indicates the timing of the storm. C: control quadrats; AR: autumn canopy removal quadrats. SR: spring canopy removal quadrats. Different letters were attributed to significantly different points within each type of quadrat. * indicates a significantly different point at that time.

DISCUSSION

After canopy removal, *L. ochroleuca* recruits rapidly. However, despite substrate availability, individuals' density is lower after than before removal, indicating that recruitment and survival was not sufficient to recover the initial density. Since individuals in the intertidal are younger and shorter than in the subtidal, they are able to quickly grow to the initial state in case of small scale canopy removal. In contrast, extensive destruction, as caused by the storm, are likely to impair recovery due to lack of protection by surrounding canopy, leaving the new and fragile individuals exposed to stronger hydrodynamics and higher exposure to light and UV (Jackson & Winant 1983, Løvås & Tørum 2001).

Besides *L. ochroleuca*, other canopy forming macroalgae occupy intertidal pools. However, their presence, size and density vary seasonally and they pose no impairment for *L. ochroleuca* recovery. As such, with increased storm frequency, failure to produce spores locally will become more evident and intertidal populations are likely to be increasingly dependent on dispersal from the subtidal and lose resilience.

The state of *L. ochroleuca* 6 months after single removal indicates that the species was able to rapidly recruit and individuals recovered almost to the initial state. Only stipe size took longer to recover. Plant size, on the other hand, varied with time as a result of seasonal growth and apical loss. After canopy removal, resources availability allowed an increase in recruitment. Lower density after removal, despite increased recruitment, indicates a high mortality of recruits. Furthermore, 18 months after removal, recruitment was higher at the control quadrats, which indicates a dependency on local spore production, as individuals at the removal quadrats were not big enough to reproduce.

At the storm site, 6 months after the destructive storm, no significant difference in size between quadrats could be observed as individuals seem to have been rapidly originated from microscopic stages, likely produced before the storm in all of them. Also, it is possible that the storm scoured, buried and transported spores, homogenizing their availability. Still, enough spores were present to rapidly recolonize the area. However, mortality after the storm was also high, most likely due to lack of protection by the absent surrounding canopy. As no significant differences in recruitment, mortality or individual size could be observed between quadrat types after the storm, extension of canopy removal seems more relevant than the recurrence of low scale disturbances.

Besides *L. ochroleuca,* other canopy forming species, such as *Cystoseira sp*, *S. muticum* and *S. polyschides,* occupy deep intertidal pools. Our results show that their presence and density varies seasonally and poses no impairment for *L. ochroleuca* recovery.

SINGLE DESTRUCTION

In the no-storm population, while the new individuals in the removal quadrats, six months after removal, had reached a total length similar to the control, their stipes were still significantly shorter. In the following census, 18 months after the destruction, no significant difference in size, either stipe or total, can be observed between the two types of quadrats, indicating that the individuals were able to grow to the initial state. This is a quick recovery when compared with other harvest experiments. *Laminaria longicruris* from Nova Scotia took one year to regrow cropped biomass and two to reach pre-harvest maturity (Smith 1985). In Brittany, France, in an area with *Laminaria digitata* species structure was recovered only after 18 to 24 months (Engelen et al. 2011). It's however important to note that, as this study focused on range limit populations, individuals are smaller and probably have a short life expectancy when compared with subtidal ones, since hydrodynamics cause higher mortality resulting in younger populations (Norton et al. 1982) . Also, the effect of tides and waves makes individuals grow more robust and causes higher erosion of the blades (Norton et al. 1982). In fact, no significant difference in total length could be found between types of quadrats at any time. Rather, total size varied with time, being significantly longer in the spring months. This variation is in accordance with the growth pattern of *Laminaria* species, growing faster during the winter, achieving maximum size in spring, when growth rate decreases and energy is allocated to reproduction (Kain (Jones) 1989). The lack of significant difference in total size between individuals in the control and in the removal quadrats indicates that, after the canopy removal, the newly available substrate was readily occupied by new individuals, as observed in previous removal experiment (Barradas et al. 2011). Since canopy removal was performed in September, spores produced by the removed individuals were most likely released prior to removal. As such, this fast recovery might have been due to either the newly released spores and/or a previously settled bank of microscopic forms in a slow development state (Chapman 1987, Hoffmann & Santelices 1991, Vásquez et al. 2006). The number of new individuals six months after the beginning of the experiment was, as expected, significantly higher at the removal quadrats than in the control, due to lack of inhibition by shade. However, the lower density after canopy removal hints for high recruit mortality, since with newly available substrate, an increase in density was to expect. 18 months after removal the number of new individuals was significantly higher for the control quadrats and cannot be directly related to canopy density, since there is no significant difference between the two types of quadrat in individuals' density or total length. As such, the most likely cause for the differences in number of new individuals is local spore availability, since in the removal quadrats individuals were younger and thus able to produce less spores.

No significant difference in density of other canopy forming species was found between types of quadrats. This varied with time, and was higher during the spring censuses, indicating that their presence was not a result of substrate availability but rather a seasonal variation. As such, these species don't seem to compete efficiently with *L. ochroleuca* and are unlikely to impair its recovery.

MULTIPLE DESTRUCTION

In the storm site, experimental destructions in autumn and spring were followed by a late winter storm that caused an almost total destruction. Since no census could be made 6 months after the spring destruction, the effects of removing the canopy at different times of the year could not be assessed. However, the effect of destruction repetition within a year and a year and a half interval was studied. Stipe and total length were not significantly different between types of quadrat, hinting that recruitment took place during a similar period. The fact that density of recruits did not vary significantly between types of quadrats after the storm indicates that the availability of microscopic forms was similar, and the lower density after removal, despite substrate availability, hint for a shortness of spores (Gaylord et al. 2006). Since the storm took place in February, the reproductive season had already taken place and it is expected that spores were already released into the substrate. Individuals in the control quadrats and areas where canopy was removed in the autumn were big enough to become reproductive before the storm. Individuals in the quadrats destroyed in the spring, however, were most likely unable to produce the same amount of spores. As such, most spores that gave rise to the new individuals are expected to be either from the previous year of dispersed from the surrounding areas. The storm might, thus, have leveled spore availability by scouring them(Gaylord et al. 2006). Although it's well known that kelp microscopic forms have the ability to stay in an arrested development state in culture, it's very unlikely that this happens in the field, since they do not have any structures to protect them from grazing and can become buried easily (Hoffmann & Santelices 1991). Although many authors defend that kelps have low dispersal ability, studies have shown that some species have mechanisms for short and long distance dispersal. Though spores are usually involved in mucus that allows them to sink faster and agglomerated, some species have the ability to disperse spores for hundreds of kilometers. *L. hyperborea*, for instance, has been reported to disperse as far as 200 m (Reed et al. 1992), population genetics analyses show that populations are differentiated at the scale of 10–15 km in both L. digitata and *L. hyperborea* kelp species (Robuchon et al. 2014). Although genetic markers are already available*,* this isn't yet known for *L. ochroleuca* (Coelho et al. 2014). Although the storm impact on the subtidal kelp populations is unknown, previous studies show that storm driven mortality decreases with increasing depth (Dayton & Tegner 1984).

Since the storm caused the death of almost all individuals, after this, most individuals were young. The lack of canopy in the quadrat and surroundings to protect the fragile juveniles from wave action might be the reason why, after the storm, mortality was almost total for all the quadrats.

Density of canopy forming species, other than *L. ochroleuca*, varied through time and between quadrat types. Their presence couldn't be related to season or substrate availability and posed no threat to *L. ochroleuca* recovery.

CHAPTER 3

CONTRASTING TIMING OF LIFE STAGES ACROSS LATITUDES – A CASE OF A MARINE FORESTS FORMING SPECIES

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ABSTRACT

In this paper we combine field demography surveys with controlled culture experiments, to understand if temperature might be the factor leading to differences in life expectancy and seasonality across different latitudinal ranges in the kelp *Saccorhiza polyschides*, This species overwinters as a macroscopic sporophyte in central populations, like Brittany, Northwestern France, while in Northern Portugal, the southern range of its distribution, only microscopic forms are present through winter. Our results did not support temperature as the cause for the different life histories observed in central and southern ranges. We found that sporophyte recruitment occurs year-round in Brittany while farther south it is limited to spring. Our results show that this seasonal recruitment is not due to winter temperature as zoospores were able to develop between 5 and 20°C with an optimum at 10-15 °C, placing the Portuguese winter temperatures within the optimum range. In addition, we showed that the synchronized release of zoospores in early fall in the southern range does not explain the absence of recruits throughout winter, as these took a maximum of 62 days, at 5 °C, to develop into a sporophyte visible in the field (5 cm) and only 20 days at 10 °C. An alternative hypothesis that could explain the different life histories is the seasonality of high seawater nutrient levels occurring in the southern but not in the central-northern range, where permanently higher nutrient concentrations might allow sporophytes to occur at any season. This hypothesis is supported by the coincidence of the presence of macroscopic sporophytes with the seasonal upwelling period in the southern range. Still, since this was not our initial hypothesis, it requires further testing.

Keywords: *Saccorhiza polyschides*, *Laminaria ochroleuca*, *Laminaria digitata*, *Laminaria hyperborea*, seasonality, upwelling

INTRODUCTION

Climate change over the last few decades has been impacting the distribution of species across many habitats, both terrestrial (e.g., Brommer 2004; Chen et al. 2011) and marine (e.g., Fernández 2011; Nicastro et al. 2013; Smale et al. 2013). Before lethal effects cause shifts in distributional ranges, changes in vital rates and timing of demographic events within populations, are likely to happen (Bates et al. 2014). However, changes in these traits have received less attention than changes in production and species distributions (Edwards & Richardson 2004). Besides the major effect of temperature patterns, one of the predicted, and already observable effects of global warming is a change in wind speed and direction, which greatly affects currents (Bakun 1990, Lemos & Pires 2004, McGregor et al. 2007, Gómez-Gesteira et al. 2008, Narayan et al. 2010). This will in turn affect seawater temperatures by altering upwelling regimes, deregulating their seasonality and intensity. Models predict not only a delay in these events but also a culmination in stronger and later upwelling (Barth et al. 2007). In addition to major temperature changes, such changes in timing and volume of colder and nutrient rich water transported near shore greatly impact local communities and may dissociate nutrient availability from demand (Chen 2000, Barth et al. 2007). Moreover, the effect of environmental factors may be coupled, as has been observed for temperature and light exposure (Staehr & Wernberg 2009) and for nitrogen and temperature (Gerard 1997, Johnson et al. 2011). Also, changes in physical conditions may decrease resistance to strong hydrodynamics (Wernberg et al. 2011) and have cascading effects on ecosystems by making conditions suitable for introduced species to proliferate or unsuitable for ecologically important ones (Johnson et al. 2011).

Kelp forests, which dominate most temperate to cold coastal areas, constitute one of the richest marine habitats upon which several species depend. Geographical shifts in kelp distribution have been observed globally, most likely due to global warming, pollution and overexploitation of marine habitats (Müller et al. 2009, Wernberg et al. 2010, Assis et al. 2013). In the Northeastern Atlantic, the annual kelp *Saccorhiza polyschides* (Lightfoot) Batters is one of the dominant forest-forming species (Smale et al. 2013). It can be found from Norway to Morocco, in the Azores and Strait of Messina, and has been reported to survive only in areas with a narrow temperature range (Ardré 1970, Lüning 1990). In Morocco, the Galician coast and the Bay of Biscay its presence is believed to depend on the typical summer upwelling, which lowers water temperature (Fernandez et al. 1988, Lüning 1990). In these areas, individuals are only observable in the field from spring to early autumn (Norton 1977, Fernandez et al. 1988, Lüning 1990). In contrast, in several areas, from Brittany northwards, they are able to overwinter (Norton & Burrows 1969, Jensen et al. 1985). Although *S. polyschides* sporophytes are known to be able to live at temperatures between 3 and 24 °C, at lower latitudes the end of the typical upwelling season (in the beginning of autumn) accompanies the decay of the individuals (Norton 1977, Fernandez et al. 1988, Lüning 1990).

The goal of this work is to understand the processes mediating life history variations across different latitudinal ranges in the kelp *S. polyschides*, by studying reproduction and recruitment in the field and conducting laboratory experiments to test hypotheses related to temperature effects on microscopic life stages. Since a myriad of species depend on these marine forests, it is important to understand what might limit this species' presence and, thus, how their populations might adapt in the future. Seasonality of reproduction and recruitment were studied in two contrasting populations: from Northern Portugal, where macroscopic *S. polyschides* sporophytes occur in spring/summer, and from Northwestern France, in Brittany, where they are found all year long. In both regions the sporophytes are annual, but they are only seasonal in the south. To serve as a control for adequate environmental conditions for kelp occurrence, the main co-sympatric perennial forest-forming kelp species were also studied in both areas. *Laminaria ochroleuca* Bachelot de la Pylaie, which has a southern distribution limit similar to *S. polyschides*, was studied in Portugal. *Laminaria hyperborea* (Gunnerus), which has the same northern limit as *S. polyschides*, and *Laminaria digitata* (Hudson) Lamouroux were studied in Brittany, where the latter finds its southern distribution limit (Lüning 1990, Tuya et al. 2012). To understand differences in the seasonality of recruitment of *S. polyschides* between latitudes, the microscopic stages of these species from both Northern Portugal and Brittany were studied in culture under different temperature conditions, until small sporophytes were obtained.

Understanding why *S. polyschides* is able to overwinter as a macroscopic sporophyte in some areas but not in others will give information on the environmental limitations and triggers of its life cycle. It might, also shed some light on the conditions affecting its distribution and help understand why they are absent from areas that seem suitable for their settlement. This information will also help predict how its distribution may vary in the near future.

MATERIAL AND METHODS

SPECIES MODELS

Although the word kelp originally designates the ashes of brown algae, here we apply the ecological definition of “kelp” as large brown algae capable of forming marine forests. A typical kelp life cycle, as seen in the orders Tilopteridales, Laminariales and Desmarestiales, consists of an alternation between two generations: macroscopic sporophytes and microscopic gametophytes (but not for kelp in the order Fucales, such as those in the genus *Durvillaea*). Species are designated as annuals or perennials according to their macroscopic sporophyte phase only. Sporophyte reproduction occurs via haploid zoospores formed in sporangia, in specialized tissue called sori. When mature, the tissue disintegrates releasing the zoospores to settle on the substrate. After settling, zoospores germinate and develop into female and male gametophytes. Eggs on female gametophytes trigger the release of spermatozoids from male gametophytes close enough to detect the signaling pheromone (Maier & Muller 1986, Mohring et al. 2013). Since pheromones are rapidly diluted, especially in areas with strong water motion, synchrony in zoospore release increases their density, which is fundamental to ensure fertilization success (Maier & Muller 1986, Norton 1992, Reed et al. 1997). This synchrony in life cycle is controlled by environmental cues such as photoperiod and temperature (Norton 1977, Kain (Jones) 1989). The zoospores and gametophytes have the potential to arrest their development or develop slowly during the most unfavorable period and to accelerate their growth when conditions become conducive (Hoffmann & Santelices 1991, Kinlan et al. 2003, Carney & Edwards 2010, Carney 2011).

CENSUS SETUP

In 2005, two 3 m^2 areas with *S. polyschides, L. digitata* and *L. hyperborea* were chosen for a two year survey. Surveys in Brittany were conducted at two sites 2 km apart: Ar Pourven (48º42’43.8”N; 3º57’30.0”W) and Duons (48º 43’ 40.8”N, 3º55’32.4”W). In Northern Portugal, a 4 m^2 area with *L. ochroleuca* was delimited in 2008 for a two year survey in Viana do Castelo (41º41’44.80”N; 8º51’8.40”W) and in 2010, another 4 m^2 area was chosen in Areosa (41°42'33.56"N; 8°51'48.81"W) for a one year survey of *S. polyschides,*. All delimited areas were above the hydrographic zero, at the shallowest area these species inhabit, being out of water seldom and for short periods during the lowest tides. The surveys were done by tagging all individuals with stipes longer than 2 cm, in a predetermined area, with cable ties. On a monthly basis, new individuals were tagged and stipe and blade length and reproductive status were recorded for all of them.

CULTURE EXPERIMENT

Reproductive tissue was collected from 40 *S. polyschides* individuals from Viana do Castelo, North Portugal (41°42'33.56"N, 8°51'48.81"W) and Roscoff, North Brittany, France (48°43'8.76"N, 3°57'55.08"W) in early September 2011. Zoospore release and initial culture was performed following the methods of Oppliger et al. (2011). Zoospore release was induced from 1.5 cm^2 of reproductive tissue from 5 different random individuals per Petri dish. Tissue from each individual was used in at most 2 replicates, but combined with different random individuals. Sets of 3 replicates from each site were set at different temperatures: 5, 10, 15, 20 and 25 °C at a photon irradiance of 40 µmol photons m^{-2} s^{-1}, in a 12 hour day^{-1} photoperiod and in 0.2 µM Provasoli enriched seawater. Three fields of view were defined for each replicate and counting was always done in the same areas, to allow following the fate of each stage. Counts were done at days 0, 1, 3, 5, 8, 11, 15, 20, 25 and 30 and included the following development stages: non germinated zoospores, germinated zoospores, male gametophyte, female gametophyte and sporophytes. Morphology of the gametophytes and number of sporophytes per female gametophyte were also recorded. As soon as sporophytes appeared in the cultures, photographs of 20 sporophytes per replicate were taken and their length measured with GIMP (GNU image Manipulation Program). When their size reached about 0.02 mm, 20 individuals from each original Petri dish were transferred into a new one, to make sure their density was similar and to follow growth and survival. The experiment ended when sporophytes reached 0.5 cm in length.

FIELD DATA ANALYSIS

Individuals were divided into recruits and adults according to their total length, which is the best predictor of the individuals' ability to become reproductive (Chapman 1987).

Since day length and temperature are considered as the main environmental conditions influencing species life cycle and seasonality, population structure, percentage of reproductive individuals and recruit density were compared with day length and sea surface temperature (SST) data to infer if these can explain the differences in life history. Daily values of SST recorded over the census year in the sampling area (4 km grid) were used, to allow a comparison between them. All SST data was provided by NASA (Aqua MODIS SST, NASA). Recruits were not considered in calculation of either population structure or percentage of reproductive individuals to avoid bias due to recruitment seasonality.

CULTURE DATA ANALYSIS

Data was analyzed with PERMANOVA and PERMDISP, with Primer + Permanova 6 software (Anderson et al. 2008). Pair-wise comparisons were used whenever a significant difference between treatments was found. Analyzes were performed with 9999 permutations. Germination ratio was analyzed considering the factors time and temperature and site of zoospore origin. Sex ratio was calculated as the number of males per female, when 100% of zoospores had developed into gametophytes and a binomial comparative trial for the null hypothesis of a 1:1 sex ratio was used. Mortality of microscopic stages was calculated for the period zoospores took to produce sporophytes seen with the naked eye (0.02 mm in length). Sporophyte mortality was calculated for the period sporophytes took to grow from 0.02 mm to 0.5 cm in length. Mortality of microscopic stages and sporophytes, sex ratio, and the number of sporophytes obtained from each 100 initial zoospores were analyzed considering temperature and zoospore origin. Since no sporophytes were produced at 25 °C, this temperature was not considered in the analysis.

Growth rate analysis was done considering the point of appearance of the first sporophytes as time 0. Since death rate of the Portuguese set at 20 °C was very high, these could only be kept until 12 days after their appearance. As such, 20 °C sets at day 18 were not included in the growth rate analysis.

Based on field work experience, 5 cm was considered as the minimum size for a sporophyte to be noticed in the field. Linear regression of sporophytes' size was used to estimate how long it would take for the sporophytes to reach 5 cm. Data were expressed in days since the zoospore release was induced. Data were analyzed with PERMANOVA and PERMDISP. The Portuguese set at 20 °C was not included since mortality was too high for them to reach this size.

RESULTS

CENSUS AND ENVIRONMENTAL CONDITIONS

Overall the number of individuals surveyed in the field in Roscoff reached 273 *L. digitata*, 51 *L. hyperborea* and 58 *S. polyschides* over a surface of 3 m²; and in Northern Portugal 82 *S. polyschides* and 125 *L. ochroleuca* over 4 m².

In Northern Portugal, populations of *S. polyschides* were seasonal, with no overwintering macroscopic sporophytes (Fig. 1A). All individuals recruited within four months (April to June, Fig. 1A) and sporophytes were observed until the end of September (Fig. 1A). They reproduced only once and those present in August 2010 and September 2011 all died at about the same time, with the first storms (Fig. 1A). Still, 2010 was a distinctive year for *S. polyschides*: recruitment occurred late, around May, and the sporophytes did not reach maturity before breaking with the first autumn storms (Fig. 1A). In Northern Brittany, on the contrary, *S. polyschides* individuals were able to overwinter and no seasonality could be identified in the two year survey data (Fig. 1B). Also, both recruitment and reproduction occurred throughout the year (Fig.

1B). Even though some individuals lived longer than 14 months, they were able to reproduce only once through the course of their life.

In contrast, *L. ochroleuca*, the main perennial forest forming kelp in North Portugal, which shares the habitat with *S. polyschides* and has the same southern distribution limit, was able to survive, recruit and reproduce all year long, indicating that suitable conditions for kelps can be found throughout the year (Supplementary material, Fig. S1A). The same was observed for the main perennial kelp species from Northern Brittany (*L. digitata*, sup mat, Fig. S1B and *L. hyperborea* sup mat, Fig. S1C).

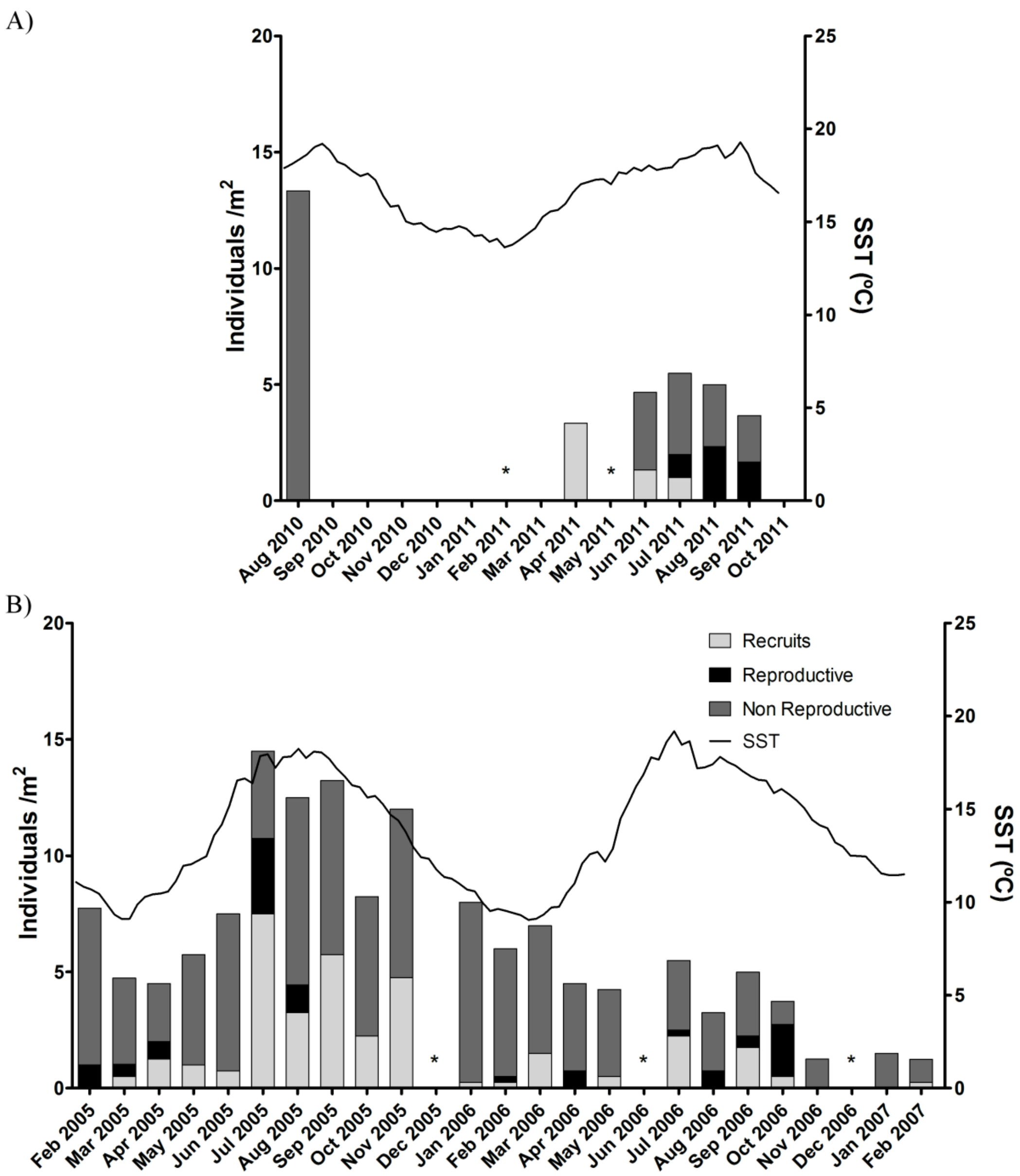


Fig. 1: Sea surface temperature and number of recruits, reproductive and non reproductive individuals per square meter for *S. polyschides* populations from (A) North

Portugal and (B) Brittany, France. * indicates a lack of data for that month, due to storms precluding field work.

CULTURE

Zoospore germination varied significantly with time (PERMANOVA, time, $p < 0.0001$, PERMDISP, time, $p < 0.0001$, sup mat, Fig. S2) apart from site of origin and temperature conditions (PERMANOVA, temperature and site, $p = 0.8333$). Maximum germination (27.7%) was observed between day 0 and 1. Most germination took place until day 3, after which it decreased below 0.1%. No significant effect of temperature or site of origin on germination was observed (PERMANOVA, temperature, $p = 0.8526$ and site, $p = 0.8796$; PERMDISP, temperature, $p = 0.8968$ and site, $p = 0.7597$). Mortality of microscopic stages increased significantly with temperature (PERMANOVA, temperature, $p = 0.0111$; PERMDISP, temperature, $p = 0.098$, sup mat, Fig. S3). This was maximal for 25 °C, where no sporophytes were obtained due to mortality. At day 11, this reached 95% and 90% for the French and Portuguese sets, respectively. Mortality of sporophytes larger than 0.02 mm was significantly different between sites and temperatures (PERMANOVA, site, $p = 0.0016$ and temperature, $p = 0.0022$; PERMDISP, site, $p = 0.0786$ and temperature, $p = 0.428$ sup mat, Fig. S4). 60.6% of sporophytes from Northern Portugal died while for Northern Brittany mortality of sporophytes was about 32.8%. Sporophyte mortality was significantly higher at 20 °C, reaching 75.5%. No significant difference was found for the remaining temperatures. Zoospores from Brittany developed into sporophytes more successfully (21.8%, PERMANOVA, site, $p = 0.0224$, PERMDISP, site, $p = 0.5714$, sup mat, Fig. S5) than those from Portugal (9.7%), but no temperature effect was detected (PERMANOVA, temperature, $p = 0.1508$; PERMDISP, temperature, $p = 0.087$). Sex ratio was on average 0.5 (males per female), significantly different from 1 ($p = 0.0381$) and was independent of site of origin (PERMANOVA, site, $p = 0.5097$; PERMDISP, site, $p = 0.985$) and temperature (PERMANOVA; temperature, $p = 0.4609$; PERMDISP, temperature, $p = 0.435$).

Although sporophyte growth rate for Northern Portugal and Brittany varied significantly with temperature for day 6 (considering sporophyte formation as day 0), (PERMANOVA, temperature, $p = 0.0002$, Fig. 2), such differences might have been due to dispersion (PERMDISP, temperature, $p < 0.0001$). From day 6 to 12, sporophyte growth rate varied significantly with site and temperature (PERMANOVA site x temperature, p = 0.0223, Fig. 2) but differences in dispersion were also significant between sites (PERMDISP, site, p = 0.2234). Significant variation was, however, found between temperatures for this period (PERMANOVA, temperature, p = 0.0015; PERMDISP, temperature, p = 0.0044) and from day 12 to 18 with site and temperature (PERMANOVA, site, p = 0.0135, and temperature, p < 0.0001) significant difference among temperatures may however be due to dispersion (PERMDISP, site, p = 0.6093; temperature, p = 0.0096; Fig. 2). Optimum temperatures for sporophyte growth were found between 15 °C and 10 °C. The time needed for a sporophyte to reach 5 cm, big enough to be identified in the field, varied significantly with temperature only (PERMANOVA, Temperature, p = 0.0002). 10 °C was the temperature at which they grew faster, reaching 5 cm in about 20 days. The lowest growth rate was found at 5 °C, at which zoospores needed on average 62 days to develop into a 5 cm sporophyte (Fig. 3).

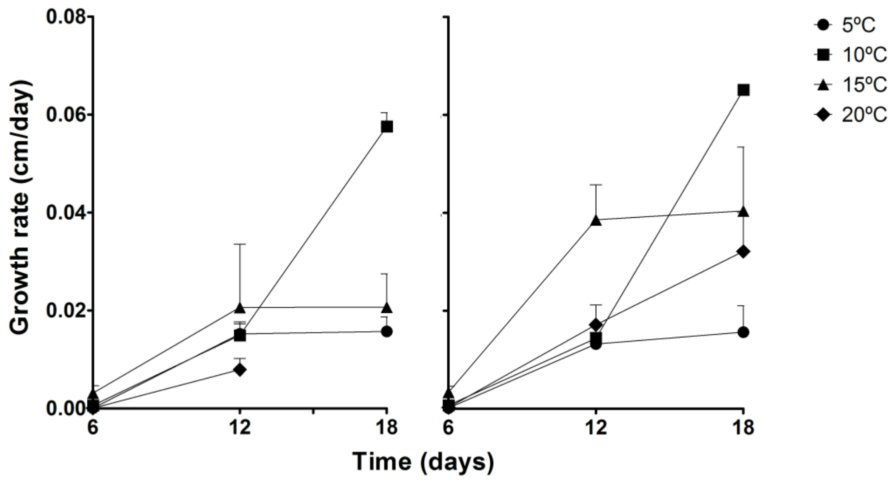


Fig. 2: Growth rate through time for sporophytes from Portugal (right) and France (left) at the different culturing temperatures. There is no 20 °C point at day 12 in the Portuguese set due to 100% mortality.

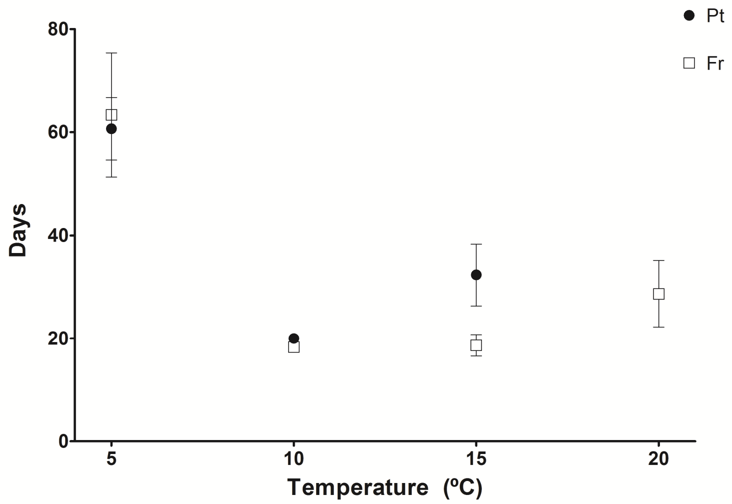


Fig. 3: Number of days needed, from the time of induced zoospore release, to originate a sporophyte big enough to be observed in the field (5 cm) for the populations from North Portugal (Pt) and Brittany, France (Fr) at the 4 temperatures where sporophytes were formed. Significant difference was found between all temperatures. No data for the Portuguese set at 20 °C is displayed due to 100% mortality.

Discussion

In this study, we found that life history differences between southern and central populations was determined by sporophyte recruitment timing, which took place year-round in Brittany whereas farther south, sporophytes only developed during spring. Our results show that seasonal recruitment in southern populations is not due to temperature limitations for microscopic development of the phases prior to macroscopic sporophyte recruitment. We also showed that the delay of recruitment until spring is not due to the synchronized zoospore release in early autumn, as in our experiments zoospores were able to complete all stages of development into gametophytes and new sporophytes in less than a month. These results raised the novel hypothesis that recruitment is delayed until spring due to nutrient limitation in southern areas. This hypothesis is supported by the observation that sporophyte development is coincident with the upwelling season in the southern range, whereas in the central range nutrient levels are higher year-round (sup mat, Fig. S6).

The results from this study on marine forests are in accordance with previous studies relating seasonality of life history parameters with the typical upwelling at low latitudes (Fernandez et al. 1988, Lüning 1990). Our results show that on the

Portuguese coast, populations of *S. polyschides* have strict seasonality, with most individuals recruiting synchronously in early spring. The population structure surveys revealed that individuals grow rapidly and by the end of the summer most are reproductive. As such, by the beginning of autumn, when decay becomes evident and their stipes break with the first storms, most individuals were already able to produce zoospores. On the contrary, although in Northern Brittany this population is semelparous, sporophytes are able to survive, reproduce and recruit over the winter, like the dominant perennial kelp species. This indicates that the synchrony in life cycle in southern areas is caused by their absence for part of the year. Although monitoring was done in limited areas, these observations were consistent both with previous years and the surrounding areas: the whole Northern Portuguese Coast and between the 3 Km apart populations in Roscoff.

One of the reasons that could explain the inability of *S. polyschides* to recruit year-round on the Portuguese coast would be inadequate conditions for kelp recruitment. However, year-round recruitment in *L. ochroleuca* on this coast suggests that this is not the case. Although these two species have very different vegetative and holdfast structures that secure them to the substrate, in small recruits these are quite similar; both adhere to the substrate via rhizoids, and only later the attachment disc in *S. polyschides* develops into a bulb, as an additional attachment structure. Therefore, based on the lack of evidence for ecological differences between recruits of both species, we hypothesize that while their ability to support hydrodynamic forces might differ in adults, small recruits are likely to have similar resistance. Temperature is also unlikely to be a cause for the differences in recruitment between these species since they share a similar southern distribution limit and have similar upper temperature limits (Norton 1977, Lüning 1990).

The presence of *S. polyschides* sporophytes in the Moroccan, Galician and Bay of Biscay coasts is usually thought to be limited by temperature; it varies between years and is dependent on upwelling (Fernandez et al. 1988, Lüning 1990, Fernández 2011). Notwithstanding, sporophytes survive during August, when SSTs may reach 23 °C in the Bay of Biscay and 25 °C in Casablanca. In these regions, although populations are exposed to higher temperatures for several months, their decay does not occur midsummer but with the first winter storms, like in North Portugal (Fernandez et al. 1988). Also, the fact that they usually break with the first autumn storms indicate that the end of their life cycle is caused by excessive hydrodynamic force. While this explains why individuals decay is a synchronized event, it does not offer an explanation for the synchrony in recruitment. Elevated temperature cannot be an explanation for the different life histories between populations from Brittany and Portugal either, since they reach similar maximum values (19 °C in summer). Although exposure to the maximum temperatures is longer in North Portugal, these observations do not support the hypothesis that high temperature is the cause for macroscopic sporophyte decay in Portugal by the end of summer. While satellite data has its limitations when studying coastal areas, as local extreme conditions are not recorded (Smale & Wernberg 2009, Smit et al. 2013), such short term extreme temperatures are probably not the key limiting factor. Seasonality of *S. polyschides* is common to the whole Northern Portuguese Coast and is not a local event, and the fact that it occurs on the Moroccan coast supports the theory that their decay is unrelated to temperature. Even at the edge of geographical distribution individuals are reported to decay by the end of summer and not during midsummer's warmer days (Norton & Burrows 1969, Fernandez et al. 1988).

Since the environmental requirements of kelp's macroscopic and microscopic parts of the life cycle may differ, we hypothesized that responses of microscopic stages to temperature might differ between populations from Portugal and Brittany and thus explain why recruitment is synchronous at low latitudes but occurs all year long at higher ones. Germination rate and sex ratio did not vary with site of origin or temperature, thus indicating no differences in adaptation to the different conditions between the two populations. Sex ratio results were however unexpected as twice as many females than males were found. Although it is commonly agreed that sex ratio in kelps is mainly genetically determined (Evans 1963), some dissimilarity in number of males and females may occur due to stressful environmental conditions (Funano 1983, Lee & Brinkhuis 1988, Nelson 2005, Oppliger et al. 2011). In a previous report, it was shown that in this species the frequency of females, higher during the first 10 days of germination due to their faster development, decreased to reach a similar number of males and females one month after germination (Pereira & Engelen 2011). As culturing conditions (10 and 15ºC) were similar to those in Pereira *et al.,* 2011, we hypothesize that sex ratio might be affected by seasonal or interannual variation, which in the present study resulted in rapid fertilization associated with reduced vegetative growth of the gametophytes. Our data on zoospore development into 0.5 cm sporophytes showed no significant differences between the two populations in mortality of microscopic stages. For both populations, mortality of microscopic stages was, however, significantly higher at 25 °C, causing the death of all zoospores before any gametophytes were formed. At the remaining temperatures there were no differences in sporophyte production, neither within nor between populations; sporophytes were successfully produced in both, indicating that temperatures between 5 and 20 °C are suitable for the development of the gametophytic phases of *S. polyschides*. However, 20 °C was not suitable for development of sporophytes larger than 0.02 mm for the population from Portugal for which mortality was absolute in such conditions. These mortality differences at 20 °C may cause lower summer recruitment, but cannot explain the lack of recruitment during winter in Portugal. However, the lack of survival of young sporophytes at 20 °C can explain why recruitment takes place only in spring and no new recruits are found throughout summer.

Since, in Portugal, most zoospores are released at about the same time, one possible explanation for the absence of recruitment for most of the year and a synchronous event around April/May would be the time lapse between zoospore release and recruitment. This could potentially be the time needed for zoospores to develop through the gametophyte stages into a sporophyte big enough to be observed in the field. However, the synchronous recruitment peak in spring cannot be explained by the synchronized zoospore release event at the end of summer. Although no significant differences in growth rate of sporophytes could be found between temperatures, this reached a maximum for 10 °C and a minimum at 5 °C. This indicates that temperature has a greater impact on the rate of gametophyte development. After sporophytes are formed, growth rate does not vary significantly with temperature. 10 and 15 °C represent the average seawater winter temperature in France and Portugal, respectively. Zoospores developing in these conditions needed on average 19 and 26 days, respectively, to develop into visible sporophytes (5 cm). Such time lapse is not sufficient to explain why no recruitment is observed between August and April in

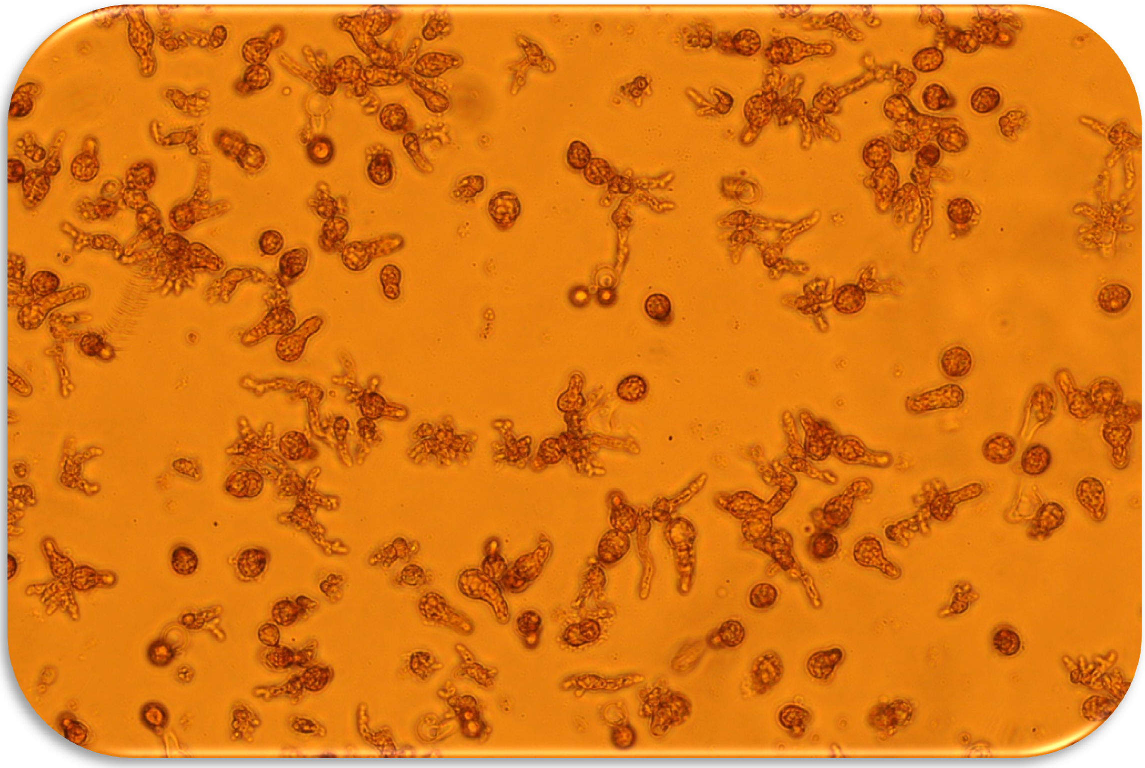
Portugal. It is important to keep in mind that growth rate is likely to vary with individual size, and our results show that they are likely to increase over time (Fig. 3). Nevertheless, many factors influence the development of the microscopic stages: shading is known to inhibit growth, which, in the field, is expected to be halted with sporophyte decay; natural light and water motion may result in faster development; and lower nutrient concentration, when compared to the enriched culture medium, may be limiting to growth (Edwards 2000, Andersen 2005, Pedersen et al. 2012, Carnell & Keough 2014). As such, extrapolation of the results obtained in controlled conditions to the field should be taken with caution.

Despite the poor correlation of autumn decay of southern populations with temperature exposure, the life cycle of *S. polyschides* seems highly correlated with upwelling events, in agreement with previous studies (Fernandez et al. 1988, Lüning 1990, Fernández 2011). In Northern Portugal, the typical upwelling season begins in April, when the species starts recruiting, and ends abruptly in September, when sporophytes decay and break with the first autumn storms (Vitorino et al. 2002, Lemos & Pires 2004). Since temperature does not seem limiting, we hypothesize that the life cycle in these regions might be more closely related and limited by nutrient concentrations, which increase during the upwelling period. Although the available information is limited, this hypothesis is supported by the comparison of average nutrient concentrations off the coastlines where *S. polyschides* occurs, which present a trend relatable to the presence or absence of overwintering populations (sup mat, Fig. S6). In the areas off Ireland, Isle of Man and Brittany, where higher concentrations of both phosphates and nitrates are found throughout the year, individuals are able to overwinter. In contrast, in areas with low nutrient concentrations, the occurrence of the macroscopic part of the life cycle coincides with the typical upwelling season, when nutrient levels rise. In these areas, from the Bay of Biscay to Portugal and in the upwelling areas of Morocco, there is typically no upwelling during roughly half of the year, coincident with the absence of sporophytes of *S. polyschides*. As such, we propose that the differences in life histories between northern and southern ranges are due to seasonal nutrient limitation in the southern but not in the northern range, keeping in mind the possibility of this resulting from the interaction with other environmental factors (Gerard 1997). We hypothesize that in Portugal, the life cycle of

S. polyschides is limited by nutrient concentration and, as such, dependent on upwelling events that seasonally bring high nutrient concentrations. Therefore, global warming and associated geographical distribution shifts might not be due to temperature increase alone but also to the associated nutrient limitation due to interruption or weakening of upwelling systems (Johnson et al. 2011). This is supported by several studies that indicate that the high rate at which the North Atlantic has been warming during the past 40 years, has been especially affecting winter temperatures and weakening upwelling systems (Hurrell 1995, Hurrell et al. 2001, Visbeck et al. 2001, Lemos & Pires 2004, Johnson et al. 2011). These alterations in the upwelling season might be predicted to deregulate seasonality of *S. polyschides.* A possible consequence that is a serious concern would be that in some areas these might recruit later than usual and be unable to develop to reproductive age before decaying by the end of summer, as was observed in 2010. Such an impact on structural species may cause their local extinction and subsequent disappearance of several species that rely on them for food and protection.

CHAPTER 4

TEMPERATURE EFFECTS ON THE MICROSCOPIC HAPLOID STAGE DEVELOPMENT OF *LAMINARIA OCHROLEUCA* AND *SACCORHIZA POLYSCHIDES*, KELPS WITH CONTRASTING LIFE HISTORIES



CHAPTER 4

TEMPERATURE EFFECTS ON THE MICROSCOPIC HAPLOID STAGE DEVELOPMENT OF *LAMINARIA OCHROLEUCA* AND *SACCORHIZA POLYSCHIDES*, KELPS WITH CONTRASTING LIFE HISTORIES

ABSTRACT

Kelp forests are one of the most diverse and productive ecosystems worldwide. Global climate change and human exploitation threaten the stability of many of these ecosystems. In this study we compare differences in temperature responses during the microscopic haploid stage development between two kelp species in order to test if the annual *Saccorhiza polyschides* outperforms the perennial *Laminaria ochroleuca* at the northern limit of range distribution of *L. ochroleuca*, in Northern Brittany. Germination and mortality, sex ratio, fecundity and reproduction were measured in culture for the two species and under three different temperatures (10°C, 15°C and 25°C). An effect of temperature was found for all traits except the sex ratio. Both species showed no ability to develop gametophytes at the highest temperature of 25ºC and were more tolerant to lower temperatures. *S. polyschides* showed higher germination rate, higher fecundity and lower mortality than *L. ochroleuca* during the period of the experiment. In addition, its gametophytes developed earlier than those of *L. ochroleuca,* a competitive advantage found in all temperature conditions. Germination rate, mortality and fecundity were significantly different between the two species. In addition, the two species showed a structural difference in the development of microscopic stages, with *S. polyschides* gametophytes occupying a larger area, which is suggested to result in a greater adhesion capacity. In conclusion, the microscopic stage of the annual species *S. polyschides* had a significant advantage in fitness compared to the perennial *L. ochroleuca*. This annual opportunistic species may outcompete *L. ochroleuca*, at least in Brittany, the study region, corresponding to its northern limit, in areas where they share habitat.

INTRODUCTION

Changes in climate can strongly alter ecosystems; including shifting species distribution ranges (Spooner 1950, Steneck et al. 2003, Hiscock et al. 2004, Hampe & Petit 2005, Farrell & Fletcher 2006, Müller et al. 2009, Wernberg et al. 2010). In seaweeds, temperature is recognized as the major environmental factor controlling geographic range and depth distribution (Breeman 1988, Izquierdo et al. 2001, Steneck et al. 2003, Müller et al. 2009).

Kelp forests are known to be one of the most diverse and productive ecosystems worldwide (Mann 1973, Steneck et al. 2003, Wernberg et al. 2010). These large brown seaweeds play a fundamental role as structural ecosystem components, serving as food for herbivores and detritivorous animals, and as shelter and nursery for a variety of species (Steneck et al. 2003, Leblanc et al. 2011). Their canopy reduces the light, creating favorable conditions for shade-adapted species, while by reducing water flow, they also influence sedimentation and erosion rates (Steneck et al. 2003). Finally, kelps are, in general, an economically important human resource, as food, fertilizer in agriculture and mariculture, and nutrition for cattle (Braud 1974, Peteiro et al. 2006, Araújo et al. 2006). Kelp extracts are used in dye, textiles, cosmetics and pharmaceutics, and as a thickener in food preparation (Braud 1974).

The kelp life cycle consists of a microscopic haploid gametophyte phase, alternating with macroscopic diploid sporophytes. These release meiotic spores that settle on the substrate. Spores germinate and develop into dioecious male and female haploid gametophytes, producing antheridia and oogonia, respectively. After fertilization and syngamy, the diploid zygote originates a new sporophyte (Dayton 1985). The gametophyte phase and subsequent sporophyte embryos are thought to be part of a "bank of microscopic forms" that will, in the following favorable period, originate the macroscopic sporophytes (e.g., Kinlan et al. 2003; Barradas et al. 2011, also reviewed by Carney & Edwards 2006).

Kelps have been classified as annual or perennial based only on the macroscopic sporophyte life span. In annual species, sporophytes are only found during a portion of the year. In contrast, perennial species have macroscopic sporophytes with a life expectancy greater than one year and that reproduces for at least two years, varying between 2 and 25 years depending on species (Birkett et al. 1998, Steneck et al. 2003). In both annual and perennial species, the life cycle is completed by microscopic forms of unknown longevity. After the decay of annual sporophytes, survival of microscopic stages during the sporophyte resting season is critical for the maintenance of the population, while in perennial species the survival of microscopic stages may become critical only in case of massive destruction. Such a case was reported in 1992 and 1997/98 in Chile, when the ENSO (El Niño Southern Oscillations) caused a rise in seawater temperature, leading to a decrease in reproduction and recruitment of *Lessonia nigrescens* Bory de Saint-Vincent 1826 (Martinez et al. 2003). When such events take place, surviving microscopic stages may allow rapid recolonization of the affected areas (Ladah & Zertuche-González 2007).

Analogous to recolonization of open gaps in a forest, annual species are expected to be the pioneer colonizers, playing the role of opportunistic species as shown by Peteiro et al. (2006), who highlighted that *S. polyschides* was the fastest-growing alga and was able to colonize newly available space in a biennial culture of *Saccharina latissima*. Competitive interactions of this nature raise concerns for the resilience of such a complex ecosystem in places where human activity and climate change might increase disturbance levels, causing shifts in species composition. Problems include kelp species unable to recolonize areas along disturbed coasts (e.g., Coleman et al. 2008); or after repeated harvesting at the same location (Engelen et al. 2011) and regression of kelp distributional limits (e.g., Assis et al. 2009; Wernberg et al. 2010).

This study was developed with two species with contrasting sporophyte life spans, but similar geographical and depth distributions: *Laminaria ochroleuca* De La Pylaie with perennial sporophytes and *Saccorhiza polyschides* (Lightfoot) Batters with annual sporophytes. Their upper temperature limits are very close, 25ºC for *L. ochroleuca* and 24ºC for *S. polyschides* (tom Dieck & de Oliveira 1993). Data for minimum survival temperature were not found in earlier reports. However, it is reported that *S. polyschides* and *L. ochroleuca* are exposed to an average winter temperature of 4ºC in Norway and 10ºC in the English Channel, respectively (Braud 1974, Norton 1977, Hoek 1982). Taking this into account, it is expected that *S. polyschides* has a more efficient development at low temperatures than *L. ochroleuca*.

This experimental work aimed to predict what would be the future composition of the kelp forests in the study area in case of a massive destruction or a rise in water temperature. As such, differences in development and fitness were sought in two species that share this same habitat but have contrasting life histories: *L. ochroleuca*, a perennial species, and *S. polyschides*, an annual. To answer this question, (1) differences in development between the two species were investigated, (2) developmental rates of both species at temperatures within the upper range of natural exposure were estimated, (3) the responses of each species to the highest temperature at their geographical southern limit were assessed and, finally, (4) differences in fertility were quantified.

MATERIALS AND METHODS

BIOLOGICAL MATERIAL

Growth of *L. ochroleuca* sporophytes is seasonal and usually takes place during the winter/spring. In summer/autumn, blade growth is reduced and spore production is maximum (Lüning et al. 2000). The sporophyte of *S. polyschides* is annual. The growth of the juvenile blade starts in the spring and maximum size is reached in the summer. In the early autumn, sporophytes reach reproductive maturity. Unlike *L. ochroleuca*, the spores are generated at the base of the frond, which breaks with the first winter storms. Microscopic gametophyte stages develop from the spore, overwinter, and generate macroscopic sporophytes in the following spring (Mann 1973, Hoek 1982).

L. ochroleuca is a Lusitanian species, found very deep in Azores (Tittley & Neto 2000) and the Gorringe submerged bank, and in the warm and temperate waters from Morocco to the Portuguese and North-West Spanish coasts, as well as very deep in some areas in the Mediterranean. It can also be found from Brittany (France) to the English and Bristol channels (Braud 1974, Hoek 1982, Birkett et al. 1998, Bartsch et al. 2008). *S. polyschides* shares this distributional range, but also extends further north along the South and West coasts of England, Wales, Scotland and Ireland, reaching the west coast of Norway (Norton 1977, Birkett et al. 1998). The study site is, thus, the northern geographical limit of *L. ochroleuca* and the central distributional range of *S. polyschides*.

The depth at which kelps are able to live varies widely with the local conditions, particularly temperature (kelp are typically cold-adapted species) and light. Their deeper limit is usually determined by light availability. In clear waters, as the whole light spectrum is able to reach deeper areas, kelps may grow below 35m. More turbid waters will reduce light penetration and change its spectral composition, preventing the kelp from developing at higher depths (Hoek 1982, Birkett et al. 1998).

CULTURE

Reproductive tissues of both *L. ochroleuca* and *S. polyschides* were collected at Morlaix Bay (Brittany, France) in July 2009, from depths around 5 m. On the day of collection, the 15 most reproductive individuals of each species were chosen, and divided in three sets with 3 tubes each. This way, all tubes in the same set had spores from the same 5 individuals. After washing the tissue with filtered seawater (SW), sporulation was initiated and lasted 15 to 18 h during which the spores settled on a microscope glass slide placed inside a 50 mL falcon tube filled with filtered SW (Oppliger et al. 2011), on a shaker at 100 rpm.

The following day was considered the first day of culture. The SW was replaced by 0.2 µM Provasoli Enriched Seawater (Andersen 2005), with medium changes every five days. One tube from each set was placed at 10 ºC, 15 ºC and 25 ºC. A 12:12 h light:dark photoperiod and photon fluency rate of 30 µmol m^{-2} s^{-1} were used. The experiment lasted 26 days and counts were made on day one, two, and every two days subsequently. Each slide had reference points to ensure that the same area was always studied.

To assess spore development, the following different life history phases were distinguished based on their morphology (Fig. 1 and 2): non germinated settled meiospores (Ng), germinated spores with one cell (identified by the presence of a germination tube) (1C), germinated spores with two cells (2C), germinated spores with more than two cells (+2C), male gametophytes (M), immature female gametophytes (Fg), mature female gametophytes (Fm) and female gametophytes with sporophyte

(Fs). Unicellular or multicellular stages were distinguished as the growth may be influenced by environmental factors, such as temperature (Izquierdo et al. 2001).

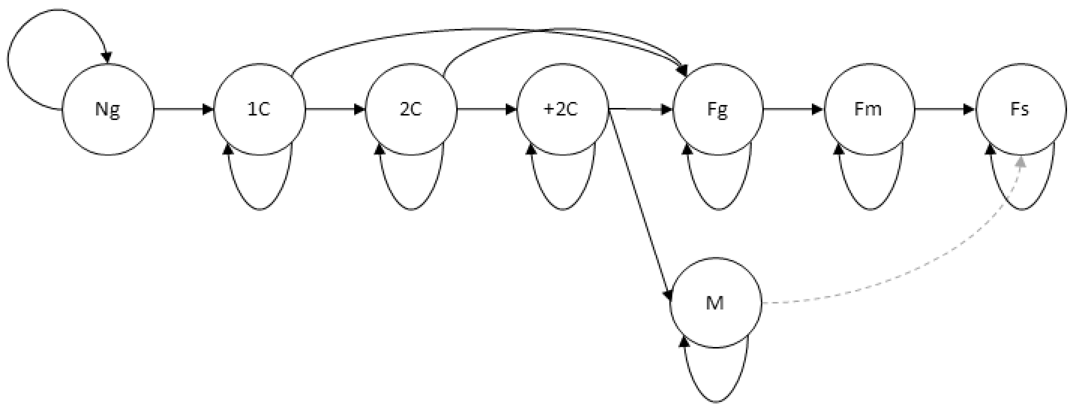


Figure 1: Overall scheme of spore developmental phases, based on morphology. The non dashed arrows indicate the transition that may occur between observations. Ng: non germinated settled meiospores; 1C: germinated spores with only one cell; 2C germinated spores with two cells; +2C: germinated spores with more than two cells whose gender was not recognizable yet; M: male gametophytes; Fg: female non mature gametophytes; Fm. Female mature gametophytes; Fs: female gametophytes with sporophyte. The dashed arrow indicates the contribution of the male gametes to originate the sporophyte that is adhering to the female at the Fs stage.

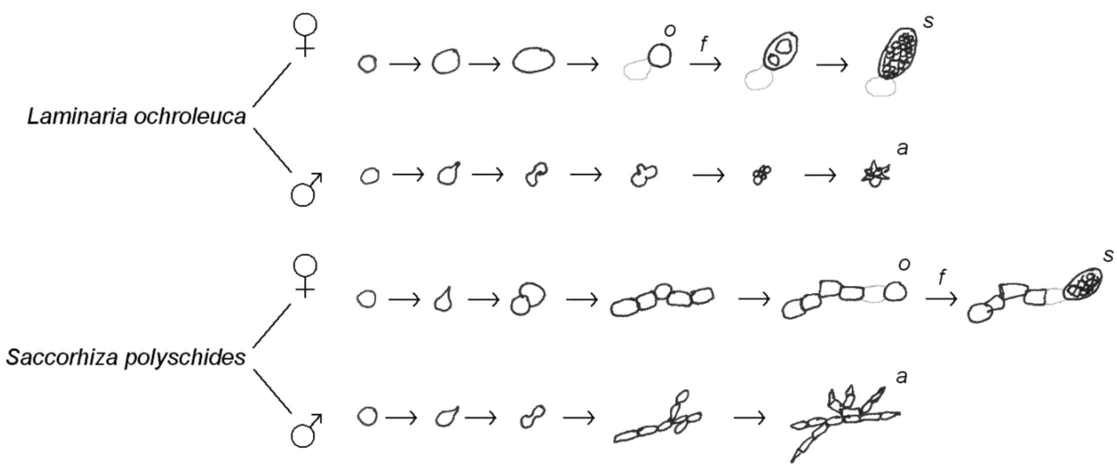


Figure 2: Distinct developments of *L. ochroleuca* and *S. polyschides*: o oogonia; a antheridia; f fecundity; s sporophyte (Not at scale).

STATISTICAL ANALYSIS

Frequencies of all stages of development were calculated relative to the total number of spores at day 1. Fecundity and reproductiveness were calculated as follows:

$$\text{Fecundity} = \frac{\text{Mature females} + \text{Females with sporophyte}}{\text{Total females}}$$

$$\text{Reproductiveness} = \frac{\text{Females with sporophyte}}{\text{Total females}}$$

$$\text{Sex ratio} = \frac{\text{Total males}}{\text{Total females}}$$

Frequencies of all stages, germination, death and sex ratio, fecundity and reproductiveness from the two species and under different temperatures were subjected to a general linear model (3-way ANOVA) to test whether they differed significantly between species (fixed factor, 2 levels), temperature (fixed factor, 3 levels) or time (fixed factor, 14 levels: 1, 2, 4, 6, 8, 10... 26). Significance was considered for p-values < 0.05. Pairwise differences for significant interaction terms were analyzed using Tukey tests.

Death and germination rate can only be considered while there are still live forms and spores available for germination, respectively. Thus, to study these parameters, only the days satisfying these conditions for both species and all the temperatures were considered. All statistical analyses were carried out with Minitab (State College, PA, USA).

RESULTS

GERMINATION

Germination rate varied temporally between species and among temperatures (3-way ANOVA, species * time, $p < 0.001$ and temperature * time, $p < 0.05$, Fig. 3 and 4, respectively and Table 1). At day two, *S. polyschides* showed a significantly higher germination rate than *L. ochroleuca* (Tukey test, $p < 0.001$) and both species had a higher germination rate compared to the following days (Tukey test, species * time $p < 0.001$ and $p < 0.01$, *S. polyschides* and *L. ochroleuca*, respectively, Fig. 3). No significant difference was found between days 4 and 6 and between both species (Tukey test, species*time, $p > 0.665$).

On day two, germination rate of both species, combined, was significantly higher at 15ºC (Tukey test, $p<0.001$).

Table 1: 3-way ANOVA and significance values for the effect of species, temperature, time and interactions between these on development traits

Variables	germination rate	mortality	male/female	fecundity	reproductiveness
Species	<0.001	<0.001	0.403 ns	0.002	0.133 ns
Temperature	<0.001	<0.001	0.107 ns	0.006	0.002
Time	<0.001	0.967 ns	<0.001	<0.001	<0.001
Species * Time	<0.001	0.742 ns	0.932 ns	<0.001	0.346 ns
Temperature * Time	0.042	0.268 ns	0.153 ns	0.126 ns	0.071 ns
Species * Temperature	0.092 ns	<0.001	0.141 ns	0.745 ns	0.459 ns
Species * Temperature * Time	0.168 ns	0.650 ns	0.203 ns	0.542 ns	0.998 ns

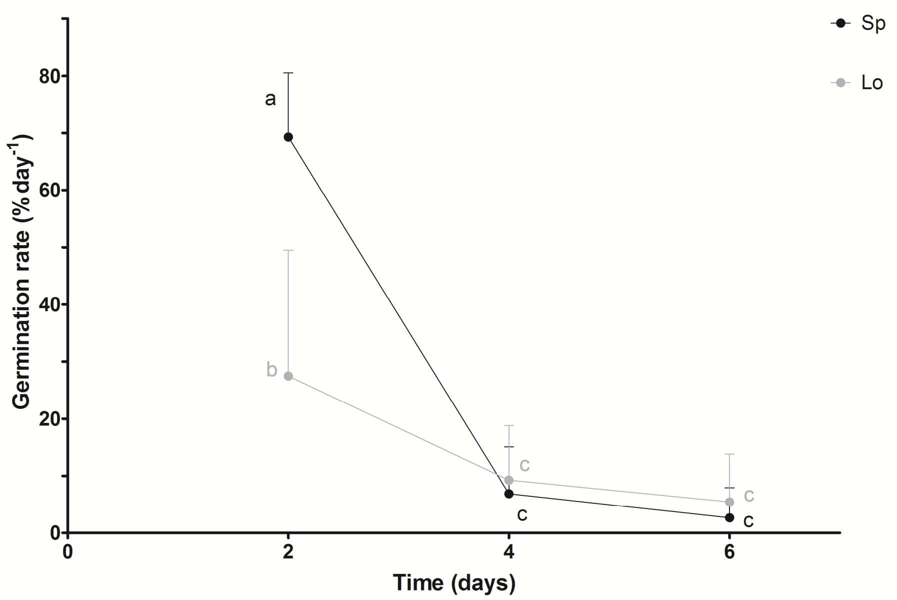


Figure 3: Germination rate of *S. polyschides* (Sp) and *L. ochroleuca* (Lo) over time (combined temperatures). Letters were attributed based on the results of Tukey test, and refer to significant differences between mean values ($p < 0.05$)

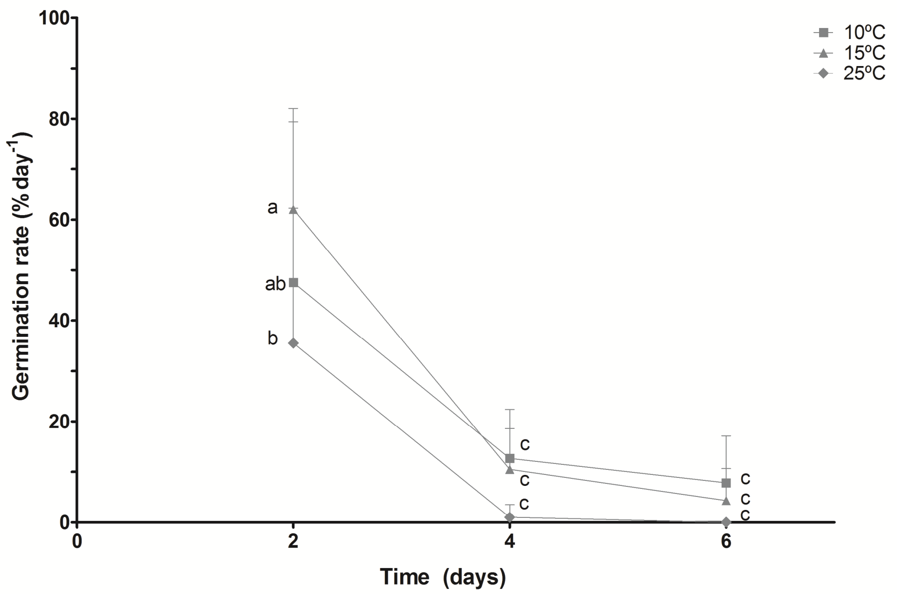


Figure 4: Combined germination rate of *S. polyschides* and *L. ochroleuca* at 10, 15 and 25ºC at days 2, 4 and 6. Different letter refer to significant differences between mean values. Based on Tukey test ($\alpha = 0.05$).

<u>Mortality</u>

Mortality differed between species depending on temperature (3-way ANOVA, species * temperature, $p < 0.001$, Table 1). However, this difference was due to the higher mortality at 25ºC of *L. ochroleuca* showing 4 times higher death rate than *S. polyschides* (Fig. 5; Tukey test, $p < 0.001$). For *S. polyschides*, mortality increased with increasing temperature with 4 times higher death rate at 25 ºC compared to 10 ºC (Tukey test, $p < 0.05$), whereas for *L. ochroleuca* mortality was not significantly different between 10 and 15 ºC (Tukey test, $p = 0.98$), but about 7 times higher at 25 ºC (Fig. 5; Tukey test, $p < 0.001$). No temporal variation was detected (3-way ANOVA, time, $p = 0.967$). At 25ºC, mortality was complete after spore germination for both species obstructing the formation of gametophytes. Consequently measures such as sex ratio, fecundity and reproductiveness of gametophytes were only analyzed for the two lower temperatures.

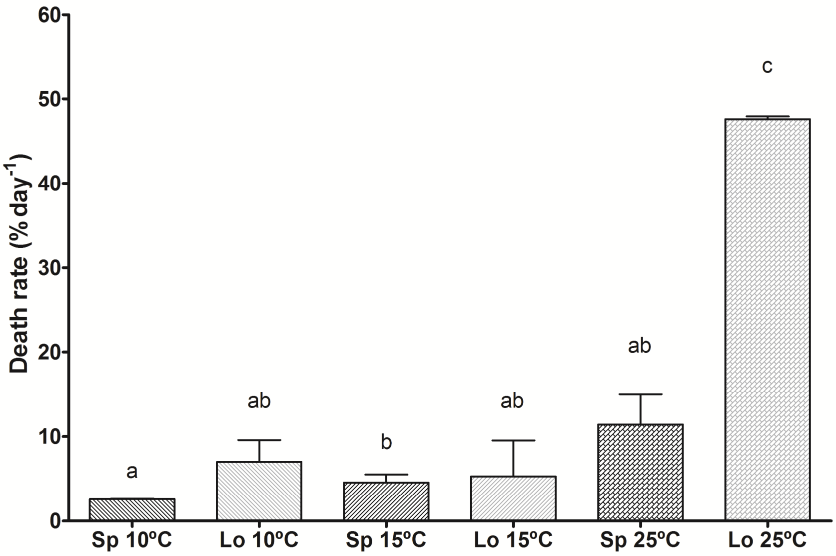


Figure 5: Mortality of *L. ochroleuca* (Lo) and *S. polyschides* (Sp) at 10, 15 and 25ºC. Different letter refer to significant differences between mean values. Based on Tukey test (α = 0.05).

Male and Female gametophytes

In both species and for both temperatures, male gametophytes appeared at the same time as female, as such, when the female gametophytes became mature, there were already males present. Gametophytes of *S. polyschides* appeared earlier (day 12 at 10ºC and day 10 at 15ºC) than for *L. ochroleuca* (day 16 at 10ºC and day 12 at 15ºC, Fig. 6). Sex ratio showed no differences between species or among temperature (3-way ANOVA, species, $p = 0.403$ and temperature, $p = 0.107$, table 1), but significant temporal variation was detected (3-way ANOVA, time, $p < 0.001$). Post-hoc comparisons failed to detected temporal differences (Tukey test, times per species*temperature $p = 0.074$), but the general trend showed increasing sex ratio over time from 0.16 at day 10 to 1.12 (mean of the two species and two temperatures) at day 26, when only gametophytes were present (data not shown).

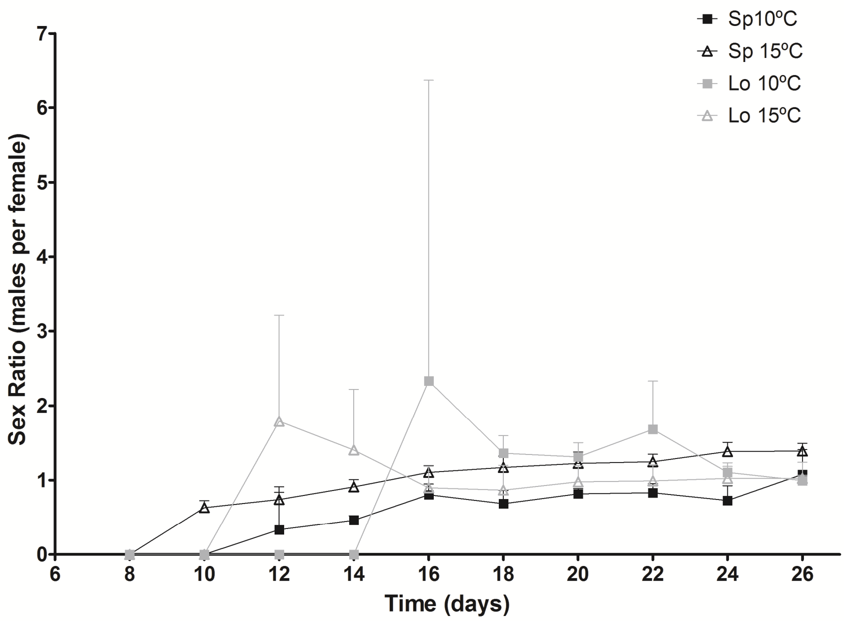


Figure 6: Ratio between males and females over time for *S. polyschides* (Sp) and *L. ochroleuca* (Lo) at each temperature conditions.

<u>FECUNDITY AND REPRODUCTIVENESS</u>

Fecundity varied between species over time (3-way ANOVA, species * time, $p < 0.001$, table 1; Fig. 7), with higher fecundities for *S. polyschides* than for *L. ochroleuca* from day 18 to day 22 (Tukey test, $p < 0.05$), after which there were no significant differences (Tukey test, $p = 0.218$). In addition, fecundities were 1.4 times higher at 15ºC than 10ºC (3-way ANOVA, temperature, $p < 0.01$).

Reproductiveness was 9 times higher at 15ºC compared to 10 ºC (3-way ANOVA, temperature, $p < 0.01$) and showed temporal variation. (3-way ANOVA, time, $p < 0.001$), with no interaction between the main effects (3-way ANOVA, temperature * time, $p = 0.071$). No difference between the species was detected (3-way ANOVA, species, $p = 0.133$).

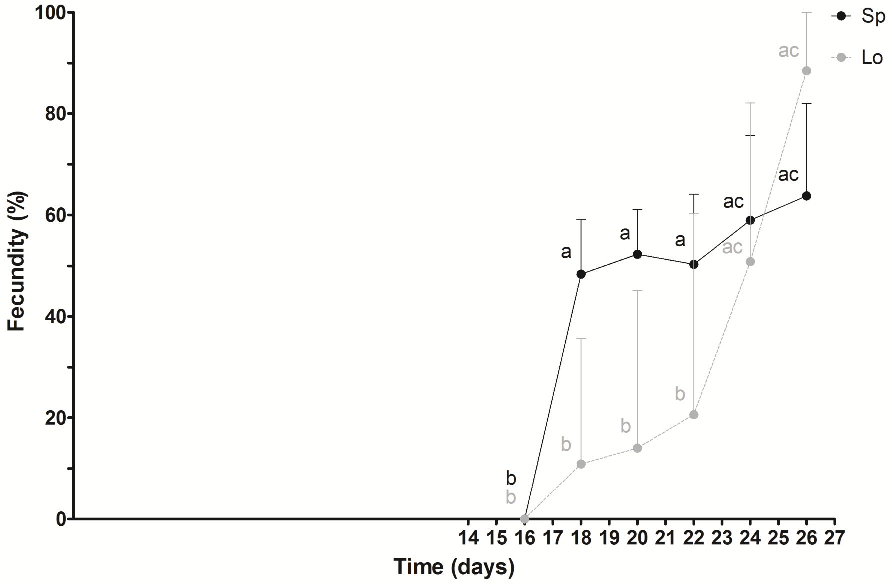


Figure 7: Fecundity of *S. polyschides* (Sp) and *L. ochroleuca* (Lo) (combined temperatures) over time

<u>GAMETOPHYTE DEVELOPMENT</u>

The life cycle differences between *L. ochroleuca* and *S. polyschides* extend to their gametophyte development. Male gametophytes were multicellular for both species. However, the male *S. polyschides* had a larger surface area per volume contacting with the substrate when compared with *L. ochroleuca*. This was mainly due to differences in the arrangements of the cells. *S. polyschides* males have a predominantly linear arrangement of cells, whereas *L. ochroleuca* males are aggregated in a ball-like shape (Fig. 2). Female *S. polyschides* gametophytes developed a multicellular structure with large cells, in contrast to *L. ochroleuca,* in which female gametophytes were composed of a single large cell (Fig. 2). Female gametophytes of both species produced only a single sporophyte.

DISCUSSION

<u>TEMPERATURE EFFECT</u>

L. ochroleuca is known to have a northern distributional boundary caused by the 10ºC winter isotherm (Hoek 1982). It has also been reported that this species has a temperature optimum for spore development between 12 and 18ºC (Izquierdo et al. 2001). Thus, a better development at 15ºC was expected.

Contrarily, the distribution of *S. polyschides* is delimited in the North by temperatures of 3ºC and its spores seem to develop more efficiently at temperature between 5ºC and 17ºC (Norton 1977). It was thus not expected to find significant variation between 10 and 15ºC. The difference in development we observed for *S. polyschides* between 10 and 15ºC might be due to an adaptation to the local environment. *L. ochroleuca* spores, as well as those of *S. polyschides*, are reported to have a maximum development temperature of 23-24 ºC for the sporophytes and 25 ºC for the gametophytes (tom Dieck & de Oliveira 1993, Birkett et al. 1998). Thus, we did not expect a total absence of gametophyte development at 25°C for either species. A possible explanation for this absence is that ecotype differentiation occurs along the distribution range, as has been described for several kelp species (Bolton & Lüning 1982, Gerard & Du Bois 1988, Peters & Breeman 1993). However, to address the question of local adaptation, more than one site would have to be studied for each species, as was done by Bolton & Lüning (1982) for *Laminaria hyperborea* (Gunnerus) Foslie, 1884, *L. digitata* (Hudson) J.V. Lamouroux, 1813 and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl and G.W. Saunders, 2006, and by Gerard & Du Bois (1988) for *Saccharina latissima*.

There are comparable examples of local adaptation of kelp populations. However, in such examples, the ecotypes that were described are now recognized as different species. For example, Martinez (1999) reveals the occurrence of different ecotypes characterized by difference in survival of the microscopic stages to temperature, between the kelp *Lessonia nigrescens* located in the Central and Northern part of the Chilean Coast. However, these two ecotypes correspond probably to two different sibling species that have been shown recently to have a disjoint geographical distribution (Tellier et al. 2009). The same was found for *Saccharina latissima* (as *Laminaria saccharina*) see Gerard & Du Bois (1988).

Although these species have roughly similar southern distribution boundaries, *L. ochroleuca* was found to be less adapted to the higher temperature than *S. polyschides*, showing a 4 times higher mortality. In the Strait of Messina (Mediterranean), where the highest temperature of their geographic distribution area is felt, it is reported that these species have different depth distribution, with *L. ochroleuca* found deeper than 30 m and *S. polyschides* being able to grow almost until the surface, thus being exposed to different temperatures (Hoek 1982). Hoek (1982) proposed two explanations for this difference in depth distribution: one was that *L. ochroleuca* was forced to occur at higher depths because of occasional increase of water temperature over 23ºC. Another or additional possibility is that it is a more shade tolerant species. The precise distributional patterns of these species support the former hypothesis. Along the Portuguese coast, *L. ochroleuca* is now only found along the northern to central regions, with *S. polyschides* extending further south (Assis et al. 2009). Indeed, reports of occurrence of *L. ochroleuca* further south are mainly very deep populations, occurring below 40 m depths (e.g., Azores, Gorringe bank).

Species with the same geographical boundaries might be delimited by different factors and / or in different life phases (Breeman 1988). As such, one of these kelp species could, for example, be delimited by its winter growth requirements and the other by summer reproduction limitations (Breeman 1988, Birkett et al. 1998). In addition, kelp sporophyte's heat tolerance varies along the year (Lüning 1984).

No significant effect of temperature on sex-ratio was detected in this study while temperature-dependent sex ratio was reported in several kelp species (in *Laminaria religiosa*: Funano 1983; *Lessonia variegata*: Nelson 2005 and *Lessonia nigrescens* complex: Oppliger et al. 2011). The authors suggested that under favorable conditions, mortality will be at a minimum and sexes will be present in equal proportion, while under stress conditions the more vulnerable sex will suffer the higher mortality suggesting a difference in sex ratio. More experiments are needed to carefully address this question since interestingly the two study species might respond differently to temperature. Although not significant, a slight tendency showing an increased of males in *L. ochroleuca* and an increase of females in *S. polyschides* with increasing temperature from 10°C to 15°C was reported in our study.

DIFFERENCES BETWEEN THE TWO SPECIES DEVELOPMENT

Following the classical ecological succession model, *S. polyschides* could be considered as a pioneer and opportunistic species that is able to colonize rapidly the open gap in a dense kelp forest, as supported by its initial higher germination rate. The difference in gametophyte morphology following spore germination should reflect such a colonizing ability. Indeed, the demographic dynamics of an annual kelp species is heavily dependent on the survival of microscopic stages during the winter. The morphological characteristic of the *S. polyschides* gametophyte may correspond to a higher adhesion capacity than *L. ochroleuca*. The hypothesis is that a bigger surface area provides a superior adhesion capacity, making *S. polyschides* more resistant to the winter storms. Although no information was found about the life expectancy of these forms, due to the difficulties of studying them in the field, one hypothesis may be that this higher adhesion capacity might allow the gametophytes to remain viable for more than one year, facilitating fast recruitment.

It was reported (Izquierdo et al. 2001) that the number and arrangement of cells in the *L. ochroleuca* gametophytes can vary with temperature. This was not observed here.

Our results reveal differences in fecundity responses through time between the two species, which may reflect differences in reproductive strategies. The rapid and higher fecundity of female gametophytes (50% after day 16 of culture) in *S. polyschides* compared to the lower and progressive fecundity curve observed in *L. ochroleuca* suggests (1) faster growth rate of the female gametophyte and (2) better synchronization of reproduction in *S. polyschides*, which should minimize the likelihood of eggs not being fertilized. A possible explanation for the difference between these two species responses is a maladaptation of *L. ochroleuca* to environmental conditions at its northern geographical distribution boundary, setting its distributional limit and rendering it more vulnerable to local extinction.

CHAPTER 5

RESPONSE OF KELPS FROM DIFFERENT LATITUDES TO CONSECUTIVE HEAT SHOCK.

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ABSTRACT

Although extensive work has focused on kelp responses to constant temperature, little is known about their response to the consecutive temperature shocks they are often exposed to in the shallow subtidal and intertidal pools. Here we characterized the responses of the two southernmost forest-forming kelp species in the Northeast Atlantic, *Laminaria ochroleuca* De La Pylaie and *Saccorhiza polyschides* (Lightf.) Batt. to multiple cycles of thermal stress. Individuals from the upper vertical limit of the geographical distribution edges where the two species co-occur forming forests, France and Portugal, were exposed to 4 consecutive cycles of thermal shock simulating a spring tide. A 24 h cycle consisted of culture at 15 °C, plus 1 h heat shock at one of four levels (22.5, 25, 27.5 or 30 °C). The maximum quantum yield (Fv/Fm) of chlorophyll fluorescence of photosystem 2 (PS2) was used to detect impaired reaction centre function, as a proxy for individual fitness costs, during recovery from heat shock. Both species showed resilience to temperatures of 22.5 and 25 °C. While exposure to 27.5 °C caused no inhibition to Fv/Fm of *S. polyschides*, a threshold was met above this temperature and exposure to 30 °C caused the death of all individuals. In contrast, *L. ochroleuca* from France was damaged but able to survive 30 °C shocks and individuals from Portugal showed complete resilience to this treatment. In both species, blade elongation decreased with increasing temperature, with necrosis surpassing growth at higher temperatures. Resilience to high temperature exposure may confer an advantage to *L. ochroleuca* to colonize intertidal pools on the Portuguese coast, in agreement with the observation that both species recruit in tide pools but only *L. ochroleuca* reach adulthood. Our results indicate that as summer temperatures increase with climate change, the disappearance of *S. polyschides* from intertidal pools and a decrease in the density of *L. ochroleuca* can be expected.

INTRODUCTION

Temperature is a major factor influencing species geographical distribution, and has increased over the last decades due to anthropogenic pressure (Hampe & Petit 2005, IPCC 2007). Since 1980, mean sea surface temperature increased 0.2-0.3 °C per decade in southern Europe, and 0.3-0.7 °C in the Norwegian and North Seas (Lima & Wethey 2012). Moreover, projections indicate that warming rates may increase further in the coming decades (IPCC 2007). These changes have the potential to cause local extinctions and poleward distribution shifts for several species (Hiscock et al. 2004, Hampe & Petit 2005, Wernberg et al. 2010), as has been observed for several species of plankton (Beaugrand & Reid 2003), gastropods (Mieszkowska et al. 2006), fish (Sabatés et al. 2006) and macroalgae (Nicastro et al. 2013), including kelps (Müller et al. 2009, Fernández 2011, Díez et al. 2012, Tuya et al. 2012, Voerman et al. 2013). However, just as terrestrial species' distribution shifts often occur in altitude (Franco et al. 2006, Kelly & Goulden 2008), on marine shores the effects of climate change may be first observed in local species distribution on the shore, before geographical shifts are perceived (Bartsch et al. 2008, Cheung et al. 2009). Species populating shallower areas such as the intertidal and upper subtidal will be the first to experience the effects of global warming, as they're more exposed to temperature extremes, UV and excessive light, desiccation and osmotic stresses, and are more exposed to storm-induced wave surge. Indeed, intertidal species have been reported to suffer shifts in their geographical distribution faster than most terrestrial species (Helmuth et al. 2002). To persist as extreme environmental conditions become more frequent, populations will have to adjust to the new conditions and compensate for the elevated losses typical of populations near distributional boundaries (Guo et al. 2005). As such, the resilience of recruits and early stages to repeated cycles of heat shock is likely to be a determining factor in persistence of populations in the intertidal.

Kelps are habitat structuring species. They modify the area they populate, its environment and resources, and are fundamental for the survival of several other species (Bruno et al. 2003, Wernberg et al. 2010). Thus, variations in kelp species composition, size and canopy density will impact ecological and oceanographic processes. Although extensive work has been done on the temperature limits of kelp species (Fortes & Lüning 1980, Bolton & Lüning 1982, tom Dieck 1993, Izquierdo et al. 2001, Pang et al. 2007, Pereira & Engelen 2011), nothing is yet is known about their ability to cope with consecutive cycles of stress exposure associated with low tides. In this study, we aimed to recreate the conditions experienced by recruiting individuals during typical summer spring tides, where individuals may be exposed daily to repeated cycles of thermal stress during low tides, and which may be decisive for population persistence. Experiments were performed to compare the two southernmost kelp-forest species in the Northeast Atlantic: *Laminaria ochroleuca* and *Saccorhiza polyschides*. Since, as reported for several species of animals and plants, individuals from different latitudes may be locally adapted or acclimated to different conditions (Liu & Pang 2009, Zippay & Hofmann 2010), we compared the responses of individuals from the higher and lower latitude ranges where these two species coexist: Brittany, France and Northern Portugal. The results of such experiments can provide clues as to whether temperature is likely to be a decisive factor influencing the distribution, persistence and composition of these populations in the near future.

MATERIAL AND METHODS

MODEL SPECIES AND COLLECTION

L. ochroleuca and *S. polyschides*, a perennial and an annual species, respectively, are two important Northeast Atlantic species. They both have their southernmost populations in isolated spots in the upwelling region of west Morocco and some very deep areas in the Mediterranean and Atlantic seamounts and islets, south of their coastal distributional range which ranges from the Portuguese coast to Brittany (France), and the English and Bristol Channels (Braud 1974, Norton 1977, Hoek 1982, Lüning 1990, Birkett et al. 1998, Tittley & Neto 2000). *S. polyschides* has a wider northern distribution, found as far north as the west coast of Norway.

Sporophytes of the two species have similar upper temperature limit of around 24 °C (Norton 1977, Birkett et al. 1998). Although no records of lower temperature limits were found for either species, at their northern distribution limit average winter sea surface temperatures (SST) are 4 °C and 10 °C, for *S. polyschides* and *L. ochroleuca* respectively (Braud 1974, Norton 1977). Moreover, these two species have an overlapping range of optimum temperatures. *L. ochroleuca* is described as growing better between 15 and 18 °C, while *S. polyschides* seems to perform better at temperatures from 10 to 17°C (Norton 1977, Izquierdo et al. 2001, Biskup et al. 2014). As such, despite their distinct life strategies, the two species have overlapping niches and are potential competitors.

This study is focused on the distributional range where the two species overlap, from northwest Iberia to the English Channel. Recruits of *L. ochroleuca* and *S. polyschides* ca. 15 cm length were collected near the northern distribution limit of *L. ochroleuca*, in Brittany, France (48°41'55.26"N 3°56'28.50"W), and in Northern Portugal (41°42'27.80"N 8°51'45.30"W), the southern distribution limit where both species are able to form forests. In Brittany, the vertical distribution of both species extends as high as the upper subtidal, being out of water only during the lowest spring tides of the year. In Northern Portugal they can be found in tidal pools and although their base is usually immersed, their blades may get exposed during spring tides. In both areas, collections were made in the upper distribution limit and were transported in cold seawater inside refrigerated boxes. In Brittany, samples were collected on the 13th of July 2011, arriving in the lab within 48h of collection. In Northern Portugal, collections were made on the 29th of March 2012, reaching the laboratory within 12 hours of collection. Experiments were done at slightly different times of the year to coincide with the recruitment peak of each area. Average SST during the month prior to collection was 14.1 °C in Brittany and 13.3 °C in Northern Portugal (Aqua MODIS SST, NASA). The same protocol and experimental conditions were used for both populations.

EXPERIMENTAL DESIGN

Immediately upon arrival, each individual was placed in 0.5 L individual tanks with 0.2 μm filtered seawater (FSW) and aeration at 15 °C for 5 days of acclimation, so they could recover from eventual stress caused by collection and transportation. This temperature close to the conditions during the month of collection and small sporophytes and other microscopic forms of both species grow efficiently at 15 °C

(Norton 1977, Izquierdo et al. 2001, Pereira & Engelen 2011). These conditions were also used as control during the experiment. Throughout the acclimation and experimental periods, sporophytes were exposed to a 12h day photoperiod, with a photon flux density of 40 µmol m^{-2} s^{-1} (Bruhn & Gerard 1996, Izquierdo et al. 2001). Seawater was changed every two days during the acclimation period. During the heat shock experiment this was done every day after exposure, to make sure individuals were not exposed to metabolites, as tidal flow would quickly wash them away.

Temperature tolerance of young sporophytes (5 replicates) was tested for a series of sequential 1 hour exposures to one of four temperature treatments; 22.5, 25, 27.5 or 30 °C. Unnaturally rapid warming was avoided by sequential transfer at each temperature, in a 30 min ramp, until the target temperature was reached. Exposure to the target temperature was for 1 hour. Afterwards, individuals were directly transferred back to the control temperature (15ºC) to simulate the returning tide, and remained at the control temperature till a 24 h cycle was complete. This procedure was repeated for 4 cycles. Measurements were repeatedly performed on the same individual and each was daily exposed to the same target temperature.

PHOTOSYNTHETIC YIELD DETERMINATION

Photosynthetic activity is considered to be one of the most heat sensitive cellular processes, both by damage to the oxygen-evolving complex in PS II, damage and rapid turnover of the D1 protein, and by impairment of recovery processes by reactive oxygen species. As such, in vivo, the efficiency of reaction center functioning (controlling electron flux for downstream processes) represents a balance between damage and repair (Allakhverdiev et al. 2008). Initial values of chlorophyll maximum quantum yield (Fv/Fm) were measured for each individual (5 replicates per treatment) under control conditions. All Fv/Fm measurements were made with a portable chlorophyll fluorometer (Junior-PAM, Walz, Germany). Individuals were dark incubated for 5 minutes, after which Fv/Fm measurements were made on two different areas, in the center of the blade of each individual's. The mean of the two values was used in downstream analyses. Because small intrinsic differences in population- and/or location-specific Fv/Fm values can obscure subsequent statistical comparisons between species and/or populations, all Fv/Fm data were normalized as a proportion of the initial values (adjusted mean=1). Initial Fv/Fm values were above 0.7 to make sure the conditions used were not stressful.

During each of 4 consecutive 24 h cycles, individuals were exposed to heat shock for one hour at the target temperature, immediately after which Fv/Fm was measured. Individuals were then returned to the control temperature (15 °C) for recovery and Fv/Fm was measured again after 24 h to assess recovery. Controls remained at 15 ºC throughout each cycle, but otherwise were manipulated in same way as the other treatments. The same individuals were used throughout the experiment and were always exposed to the same target temperature.

GROWTH MEASUREMENTS

Individual growth was expressed as relative change in blade area determined from photographs taken at the beginning of the experiment, after the acclimation period, and by the end of the 4 heat shock cycles. Tissue death was considered as negative growth. The software GIMP (GNU Image Manipulation Program) 2.6.6 was used for these measurements.

STATISTICAL ANALYSIS

PERMANOVA assesses differences in distribution, which may be caused by differences in means, in dispersion or both. PERMDISP focuses only on dispersion, complementing the PERMANOVA results. Both PERMANOVA and PERMDISP routines (Anderson et al. 2008) were used to analyze the data. Maximum quantum yield was compared between species and temperatures for each location. Growth data was compared for the two species exposed at the different temperatures. No direct statistically comparisons between French and Portuguese experiments were performed because these were done in different months of different years.

RESULTS

The maximum quantum yield of photosystem II (Fv/Fm) of recruits collected in Brittany (France) varied between species, temperature and time (PERMANOVA, time * species * temperature, $p < 0.0001$). At the control temperature, 15 °C, no significant difference was observed through time for either species (Fig. 1). At 22.5°C, although significant but rather small differences were observed through time, for both species, within the temperature treatment, there was no significant difference at each time point between 22.5ºC and the control. Exposure to higher temperatures (25 and 27.5 °C) caused significant initial decreases in Fv/Fm in *L. ochroleuca* following stress exposure. However, recovery was complete within each 24h cycle. Thus, sublethal temperature stress in the Brittany *L. ochroleuca* population caused reversible reductions in Fv/Fm (photoprotection of PSII). However, at 30 °C, Fv/Fm declined following each consecutive stress cycle, indicating a breakdown of thermal resilience. Nevertheless, at the end of the experiment all *L. ochroleuca* individuals were still alive. A rather different strategy was seen in *S. polyschides* from Brittany, where almost no effect of temperature on Fv/Fm was observed between 15 and 27ºC (Fig. 1), but where resilience collapsed after repeated exposure to 30ºC. By the end of the 4th cycle no chlorophyll fluorescence could be detected for any individual, and no recovery was observed by the end of the experiment. As the threshold temperature tolerance was approached, individual variance clearly increased relative to that under control conditions (Fig. 1).

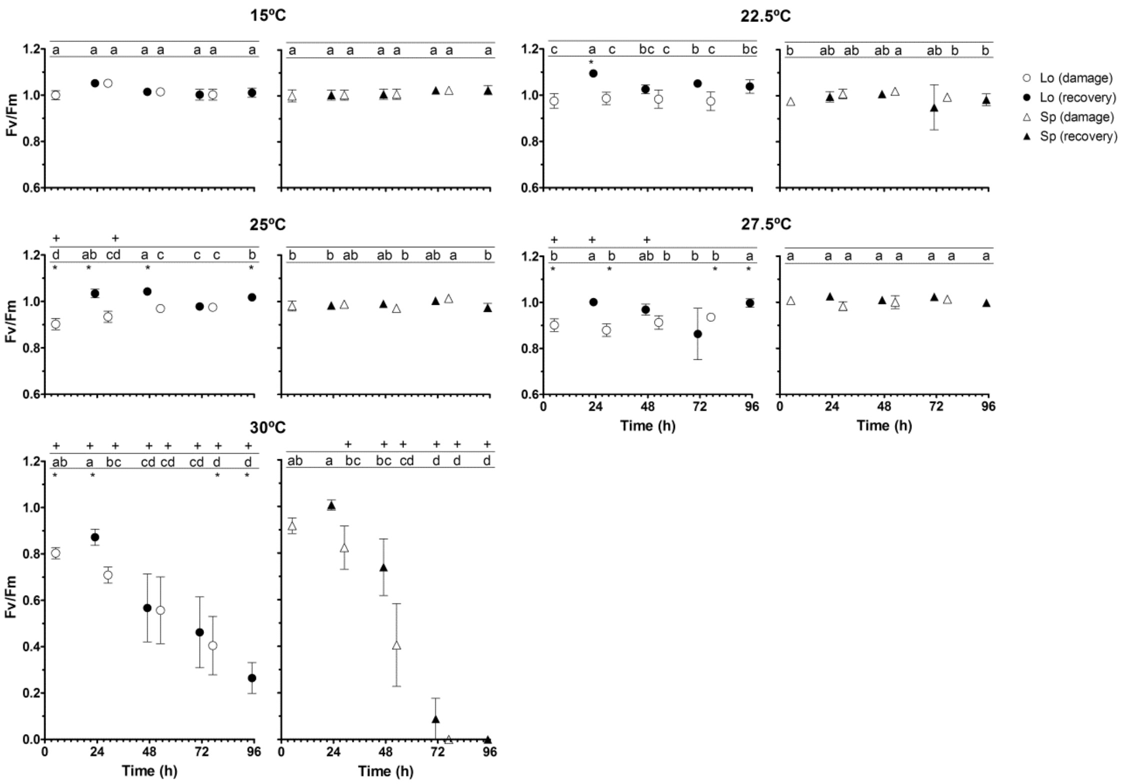
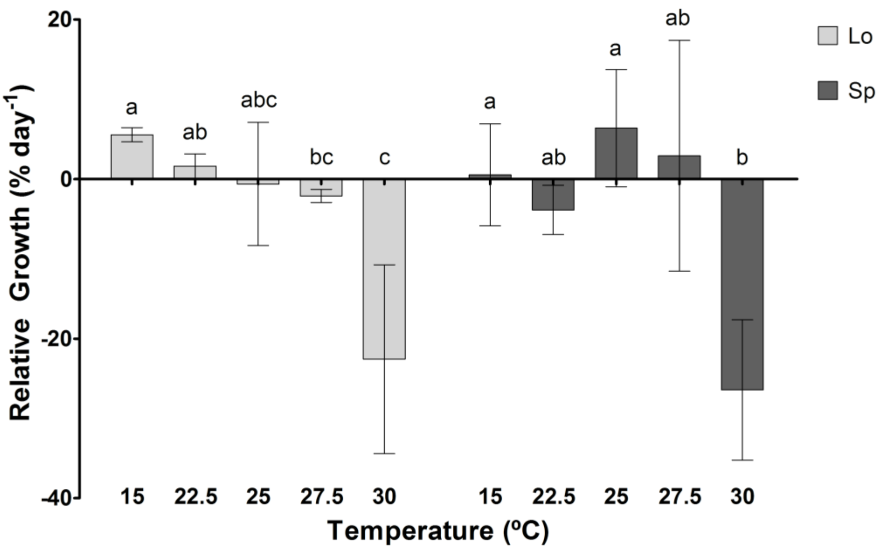


Fig.1: Fv/Fm variations of *Laminaria ochroleuca* (left, circles) and *Saccorhiza polyschides* (right, triangles) from Northern Brittany, France, in response to exposure to repeated 1 hour temperature elevation. Error bars represent standard error (n=5). Open symbols indicate measurements post-stress. Closed symbols refer to measurements after the recovery period. + indicates a significant difference from the control (15°C). * indicates a significant difference between the two species. Different letters were attributed, per species and temperature, to significantly different means.

Kelp recruits from the French populations showed no significant interspecific differences in relative growth rates. Growth rates were, however, significantly affected by temperature (PERMANOVA, temperature, $p = 0.0028$), being reduced as temperature increased to 27.5 °C for *L. ochroleuca*, and to 30 °C for both species. At higher temperatures growth was surpassed by necrosis, resulting in size decrease (Fig. 2).



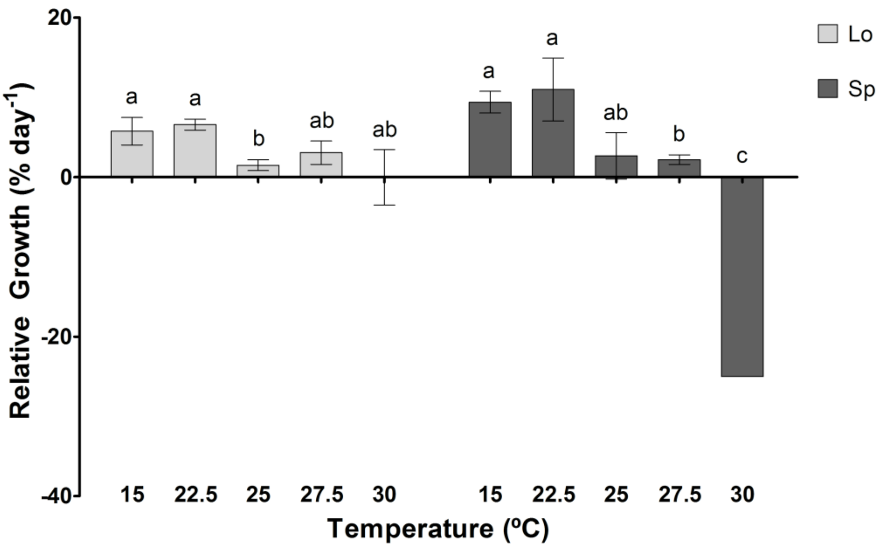


Fig. 2: Mean relative blade area growth rate (n = 5) of *L. ochroleuca* (Lo) and *S. polyschides* (Sp) from Brittany, France (top) and Northern Portugal (bottom), cultivated at 15 °C with daily recurrent exposures of one hour to 15, 22.5, 25, 27.5 and 30 °C. Error bars represent standard error. Different letters were assigned, per species, to points with significantly different means ($p < 0.05$). No significant difference was observed between species at identical temperature treatments.

Fv/Fm of recruits from Portugal varied significantly over time between species and temperatures (PERMANOVA, Time*Species*Temperature, $p < 0.0001$, Fig. 3). Data from the 15 °C treatment shows that *L. ochroleuca* and *S. polyschides* responded similarly to culture conditions, although the variations in Fv/Fm over time and between individuals were greater than observed for the Brittany populations. Significant differences between post-stress and recovery points were not observed during exposure to either 22.5, 25 °C or 27.5 °C. Exposure of *L. ochroleuca* to 30 °C revealed a clearly greater resilience in the Portuguese compared to the Brittany population (c.f. Figs 1 and 3). Although photoinhibition increased with consecutive heat-shock cycles, recovery of Fv/Fm to control levels occurred within 24 h after each exposure, indicating resilience to this level of thermal stress, as opposed to the population from Brittany. In contrast, Portuguese *S. polyschides* was even more affected by repeated exposure to 30 °C. At this temperature, no resilience was observed and cumulative damage resulted in the death of all individuals by the 3rd heat shock cycle (Fig. 3).

Relative growth of kelp recruits from Portugal varied significantly with species and temperature (PERMANOVA, species * temperature $p < 0.0001$, Fig. 2). Growth rate shows a tendency to decrease with increasing temperature for both species, but this difference was significant only for *S. polyschides*, which showed a more pronounced trend. Relative growth differed significantly between species at 30 °C only.

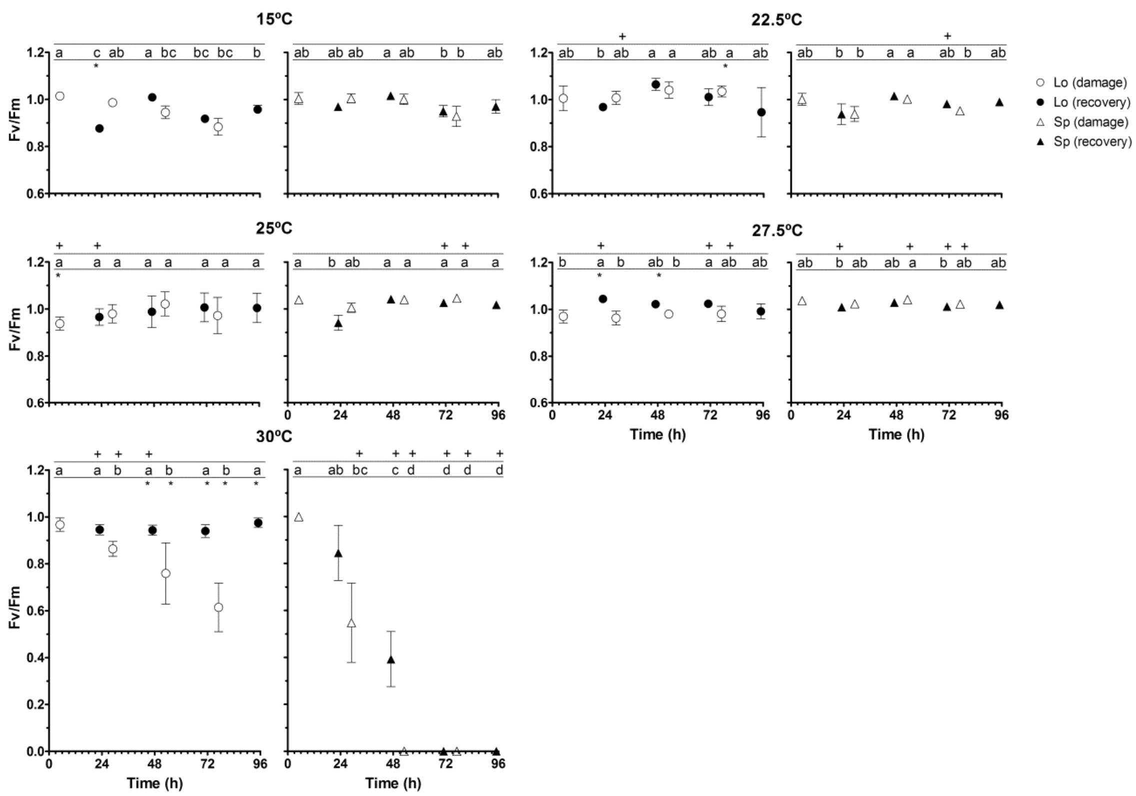


Fig.3: Fv/Fm variation with repeated exposure to high temperature in *Laminaria ochroleuca* (left, circles) and *Saccorhiza polyschides* (right, triangles) from Northern Portugal. Error bars represent standard error (n=5). Open symbols indicate measurements after the heat shock. Closed symbols refer to measurements after the recovery period. + indicates a significant difference from the control (15 °C). * indicates a significant difference between the two species. Different letters were attributed, per species, within each temperature, to significantly different points.

DISCUSSION

In this study we report lethal temperature limits and sub-lethal temperature effects for recruits of the two southernmost forest-forming kelp species in the northeastern Atlantic: *L. ochroleuca* and *S. polyschides.* We also show differences between populations of each species taken from southern and northern regions where they co-occur, with evidence that *L. ochroleuca* potentially exhibits greater thermal resilience near the southern edge of its range, which might indicate some adaptation to local conditions. Such phenotypic plasticity has been reported for *S. polyschides* (Biskup et al. 2014) and other kelp species, such as *Laminaria digitata* (Delebecq et al. 2012), *Saccharina japonica* (Pang et al. 2007) and *Ecklonia radiata* (Wing et al. 2007, Staehr & Wernberg 2009). Previous studies, based on constant growth temperature, reported that both species have a similar upper temperature limit of 23 - 25 °C (Norton 1977, Lüning 1990). These limits were, however, determined by exposure to a constant growth temperature, and nothing was known about the impact of the rapid and consecutive temperature rise they are often exposed to in tidal pools, a common habitat in Portugal. Because the species occupy similar areas on the shore, it was expected that their responses would be similar. While water temperature in Northern Portugal is usually about 15 °C, in tidal pools it may reach 20 °C on warmer summer days and blades may be subjected to temperatures as high as 30 °C near the surface (Engelen et al. 2008). Both species showed resilience to relatively short exposures to temperatures above their reported 23 -25 °C survival limit (Norton 1977, Lüning 1990). Although *S. polyschides* showed no significant response when exposed to up to 27.5 °C, when subjected to 30 °C a threshold seems to have been met as individuals were significantly damaged and died. In contrast, *L. ochroleuca* appeared more resistant to extreme temperatures; individuals from Brittany were damaged by 30 °C exposure but were still alive by the end of the experiment and individuals from Northern Portugal were even more resistant, showing total resilience. Still, temperature increase caused a tendency for blade elongation to decrease and at higher temperatures necrosis surpassed growth. This also happened for *S. polyschides* from both locations. While previous work (Biskup et al. 2014) reported a higher physiological plasticity of *S. polyschides* when compared to *L. ochroleuca*, consecutive exposure to high temperatures indicates that such disparate responses are mainly a matter of strategy, most likely associated with their distinct life history. *L. ochroleuca*, as a perennial species, benefits more from photo protective mechanisms, while for the opportunistic annual, *S. polyschides*, strategies for faster growth may be selected. Although *S. polyschides* is able to recruit in tidal pools, only few individuals can be found in the summer and the main kelp species occupying such areas is *L. ochroleuca* (personal observations; Barradas et al. 2011). The response of *L. ochroleuca* to short high temperature exposure suggests that its resilience provides an advantage to colonize such shallower areas. The inability of *S. polyschides* to cope with daily short exposure to 30 °C may offer an explanation as to why these are found in tidal pools in such low number and there they do not reach similar dimensions to *L. ochroleuca* nor to those attained by other *S. polyschides* in the high subtidal (personal observations).

Responses to change in environmental conditions are reported to be largely mandated by an individual's history (Lüning 1990). Although no significant difference in Fv/Fm between sites can be asserted for *S. polyschides*, the response of *L. ochroleuca* varied between sites at 30 °C. While *L. ochroleuca* from Portugal exposed to 30 °C suffered necrosis, individuals from Brittany showed significant blade loss at temperatures above 27.5 °C, an important difference between populations. Although blade elongation is not an expression of meristematic activity, this balance between growth and necrosis is important for population persistence, particularly since an individual's spore production depends on blade length. Our finding on differences in responses to temperature between populations of *L. ochroleuca* does not match other studies on *Laminaria sp*, where the effect of temperature on growth and survival didn't vary significantly along a latitudinal range (Jones & Kain 1967, Bolton & Lüning 1982). Such variation has, however, been observed for other kelp species (Liu & Pang 2009).

Our results suggest that, in the near future, temperature will most likely not be a limiting factor for either species in the high subtidal, at least in the moderately exposed shores where they occur and where water mixing prevents strong thermal stratification at the surface. However, in tidal pools, a temperature increase might lead to higher apical blade loss and an increase in mortality rates. Moreover, climate change is accompanied by environmental changes other than temperature increase (Fortes & Lüning 1980, Deysher & Dean 1986, Han & Kain (Jones) 1996, Müller et al. 2009) which may impair local persistence before temperature increases enough to hamper recruit survival in tidal pools. Nevertheless, our results show that temperature responses can explain subtle differences in the upper distribution of these kelp species and variation between populations along the distributional range.

DISCUSSION

DISCUSSION

The studies in this thesis have filled in some of the knowledge gaps about the biology of marine kelp populations at their southern distribution edge. By focusing on the southernmost forest forming kelp species of the northeast Atlantic, *S. polyschides* and *L. ochroleuca*, we were able to answer several questions related to population responses to climate variation. Over the last few decades, the distribution of *S. polyschides* and *L. ochroleuca* has been shifting poleward as did also the low-latitude edge of *L. hyperborea*, in Northern Portugal (Ardré 1970, Pérez-Ruzafa et al. 2003, Fernández 2011, Smale et al. 2013, 2014). These tendencies make it especially important to study populations in the southern distribution limit where they form forests as this information can help understand the causes behind these shifts and how they might evolve in the future. Special regard was given to the effect of temperature raise as the most immediate and direct climate change related alteration.

CHAPTER 1 – POPULATION DYNAMICS OF TEMPERATE KELP FORESTS NEAR THEIR LOW-LATITUDE LIMIT

LAMINARIA HYPERBOREA

In 2008 and 2009, *L. hyperborea* could be found in several intertidal pools, under the canopy of *L. ochroleuca* and in the shade side of intertidal pools in Moledo, near the Portuguese-Galician border. As *L. hyperborea* is a low temperature and shade-adapted species, it may be hypothesized that the shade provided by *L. ochroleuca* canopy was essential for their maintenance (Lüning 1986, Han & Kain (Jones) 1996). In the 2009-2010 censuses, individuals of both *Laminaria* species there were small and *L. hyperborea* sporophytes were devoid of the lamina from the previous year. This indicates that the local hydrodynamics might have caused high mortality and degradation of the older thalli parts, although this species was described to thrive in wave-exposed sites (Pedersen et al. 2012). The reduced size of the sporophytes limited their reproductive effort. Besides, during its typical reproductive season, between September and February, few individuals had sori and, throughout the monitoring period, only two new recruits were identified (Han & Kain (Jones) 1996). Fertilization in this species is known to be impaired at temperatures above 15 °C, which in intertidal pools are frequent during summer (Lüning 1980). This, together with the low local spore production, could explain the low recruitment observed.

During the monitoring period, a strong late winter storm, associated with the North Atlantic Oscillation (NAO) negative phase, caused massive destruction of kelp forests all over the northern Portuguese coast (Grumm 2010, Seager et al. 2010, Cattiaux et al. 2010). In the monitored pool, 84% and 91.5% of *L. ochroleuca* and *L. hyperborea*, respectively, were removed and *L. hyperborea* was unable to recruit back during the next six months, until the end of the monitoring effort.

Two years after the last census, in 2012, *L. ochroleuca* population had completely recovered, with a higher density of sporophytes and a denser canopy. With this, shade conditions likely needed for *L. hyperborea* were regained. However, no *L. hyperborea* individuals were observed in the pool. This indicates that the population was unable to sustain itself and the scattered populations from the subtidal were not able to disperse enough microscopic forms to allow recruitment (Reed et al. 1992, Gaylord et al. 2002).

Laminaria hyperborea is a cold and shade-adapted species that finds its southern distribution on the Portuguese northern coast. It has been reported to be disappearing for Asturias and its low-latitude distribution limit has been shifting poleward (Ardré 1970, Pérez-Ruzafa et al. 2003, Fernández 2011). In 1969 it could be found as far south as cape Mondego (40°10'49.23"N; 8°54'20.88"W) (Ardré 1970) and in 2003, as it shifted further North, in praia da Aguçadoura, Porto (41°25'40.79"N; 8°46'57.98"W) (Pérez-Ruzafa et al. 2003). Nowadays, in northern Portugal, it is still found in a limited number of locations, mostly deep in the subtidal (Tuya et al. 2012, personal observations, João Franco personal communication). These results are consistent with an ongoing retreat, which is likely to happen in depth prior to geographically.

<u>*LAMINARIA OCHROLEUCA*</u>

L. ochroleuca has been undergoing distributional changes during the last decades. Its range has extended north along SW England where it's becoming more frequent (Spooner 1950, Smale et al. 2014) and it retreated to colder regions along the north coast of Spain (Fernández 2011). In Europe, it´s coastal shallow southern limit is near Cape Espichel (central Portugal) where its progressive decline is widely reported by local fishermen.

Based on the size and population structure, *L. ochroleuca* populations occupying intertidal pools seem to be young, with high mortality and high recruitment to compensate losses, as shown by the positive population growth of the first year census (2008-2009) (chapter 1) (Norton et al. 1982, Dayton et al. 1984). The high subtidal population, on the contrary, had bigger individuals with a larger reproductive effort but reduced recruitment. This was most likely a result of canopy overshading, inhibiting the development of microscopic forms (tom Dieck 1993). In such populations, recruitment of newly available substrate can only take place when mortality locally reaches a threshold and inhibition by shading is halted. As such, this population may show a negative population growth without losing function or the ability to recover (Dayton et al. 1984). In fact, when the late winter storm of 2010 caused a destruction of 88% of the individuals, recruitment became higher there than at the intertidal pool, where destruction was only 36%. It is possible that individuals from the intertidal where less impacted by the strong hydrodynamics thanks to their reduced size.

To asses if the populations were able to recover, the state of the population two years after the last census was compared with a simulation of what the population would be like if the storm didn't occur, which is the state expected in case the population fully recovered. As such, the simulation was done as if 4 years without major events took place, considering the first census as the starting point. The simulation for the intertidal pool agreed with the smaller stages only, while there were fewer of the larger individuals than expected. This indicates that more than two years were necessary for the population to completely recover. The population structure simulated for the high subtidal, on the other hand, was in agreement with the frequency of larger individuals only. Due to the high mortality caused by the storm in the subtidal, the population became devoid of adult individuals and almost totally dependent on survival and development of microscopic stages, as the surrounding areas were equally stripped from sporophytes. As the storm took place after the reproductive season, a bank of microscopic forms was present, ensuring recruitment. However, as it takes longer than one year for a sporophyte to reach adulthood, during the following reproductive season the individuals were not big enough to produce spores. As the surrounding area was equally destroyed by the storm, a dispersal of spores to this site is unlikely (Reed et al. 1992, Gaylord et al. 2002). As such, in the fourth year, a shortness of microscopic forms is presumable. With this, the individuals that recruited in the previous year reached adulthood but no new recruits were present. This leads to the hypothesis that one year after a major destruction, population persistence depends entirely on the new individuals' ability to survive till the reproductive age, as microscopic forms are unlikely to survive for longer than a year (Chapman 1987, Hoffmann & Santelices 1991, Tegner et al. 1997, Vásquez et al. 2006).

SACCORHIZA POLYSCHIDES

In Portugal, macroscopic sporophytes of *S. polyschides* are seasonal, occurring in the spring and summer, while during winter the population is composed exclusively of microscopic forms. As such, it's likely that the 2010 late winter storm impacted the microscopic population, by scouring and burying them in sediment (Ballantine 1984, Hoffmann & Santelices 1991, Grumm 2010, Burrows et al. 2011). In the following spring, recruitment took place later than usual and the new sporophytes were scattered. As a result of this delayed recruitment, individuals were still young and died off before being able to reproduce. Since microscopic forms are not expected to survive for more than a year, and the more subtidal population was probably less impacted by wave action, the 2011 population was most likely resultant from the dispersal of microscopic forms from neighbor individuals deeper in the high subtidal

(Dayton & Tegner 1984, Gaylord et al. 2002, 2004). Still, local spore production seems to be an important factor in population maintenance, as the 2011 population density was 50 % of the previous year. Nevertheless, recruitment took place during the regular period and individuals were able to develop and reproduce before the first autumn storms. A possible result of local spore production was the significantly higher population density in 2012 than in the two previous years. These data indicate that in areas where the macroscopic phase of the population is seasonality restricted, yearly climate variations can influence its life cycle and population structure.

CHAPTER 2 – LIVING ON THE EDGE: KELP FOREST RESILIENCE AT THE EDGE OF DISTRIBUTION

With climate change, recurrence of highly energetic storm, capable of destroying large kelp forest areas, are expected to become more frequent. Kelp population ability and time needed to recover from canopy removal has been studied for *Laminaria* species such as *L. digitata* and *L. longicruris*. However, the recover ability of *L. ochroleuca* was unknown.

As canopy removal was performed by the end of the reproductive season, spores produced by the removed canopy where most likely released prior to removal. Six months later, although total length of individuals was similar to the control, population was less dense. This indicates that the species recruited quickly but, despite increased recruitment, survival was not sufficient to recover the initial density. It took 18 months for individuals in these intertidal areas to recover to the previous state. Although the dimensions of both stipe and blade were similar between experimental and control quadrats, recruitment was significantly higher at the control, hinting that local production of spores plays an important role in patch recovery, as most spores settle in the vicinity of their source (Gaylord et al. 2006). Similar effect of lack of local spore production was observed for *S. polyschides* (Chapter 2).

At the storm-impacted site, canopy removal quadrats had been prepared with a 6 months interval. One year after the last removal, the late winter storm of 2010 caused the destruction of this population (Grumm 2010). This storm took place in February, by the end of the reproductive season. As the timing of first disturbance differed between quadrat type, it is to be expected that in the spring removed quadrats reproductive effort was lesser, as a result of the younger individuals. However, 6 months after the storm, recruitment was equally quick for all quadrats. As such, it’s to be expected that the increased hydrodynamics leveled spore availability by scouring and resuspending microscopic forms (Reed 1990, Reed et al. 1992, Gaylord et al. 2002, 2004).

The storm caused the removal of all canopy from the studied quadrats and surrounding area. By the next census, new individuals had already recruited back, but their mortality was almost total. As such, one year after the storm this population was still composed of young individuals. This indicates that although canopy may overshadow the substrate inhibiting recruitment, it also provides protection from hydrodynamics for the new and fragile recruits (Jackson & Winant 1983, Løvås & Tørum 2001). The fact that one year after the storm the population didn’t yet have adult individuals is in accordance with the hypothesis for a shortness of spores one year after the storm in the subtidal area (Chapter 1).

These data indicate that the extension of the destruction may be more importance than its recurrence, as small canopy removals may be compensated by spore dispersal and canopy protection from neighboring areas. Small areas in the intertidal where canopy was removed were able to fully recover in 18 months, while both intertidal and subtidal areas where destruction was more extensive took longer than two years to fully recover. These findings are in accordance with harvest experiments performed in other *Laminaria* species. In Nova Scotia, *Laminaria longicruris* was able to regrow cropped biomass in one year while pre-harvest maturity took two years to reach (Smith 1985). Similarly, population structure of *Laminaria digitata* in the low intertidal , in Brittany, needed between 18 and 24 months to recover (Engelen et al. 2011)

With global warming, an increase in the frequency of such energetic storm is expected. Individual’s life expectancy is thus likely to decrease and prevent them from reaching adulthood. This would impair local production of spores, rendering the population dependent on spore dispersal from deeper areas, less affected by storm-associated wave action (Dayton & Tegner 1984, IPCC 2007, Byrnes et al. 2011, Hemer et al. 2013), which is expected to lead to less dense *L. ochroleuca* in shallower areas.

Density of other canopy forming species (*Cystoseira tamariscifolia*, *Cystoseira baccata*, *Sargassum muticum* and *Saccorhiza polyschides*) varied throughout the year and between treatments unrelated to *L. ochroleuca* density. While their presence couldn't be related to season or substrate availability, it also posed no threat to *L. ochroleuca* recovery.

CHAPTER 3 – CONTRASTING TIMING OF LIFE STAGES ACROSS LATITUDES – A CASE OF A MARINE FOREST FORMING SPECIES

Southern populations of *S. polyschides*, such as those in Iberia, are highly seasonal and macroscopic sporophytes are present in the field during a limited period; in contrast, central population are able to overwinter and recruit year-round. It used to be generally accepted that temperature was responsible for this limitation in the south and populations were only able to persist in the summer thanks to the temperature drop caused by the typical upwelling season. However, we found that temperature is not a limiting factor for the development of the microscopic stages. Similarly, winter temperatures of both locations are in the optimum temperature range for this species, equally offering no explanation for the lack of recruitment during winter. Based on the period needed for spores to develop into visible sporophytes, synchrony in spore release is not the cause for this seasonality.

It is very relevant that the seasonality of macroscopic sporophytes in the southern range of distribution is coincidental with the typical upwelling season. However, as temperature cannot explain this difference, these observations led to the novel hypothesis that seasonality in southern populations is caused by nutrient limitation, as nutrients increase during upwelling events, while in the central range nutrient levels are higher year-round. This hypothesis explains well the observations; however, it needs to be tested.

CHAPTER 4 – TEMPERATURE EFFECTS ON THE MICROSCOPIC HAPLOID STAGE DEVELOPMENT OF *LAMINARIA OCHROLEUCA* AND *SACCORHIZA POLYSCHIDES*, KELPS WITH CONTRASTING LIFE HISTORIES.

Microscopic forms of both *L. ochroleuca* and *S. polyschides* have been reported to have a maximum development temperature of 25 °C. However, in cultures of spores from Brittany, northwest France, neither species was able to develop gametophytes at this temperature. Also, although these species have similar southern distribution edges, mortality rate in *L. ochroleuca* was 4 times higher than for *S. polyschides.* Still, it might be proposed that ecotype differentiation along the distribution range is the cause for such high mortality in *L. ochroleuca* (Bolton & Lüning 1982, Gerard & Du Bois 1988, Peters & Breeman 1993). However, in the Strait of Messina, the geographical distribution edge for both species, *L. ochroleuca* is found in the subtidal, around 30 m, while *S. polyschides* is able to grow almost until the surface, being exposed to higher temperatures and irradiances (Hoek 1982). Likewise, in the Portuguese coast, *L. ochroleuca* occurs only in the North while *S. polyschides* can be found in sites further south. However, cultures of *S. polyschides* from other population in Northern France and from Northern Portugal also showed an inability to survive at 25 °C. As such, it can be proposed that although this species have the same southern distribution edge, as they can both be found in the Moroccan coast, this may be limited by different conditions or in different stages of the life cycle.

Although sex of gametophytes is known to be, to an extent, genetically determined, environmental conditions are also known to influence the sex ratio (Evans 1963). Such is the case of temperature (Funano 1983, Nelson 2005, Oppliger et al. 2011). However, in cultures from Northwestern France performed in 2009, sex ratio was close to 1 and temperature had no significant impact on either *L. ochroleuca* or *S. polyschides*. In cultures of *S. polyschides* from Northwestern France and Northern Portugal made in 2011 (Chapter 3), however, sex ratio was close to 2 females per male, a significant deviation from 1:1 ratio. This indicates that sex ratio may not only be affected by the conditions at which they develop but also due to seasonal and/or interannual variation. Similar effect was observed in vegetative growth as, in cultures of *S. polyschides*, in 2009 gametophytes showed considerable vegetative growth, while in 2011 reduced vegetative growth and rapid fertilization were observed (Chapter 3). Differences in vegetative growth were also observed in the 2009 cultures between the two species, with *S. polyschides* forming filamentous female gametophytes while those of *L. ochroleuca* were unicellular. This bigger surface in *S. polyschides* might provide the newly formed sporophyte, attached to the female gametophyte, a higher adhesion capacity. Temperature has also been previously reported to affect vegetative growth of *L. ochroleuca* female gametophytes. However, this was not observed.

Overall, *S. polyschides* showed higher vegetative growth and quicker fertilization than *L. ochroleuca*. The strategy of this annual opportunistic species might provide it with an advantage when competing for space with the perennial *L. ochroleuca*. However, it should be noted that this study was done near the northern geographical limit of *L. ochroleuca*, so a maladaptation to local conditions need to be considered.

CHAPTER 5 – RESPONSE OF KELPS FROM DIFFERENT LATITUDES TO CONSECUTIVE HEAT SHOCK

Although extensive work has been done on the temperature limits of several kelp species, little is known about their response to the shorter shocks they are exposed to when inhabiting intertidal pools, where environmental conditions can change quickly.

When exposed to 4 cycle with 1 hour heat stress (at 22.5, 25, 27.5 and 30 °C) simulating a tidal cycles, both *L. ochroleuca* and *S. polyschides* recruits from Northern Portugal and Northwestern France showed decreased blade elongation with increasing temperature. At the higher temperatures, blade elongation was not able to compensate necrosis. Using maximum quantum yield to detect impaired reaction center function, as proxy of individual fitness coasts, both species showed complete resilience to 1 h shock at 22.5 and 25 °C. Exposure to 27.5 °C caused no damage in *S. polyschides* while exposure to 30 °C caused the death of all individuals, independent of site or origin. Contrastingly, different responses were observed between *L. ochroleuca* from near the southern and northern distribution limits. Recruits from Northwestern France were damaged but able to survive the 30 °C shocks, while individuals from Northern Portugal showed complete resilience to this treatment. This resilience of *L. ochroleuca* from the Portuguese coast may confer an advantage to colonize intertidal pools. This is in agreement with observations in the field, where both species are able to recruit in intertidal pools but only *L. ochroleuca* is able to reach adulthood, while the few *S. polyschides* that survive remain small and unable to reach maturity. These results indicate that with increasing summer temperature, as a result of climate change, it can be expected that *S. polyschides* disappears from intertidal pools and *L. ochroleuca* individuals become less dense.

CONCLUSION

As a shade adapted species, *L. hyperborea* survival in intertidal pools seems to depend on the shade provided by *L. ochroleuca* canopy. While in 2008 and 2009, *L. hyperborea* could be found in several intertidal pools, after the 2010 late winter storm this population was unable to recover, indicating that it was not self-sustainable and not enough microscopic forms dispersed from the subtidal to allow recruitment.

Intertidal populations of *L. ochroleuca*, despite suffering high mortality, are able to compensate losses, maintaining a positive population growth. Subtidal populations, on the other hand, are able to reach bigger dimensions inhibiting recruitment due to canopy overshadowing. This may cause a negative population growth without loss of function or recover ability. After major canopy loss, intertidal and subtidal populations may take longer than two years to recover. The intertidal population seems to have continued suffering high mortality, while in the subtidal area mortality increased due to lack of protection by surrounding canopy and, after one year, this population's persistence was most likely dependent on individual's survival till maturity. These hypotheses are supported by chapter 2. Individuals present 6 months after the storm suffered total mortality and one year after the storm no adults were present at the impacted site. Also, the single canopy removal and the storm impacted site indicate that populations take longer than two years to fully recover as well. As small canopy removals may be compensated by spore dispersal and canopy protection for the surroundings, extension of canopy loss may be more important than its recurrence.

The presence of other canopy forming species posed no threat to the recovery of *L. ochroleuca*. Local spore production, on the other hand, seems to be an important factor for population maintenance of both *L. ochroleuca* and *S. polyschides* (Chapter 1 and 2).

Whereas southern *S. polyschides* population such as those in the Northern Portuguese coast are highly seasonal and macroscopic sporophytes are present in the field during a limited period, central population are able to overwinter and recruit year-round. In seasonal populations, yearly climate variations have a great impact on life cycle, and population structure maintenance (Chapter 1). Although commonly accepted, this work showed that temperature is not the reason for the difference in life history across latitudes, as it poses no limitation for southern populations (Chapter 3). As seasonality of southern populations coincides with the typical upwelling events, this led to the novel hypothesis that differences in life history are caused by nutrient availability. As such, in southern areas this species is only observed in the field when upwelling events cause a raise in nutrient availability, while this is more stable in the central range of distribution, allowing sporophytes to overwinter and recruit year-round. Still, this hypothesis needs to be tested.

Although spore of both *S. polyschides* and *L. ochroleuca* are reported to be able to develop at temperatures up to 25 °C, cultures of spores from Northwestern France of either species suffered 100% mortality in these conditions. Data from cultures of *S. polyschides* microscopic stages from different years (2009 and 2011, chapter 4 and 3, respectively) showed that sex ratio may not only be affected by the conditions at which the spore develops but also by seasonal and interannual variations. In 2009, cultures of *S. polyschides* showed considerable vegetative growth (Chapter 4) while in 2011 vegetative growth was reduced, allowing rapid fertilization (Chapter 3). Differences in development were also observed between species, with *S. polyschides* suffering low mortality, higher vegetative growth and quicker fertilization than *L. ochroleuca* (Chapter 4). This strategy of the annual *S. polyschides* may constitute an advantage when competing for space.

While no difference in temperature resistance was shown for the microscopic stages of *L. ochroleuca* and *S. polyschides*, recruits showed differences in resistance to short heat shocks. Both species were not significantly affected by 1h exposures to 22.5 or 25 °C. Between 27.5 and 30 °C *S. polyschides* seems to reach a threshold. While not affected by 27.5 °C, exposure to higher temperature caused the death of all individuals, independent of site of origin. *L. ochroleuca*, on the other hand, was able to survive exposure to 30 °C. However, recruits from Northwestern France were significantly more damaged than individuals from Northern Portugal. These showed complete resilience to this short heat exposure, which may confer an advantage to colonize intertidal pools.

With global warming, both an increase in summer temperatures and frequency of highly destructive storm are expected. This may result in the disappearance of *S. polyschides* from intertidal pools, where their growth ability is already limited, being unable to reach maturity. It may also cause a decrease in *L. ochroleuca* populations' density in shallower areas and impair local spore production. As a consequence, at the low-latitude edge where this species form forests, it seems likely that distribution shifts would occur before geographical ones can be perceived, as might be happening for *L. hyperborea*.

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SUPPLEMENTARY MATERIAL

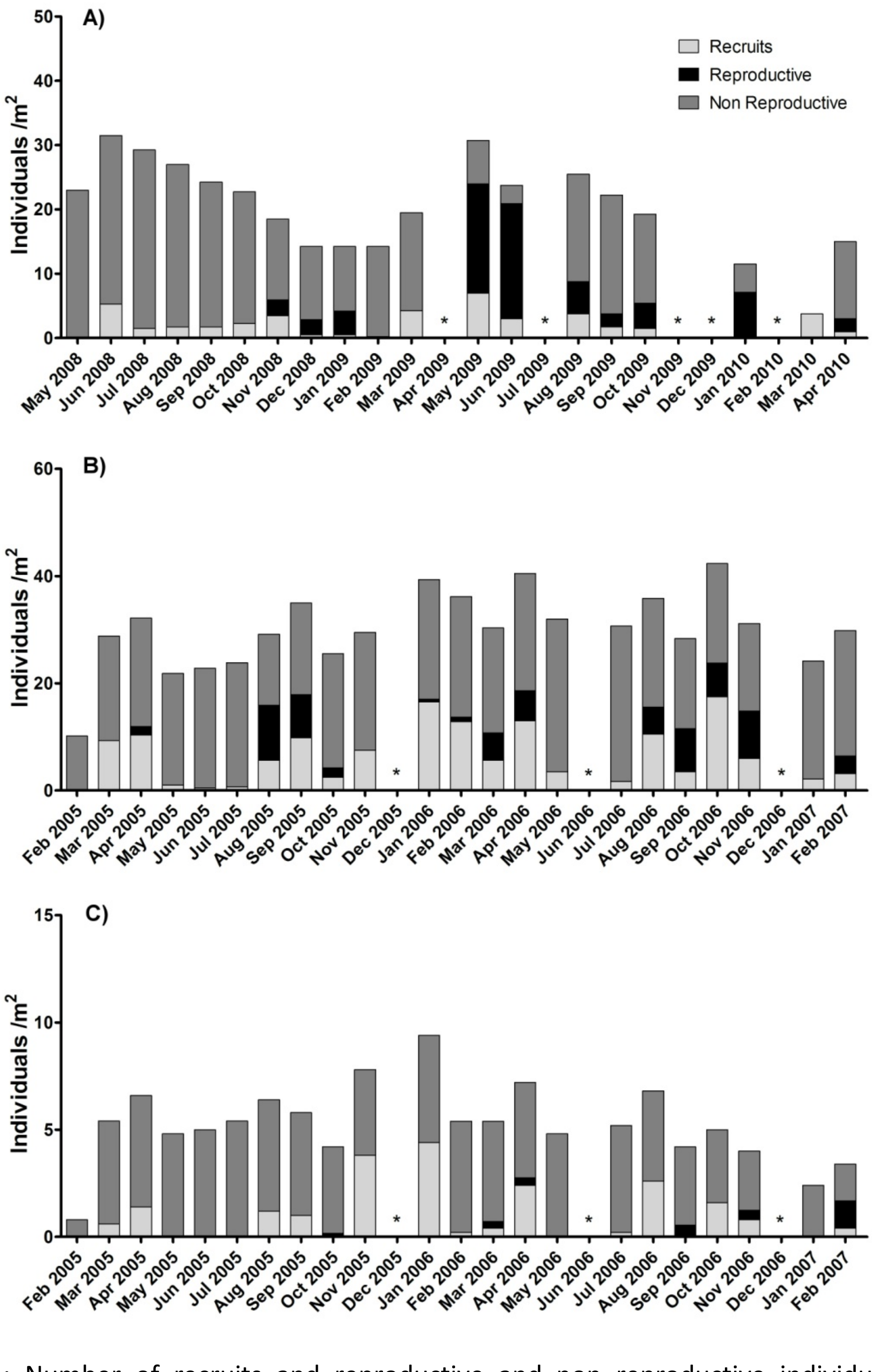

Fig. S1: Number of recruits and reproductive and non reproductive individuals per square meter for *L. ochroleuca* from North Portugal (A) and *L. digitata* (B) and *L. hyperborea* (C) from Brittany. * indicates a lack of data for that month, due to storms making field work impossible.

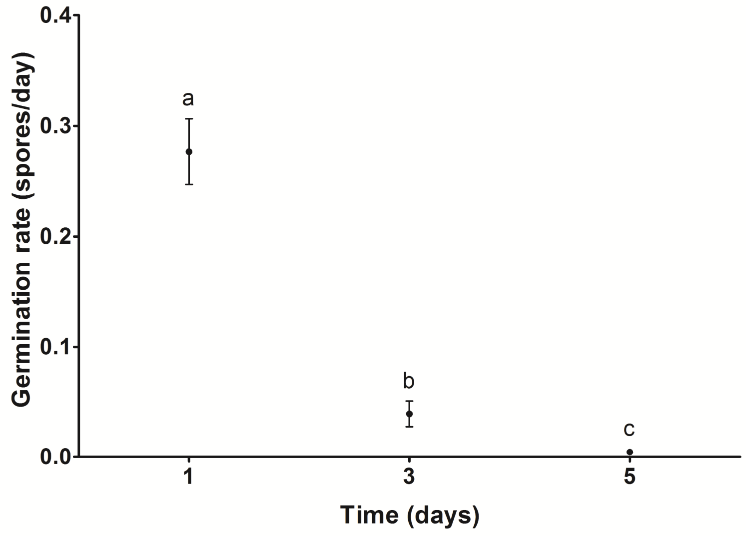

Fig. S2: Germination rate varied with time only (PERMANOVA, time, $p < 0.0001$; PERMDISP, $p < 0.0001$). Different letters were attributed to significantly different time points, based on a Tukey test.

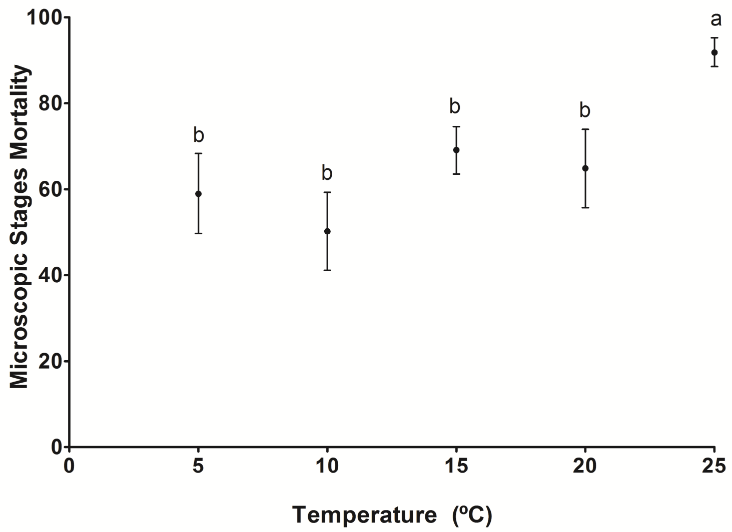

Fig. S3: Mortality of microscopic stages (spores, gametophytes and sporophytes smaller than 0.02mm) varied with temperature only (PERMANOVA, temperature, $p = 0.0111$, PERMDISP, $p = 0.098$). Different letters were attributed to significantly different points, based on a Tukey test.

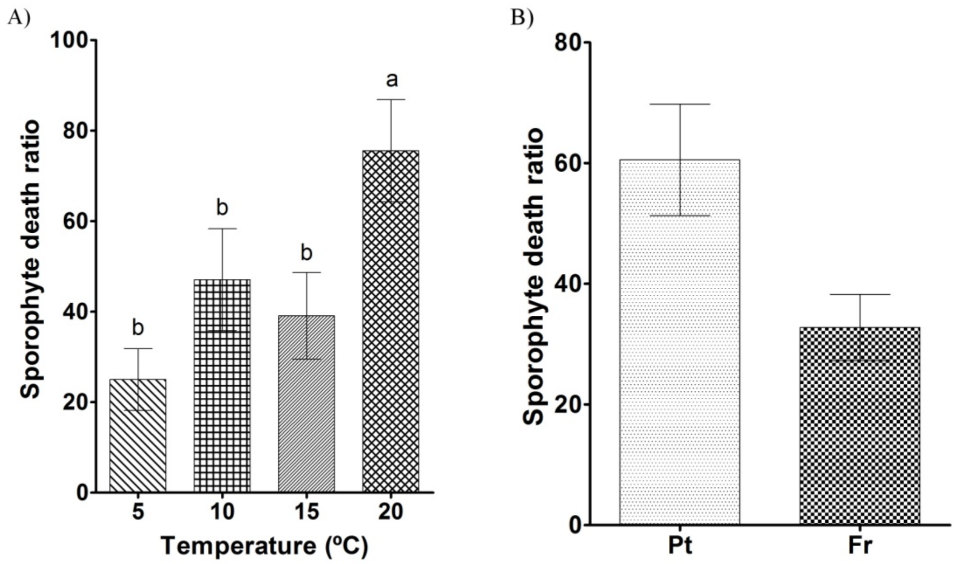

Fig. S4: A) Macroscopic sporophyte (longer than 0.02 mm) mortality varied with temperature (PERMANOVA, temperature, $p = 0.0022$; PERMDISP, $p = 0.428$) B) and between sites of origin (PERMANOVA, site, $p = 0.0016$; PERMDISP, $p = 0.0786$). Pt. Portugal; Fr, France. Based on a Tukey test results, different letters were attributed to significantly different points.

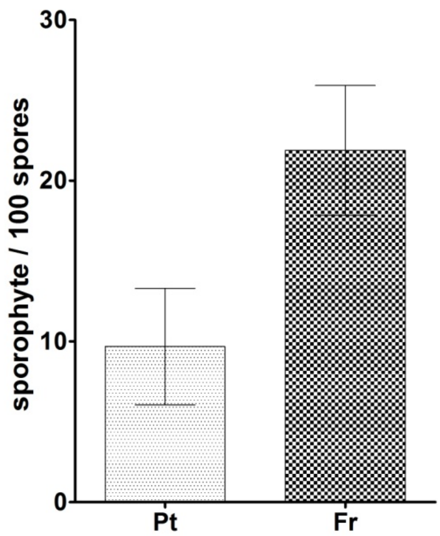

Fig. S5: Number of sporophytes obtained from a 100 initial zoospores varied between sites of origin only. (PERMANOVA, site, $p = 0.023$)

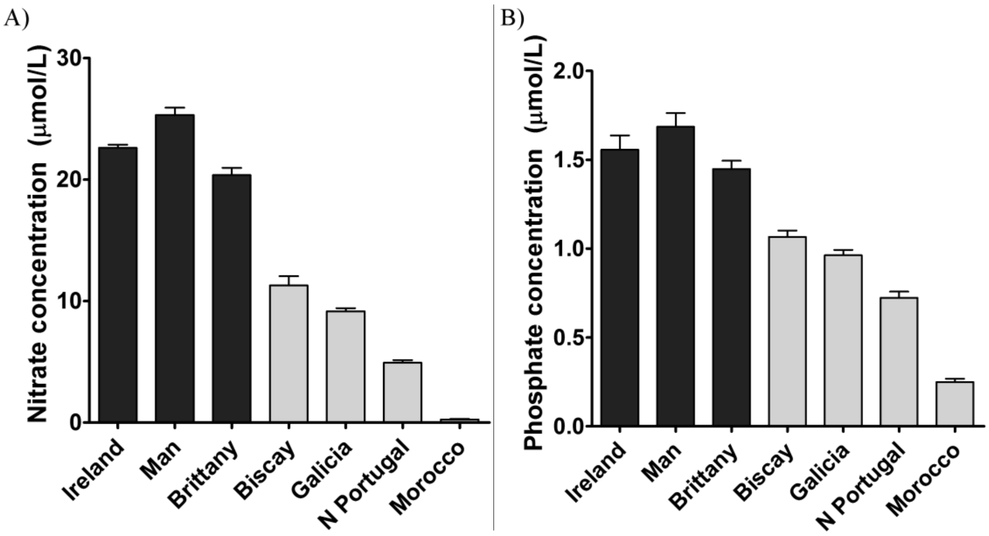

Fig. S6: A) Average yearly concentration of nitrates B) and phosphates, in μmol/L, for areas where the presence/absence of overwintering individuals of *S. polyschides* is described (NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their Web site at http://www.esrl.noaa.gov/psd/). Dark grey bars indicate the areas were individuals overwinter, while light grey bars indicate where populations are seasonal.