Developing kelp reforestation methods

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

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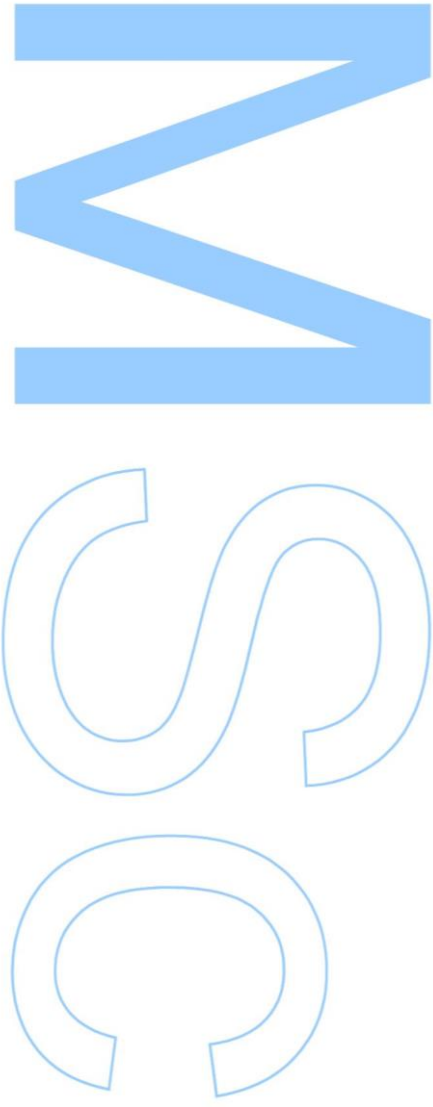

Sworn Statement

I, Tomás Fernandes Pinheiro, enrolled in the Master Degree Biodiversity, Genetics and Evolution at the Faculty of Sciences of the University of Porto hereby declare, in accordance with the provisions of paragraph a) of Article 14 of the Code of Ethical Conduct of the University of Porto, that the content of this dissertation reflects perspectives, research work and my own interpretations at the time of its submission.

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Tomás Fernandes Pinheiro

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RESUMO

Durante as últimas décadas, o declínio das florestas de algas tem sido consistentemente reportado ao longo da costa europeia. Isso resulta em decréscimos importantes na biodiversidade e nos serviços ecossistémicos. Atualmente, as ações de conservação, embora importantes, são insuficientes para manter a saúde dos nossos oceanos, e precisam ser complementados por esforços de restauração. Para isso, métodos eficientes precisam de ser desenvolvidos. Este é o principal objetivo deste trabalho.

Neste sentido, foram realizadas duas experiências laboratoriais e duas experiências de campo, utilizando abordagens diferentes, transplantação de algas e técnicas de semeio de algas. As experiências de laboratório foram estabelecidas para testar o melhor substrato de pedras (granito, calcário, quartzo ou xisto) para a técnica "green gravel" e substrato de corda biodegradável (algodão, linho e cânhamo). A primeira experiência de campo foi estabelecida para determinar qual a fase do ciclo de vida de *Laminaria ochroleuca* em que os esforços seriam mais eficazes e como a ausência de floresta de algas poderá afetar esses esforços. O segundo, baseado nos resultados do primeiro, teve como objetivo testar a capacidade de recrutamento da fase de vida microscópica em áreas protegidas (com floresta de algas) e sem proteção e validar métodos de reflorestação em áreas afetadas por elevada hidrodinâmica.

Em laboratório, ao testar diferentes substratos de pedras, o quartzo demonstrou, em geral, os resultados mais promissores. Em relação ao substrato de corda, os resultados demonstraram que o algodão, o linho e o cânhamo não foram adequados para o crescimento desta alga, pois não foi observado nenhum recrutamento. Substratos de corda alternativos sem tratamentos químicos devem ser explorados como alternativas de menor impacto, especialmente porque seriam biodegradáveis e não contribuiriam para a poluição por microplásticos.

Observou-se que o principal obstáculo aos esforços de reflorestação é a competição com espécies oportunistas, de rápido crescimento, seguido pela elevada hidrodinâmica e sedimentação que precisam ser consideradas na escolha do método de reflorestação.

Este estudo forneceu informações úteis essenciais para desenvolver esforços eficientes de reflorestação que podem ser aplicados para outras espécies de algas perenes e em outras regiões.

PALAVRAS-CHAVE

Laminaria ochroleuca; conservação; transplantação; semeio

ABSTRACT

During the past decades, the decline of kelp forests has been consistently reported along the European coastline. This results in important losses in biodiversity and ecosystem services. Currently, conservation actions, although important, are insufficient to maintain our ocean's health, and need to be complemented by restorative efforts. To achieve this, efficient methods need to be developed. That is the main purpose of this work. With this in mind, two laboratory and two field experiments were conducted, using different approaches, kelp transplantation and kelp sowing techniques. The laboratory experiments were set up to find the best gravel substrata (granite, limestone, quartz, or schist) for the "green gravel" technique and natural rope substrata (cotton, linen, and hemp). The first field experiment was set up to determine at which phase of *Laminaria ochroleuca* life cycle reforesting would be more efficient and how the absence of canopy affected these efforts. The second one, based on results from the first, aimed to test the ability of microscopic life stage to recruit in protected (with canopy) and exposed areas and to validate methods of reforestation in areas affected by strong hydrodynamics.

In the laboratory, testing different natural (gravel) substrate, quartz demonstrated, overall, the most promising results. Regarding the rope substrata, results demonstrated that cotton, linen, and hemp were not adequate for sporophytes to recruitment, as no kelps were observed growing on the ropes. Alternative rope materials without chemical treatments should be explored as lower impact alternatives particularly as these would biodegrade and not contribute to microplastic pollution.

We observed that the main obstacle to reforestation efforts is the competition by fast growing, opportunistic turf species, followed by the strong hydrodynamics and sedimentation which need to be considered when choosing the reforestation method.

This study provided useful information that is essential to develop efficient reforestation efforts that can be used for other perennial kelps species and along other coastal regions.

KEYWORDS

Laminaria ochroleuca; conservation; transplant; sowing

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INTRODUCTION

BROWN ALGAE AND KELP

The brown algae, comprising the class Phaeophyceae include 4 subclasses, 18 orders, 54 families, with approximately 308 genera (Silberfeld et al., 2014). Brown algae are the dominant seaweed species along the European coastline, mainly belonging to the Laminariales and Tilopteridales, being distributed in shallow subtidal (<30m depth) rocky habitats, depending on water conditions (e.g., clarity).

Kelps are large brown seaweeds comprising the order Laminariales. There are currently 8 accepted families of Laminariales, represented by 29 genera and 392 species. Even though some authors consider kelps as restricted to this order, some species belonging to the orders Tilopteridales (such as *Saccorhiza polyschides*) and Fucales (e.g., *Ascophyllum nodosum*) are frequently referred to as kelp due to their ecological role as forest forming species. This diverse group of seaweeds, differ remarkably in size, morphology, longevity, and habitat (Kain, 1979; Dayton, 1985; Estes and Steinberg, 1988). Regarding canopy morphologies, they can be floating (blades at or near the surface, e.g., *Macrocystis* and *Nereocystis*); prostrate (fronds lie on or immediately above the substratum, e.g., some *Agarum* and *Laminaria* spp.), and stipitate (erect understory where blade is supported by a stipe beyond the understory, e.g., *Ecklonia* and *Lessonia*) (Dayton, 1985).

Kelp species can be categorized as seasonal (e.g., *Nereocystis luetkeana*, with life expectancy generally restricted to one-year) or pluriannual/ perennial (e.g., *L. ochroleuca*, with a maximum life expectancy of about 25 years). When it comes to life cycle, kelp are considered a heteromorphic diplohaplontic that fluctuates between a macroscopic, diploid sporophyte stage and a microscopic, haploid gametophyte stage (Dayton, 1985). Sporophytes produce flagellated zoospores, in specialized reproductive structures (sporophylls) or in the blade, which settle and develop into either male or female haploid gametophytes, producing sperm cells and egg cell, respectively. Fertilization results in zygotes formation that develops into a juvenile sporophyte, completing the cycle (Pereira et al., 2011).

Kelp forests are distributed along temperate rocky coastlines worldwide (Steneck & Johnson, 2014) (Figure 1). Kelp with floating canopy are dominant in western North and South of America and Africa, and parts of Tasmania. On the other hand, kelp and fucoid species with smaller prostate, stipitate or buoyant flexible canopy, are present throughout Asia, most of Australasia, the South and North Atlantic, the Arctic, and the Mediterranean Sea coastlines.

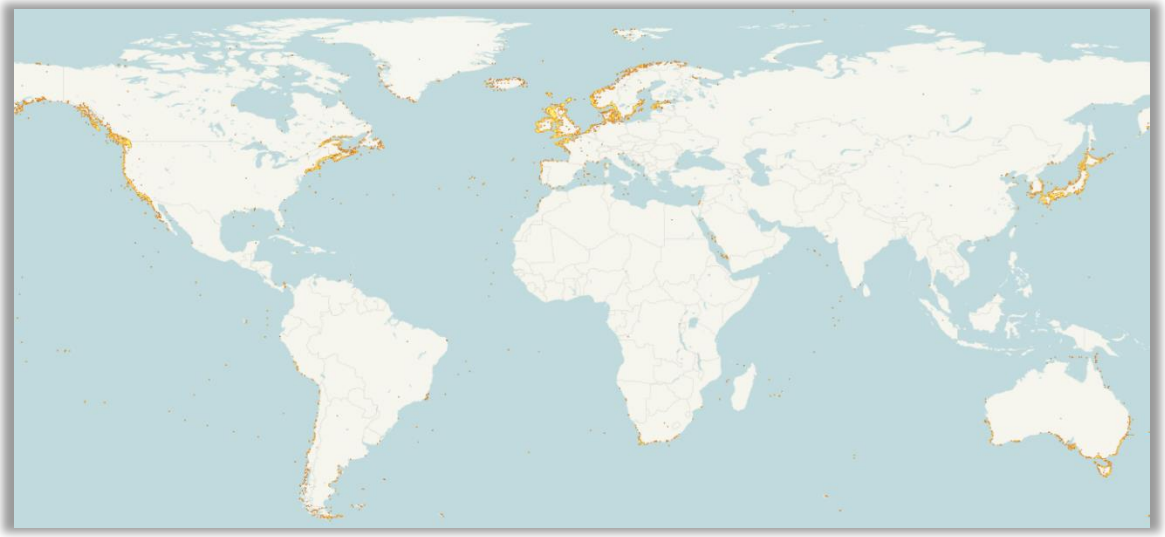

Figure 1- Kelp forests distribution worldwide (Source: GBIF).

ECOLOGICAL RELEVANCE

Kelp provides a wide variety of ecosystem goods and services, not only social, but also economic and ecological. These may be direct (e.g., kelp harvesting, commercial, subsistence, recreational fishing, and ecotourism), and indirect (e.g., coastal protection, carbon storage and sequestration, eutrophication mitigation, nutrient cycling), or non-use value (i.e., economic values assigned that are unrelated to current or future uses) of the kelp forest itself (e.g., biodiversity and scientific research or cultural importance).

BIODIVERSITY AND HABITAT

The three-dimensional structure of kelp forests provides complex biogenic habitat, providing spawning grounds, nursery, and food to countless species (Bertocci et al., 2015; Graham, 2004; Teagle et al., 2017). Kelp are habitat-forming species, also known as habitat engineers (sensu Jones et al., 1994), as they create complex habitats, having the ability to modify the local environmental conditions, facilitating, and supporting the establishment of associated local communities (Christie et al., 2009; Teagle et al., 2017; Thomsen et al., 2010). Their canopy influence light availability (Wernberg et al., 2005), sedimentation (Wernberg et al., 2005), physical abrasion (Irving & Connell, 2006), water flow (Stewart et al., 2009), substrate availability and architecture (Christie et al., 2007),

nutrient availability (Krumhansl & Scheibling, 2012), water quality (decreasing eutrophication and acidification; Jiang et al., 2020) and help prevent microalgae booms in their surrounding ecosystem.

As habitat formers, a single individual can support directly rich and diverse assemblages of algae and invertebrates. In addition, epiphytes (primarily attached to the stipe) can grow on some kelps species (e.g., *Laminaria hyperborea*), providing a secondary habitat which may well be utilized by invertebrate assemblage. Christie et al. (2003) showed that in Norway, *L. hyperborea* harboured significant biodiversity, approximately 130 species represented by almost 8000 individuals per kelp. The holdfast supports high diversity, with species richness per holdfast typically achieving 30–70 macrofaunal species (Moore, 1973; Thiel & Vásquez, 2000). The abundance of invertebrates per holdfast can almost reach 10 000 individuals (Christie et al., 2003). However, values for the richness and abundance diverge significantly between species and regions, being affected by the size and complexity of the holdfast habitat (Blight & Thompson, 2008), as well as by regional factors, such as turbidity or exposure.

PRIMARY PRODUCTION AND FOOD WEBS

Kelp are important primary producers, and their productivity is affected by many factors such as hydrodynamics (Pedersen et al., 2012), nutrient availability (Gagne et al., 1982), light and temperature (Bearham et al., 2013). Kelp detritus is generated primarily through either the dislodgement of whole individuals, or fragmentation of parts of the blade through erosion (Krumhansl & Scheibling, 2011), but this proportion varies with kelp species, morphology, age, and with local conditions, such as hydrodynamics (e.g., Bettignies et al., 2013; Krumhansl & Scheibling, 2011). This detritus is a key vector of trophic connectivity in numerous coastal ecosystems, which can improve species diversity, productivity, and reproductive yield (Krumhansl & Scheibling, 2012). Additionally, they can be a crucial source of food in kelp beds and/or forests, in sandy bottoms, beaches and in deep areas, where productivity is low (Britton-Simmons et al., 2012; Duggins et al., 1989; Filbee-Dexter & Scheibling, 2014; Vetter, 1995).

COASTAL DEFENCE

Kelp forests can help prevent coastal erosion and the movement of sediments, such as sand and pebbles, from adjacent coastlines (Løvås & Tørum, 2001; Mork, 1996). As the impacts of climate change are intensifying (e.g., sea-level rise), coastal defence will become more important along many coastlines. Kelp morphological characteristics (such as height, or individual strength) strongly influence the magnitude of wave currents and surface waves and, as such, will differ between biogeographical coastlines.

GOODS

Kelp forests are crucially important not only to marine life but also to humans. Indeed, a theory was proposed, the "Kelp Highway Hypothesis", regarding the colonization of the American continents. This hypothesis proposes that the first Americans reached the New World by sea, following the coastline along Beringia and into the American continents, suggesting that kelp forests may have offered secure anchorages for ships, reducing wave energy and providing holdfasts for boats, and assisted with navigation (Erlandson et al., 2007).

The use of kelp and other macroalgae to feed domestic animals, such as cattle, horses, and sheep, may have occurred in the 6th or even the 5th century AD (Balasse et al., 2005). There are records of the use of the kelps *Chorda filum*, *Laminaria digitata* and *Alaria esculenta,* as winter fodder (Chapman, 1970). Nowadays, kelp is consumed by humans in the most varied ways. In Asian gastronomy, *Eisenia bicyclis* (known commercially as "Arame"), *Palmaria palmata* ("Dulse"), *Porphyra umbilicalis* ("Nori"), *Laminaria sp.* ("Kombu") and *Undaria pinnatifida* ("Wakame") are important ingredients. In English, Irish, and Scottish gastronomy, *P. palmata* is also used is various forms (e.g., raw, boiled, toasted). In the Azores Islands, *P. umbilicalis* (Atlantic Nori or Purple Laver) is used, fried as an ingredient in omelettes or pies ("Tortas de Erva do Calhau").

In Portugal, the collection and use of seaweeds were firstly described in the 14th century. This collection was mainly done by hand-picked the seaweeds that stranded on the beach and used in the fields as fertilizers. This was an important economic and social activity until the 20th century (Cole, 1991), mainly in the Northern region. The "sargaço", collected between the Minho and Douro rivers, and "moliço", collected in the Aveiro region, are the main mixtures of algae traditionally used as fertilizer. "Sargaço" (also called "argaço") is a mixture of mainly brown (*S. polychides*, *Laminaria*, *Fucus*), but also red (*P. palmata* and *Chondrus crispus*), and green algae (*Codium spp.*). "Moliço", on the

other hand, is a combination of algae (such as *Gracilaria*, *Rhizoclonium*, and *Ulva*) and marine plants (*Potamogeton*, *Ruppia* and *Zostera*), collected in the Ria de Aveiro (Gaspar et al., 2019). This activity, although not common, can still be found in Viana do Castelo and Póvoa do Varzim regions.

The phycocolloid industry in Portugal, began during World War II, when there was a lack of agar from Asia (used mainly in laboratories and in jelly deserts), allowing the development of the Agar Portuguese industry (Palminha, 1971). The first agar factory in Portugal was constructed in 1947, and 24 years later the number of factories had increased to six, including two in Azores (Santos & Duarte, 1996). However, the impact of harvest being too great for population to recover, led to the loss of this industry. Nowadays, there is one company remaining: Iberagar, S.A..

SOCIOECONOMIC IMPORTANCE

Kelp forests can generate direct (e.g., commercial) or indirect (e.g., research and education) economic benefit (Blamey & Bolton, 2017). The economic value of ecosystem services is very difficult to establish, and those provided by kelp forests are no exception. However, estimates value kelp forests around €400,000–900,000 per year per kilometre of coastline. These values are most likely considerably under-rated (the true value might be three to six times higher) since they are almost exclusively based on direct-use services (e.g., Vásquez et al., 2013) barely including indirect and non-use services (Filbee-Dexter & Wernberg, 2018).

Kelp beds in Northern Chile cover 666 km of coastline, supporting biodiversity and generating and estimated value of US$540 million annually (≈ €667,000 km^{-1} $year^{-1}$) in kelp harvesting, associated-species fisheries and in scientific, biological and climate value (Vásquez et al., 2013). In the Indian and Antarctic Oceans, kelp forests cover ≈8000 km of coastline, from New South Wales, down the east coast of mainland Australia, around Tasmania, along Australia's southern coastline to Kalbarri (Western Australia), supporting biodiversity and generating an estimated value of AU$10 billion annually (≈ €803,000 km^{-1} $year^{-1}$) in fishing (commercial and recreational) and tourism (Bennett et al., 2016). In South Africa, the nearshore subtidal zone is dominated by kelp forests and associated temperate reefs, with kelp forests covering approximately 1000 km of the coastline, and with an estimated value of R 5.2 billion annually (≈ €420,000 km^{-1} $year^{-1}$) in commercial, recreational, and illegal fishing, ecotourism, and nutrient cycling and carbon sequestration (Blamey & Bolton, 2017).

THREATS AND CURRENT STATUS

OCEAN WARMING/ HEAT WAVES

In the past half-century, the quantity and severity of kelp forest threats have intensified, with an approximate decline of 40–60% of the world's kelp forests (e.g., Krumhansl & Scheibling, 2012; Teagle et al., 2017; Wernberg et al., 2019). Temperature rise is perhaps the most important change happening in marine ecosystems, as it affects fundamental processes of plant physiology, and ecology across all biological levels, from genes to ecosystems (Smale et al., 2011). This stress can lead to modify enzyme mediated processes, membrane composition, and photosynthetic and growth rate, depending on the period and intensity of exposure and its interaction with other environmental factors (e.g., light, nutrients, UVB radiation), and result in photoinhibition, oxygen demand and alterations in pigment composition (Andersen et al., 2013; Los & Murata, 2004; Mabin et al., 2019; Machalek et al., 1996; Xiao et al., 2015). Kelp are expected to become more susceptible to diseases as they are exposed to physiological stress, triggering wide-spread mortality or have sub-lethal effects (e.g., decreased growth and fecundity), and may well stimulate changes in community structure and facilitate the propagation of non-native species (Gachon et al., 2010; Wahl et al., 2015).

Climate change is driving global species redistribution, as marine species inhabit well-defined thermal niches (Pinsky et al., 2019; Pörtner & Farrell, 2008; Sunday et al., 2012). Nevertheless, climate change is also associated with extreme events, such as severe storms, which increase rupture or cause dislodgment of entire kelp plants or strands (Bettignies et al., 2013; Filbee-Dexter & Scheibling, 2012).

The North-East Atlantic represents a hotspot of warming, as since the mid-20th century the sea surface temperature has increased at levels of ≈0.3–0.8°C per decade (Hughes et al., 2010; Lima & Wethey, 2012; Smale et al., 2013). In the southwest corner of Europe, the Iberian Peninsula, is among the regions most affected by climate change, where many species persist at their thermal maxima, and the numbers of reports of kelp forest decline or loss are increasing (Belkin, 2009; Smale, 2019). In Portugal and Spain, *L. hyperborea*, *L. ochroleuca* and *S. latissima* have exhibit abundance declines, local extinctions, and range contractions (Casado-Amezúa et al., 2019; Piñeiro-Corbeira et al., 2016; Voerman et al., 2013). In the Northwest Atlantic Ocean, in Nova Scotia, kelp forest has been severely affected by warming trends, as 85–99% of kelp biomass (mainly *L. digitata* and *S. latissima*) has been lost over the past 4–6 decades (Filbee-Dexter et al., 2016).

Kelp forests in some regions have suffered discrete warming events without high damage, mainly in centre and colder limits of species' distributions (e.g., *Macrocystis pyrifera* in Washington State; *Nereocystis luetkeana* in British Columbia) (Pfister et al., 2018; Starko et al., 2019). In contrast, the most of the affected kelp forests have been detected in warming hotspots or close to the borders of their distribution (e.g., *Ecklonia radiata* in Australia) (Carnell & Keough, 2019; Wernberg et al., 2016b) (Figure 2).

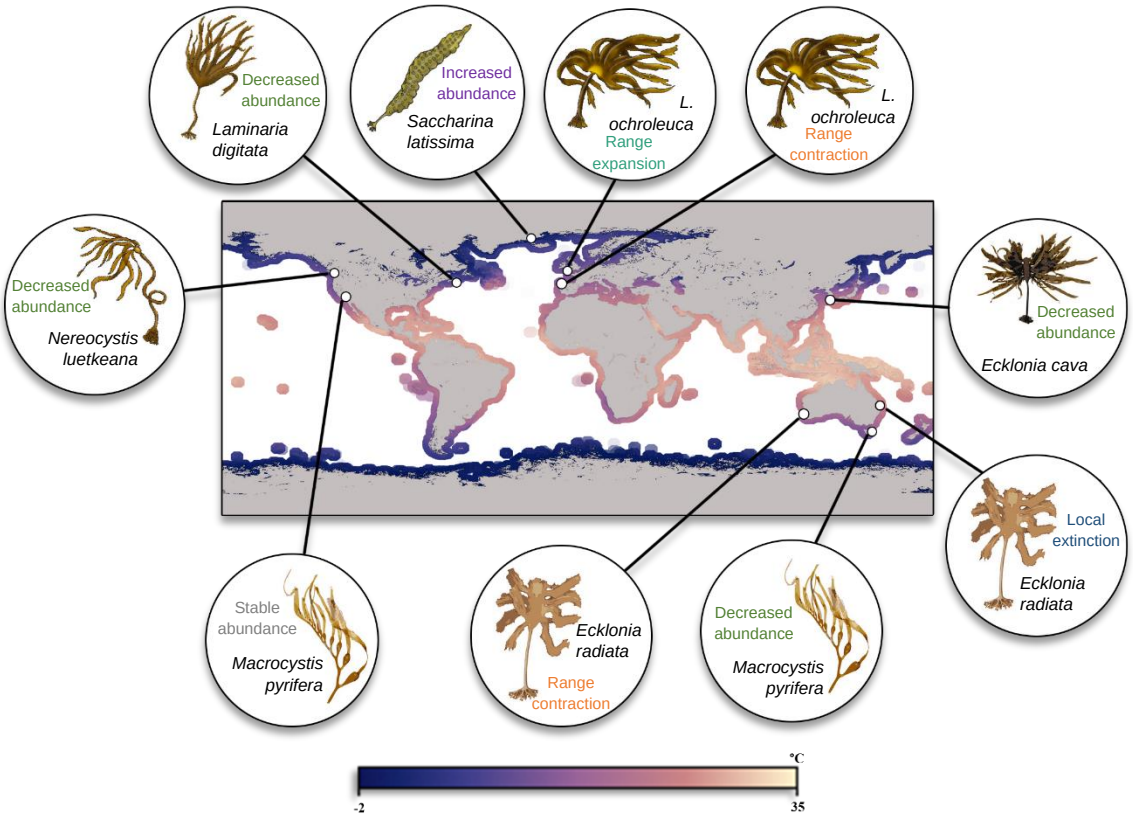

Figure 2- Selected recent examples of responses of kelp populations to ocean warming (Adapted from Smale, 2019). Examples chosen to represent a range of species, responses, and regions, not a comprehensive analysis of recent responses. Underlying global map shows rates of sea surface in coastal waters in November 2020 (≈ 500 km from land) from NASA Earth Observations (NEO) team in collaboration with Gene Feldman and Norman Kuring, NASA OceanColor Group. Coolest waters appear in blue (approximately -2 degrees Celsius), and the warmest temperatures appear in pink-yellow (35 degrees Celsius).

NON-CLIMATIC DRIVERS

Overgrazing of kelp forests by invertebrate herbivores, particularly sea urchins, can contribute to significant loss of biogenic habitat, escalating fragmentation and breakage, or by reducing the amount of kelp sporophytes through the targeted consumption of reproductive tissue, devastating kelp forests and, in extreme circumstances cause phase shifts from structurally and biologically complex habitats to depauperate “barrens” (e.g., Norderhaug & Christie, 2009; o Brien & Scheibling, 2016). Therefore, sea urchins can influence the distribution of kelp globally (Filbee-Dexter & Scheibling, 2014; Steneck et al., 2002).

The shift from kelp forests to turfs has been increasingly documented along many temperate coastal regions worldwide, in South Australia (Connell et al., 2008), in the Baltic Sea and Skagerak (e.g., Middelboe & Sand-Jensen, 2000), along Atlantic Canada coastline (Filbee-Dexter et al., 2016), Europe (e.g., Moy & Christie, 2012), and Australia (Wernberg et al., 2016a). The impacts of harvesting on kelp can be more acute during seasonal periods of low growth, or if there is stress by high water temperatures (Gao et al., 2013; Krumhansl et al., 2017). Inadequate management of kelp harvest can lead to kelp losses, but harvest can be sustainable when properly structured (Vásquez, 2007). Pollution, on the other hand, can lead to eutrophication and to an increase in the amount of sediment. Eutrophication can and the presence of sediment can reduce light penetration in water columns and bury kelp microscopic stages, preventing their recruitment (Airoldi, 2003; Gorman & Connell, 2009; Gorman et al., 2009).

CURRENT STATUS

There are very few brown algae species classified in The International Union for Conservation of Nature’s (IUCN) Red List of Threatened Species compared to other marine species, which is a hold-back in conservation programs as red lists have been developed as a set up for conservation managers to prioritize actions to protect species or habitats (IUCN, 2021).

In Europe, experts reported the existence of some conservation programs for kelp forests. In France, these conservation programs involved the establishment of a Marine Protected Area, ‘‘Parc Naturel Marin d’Iroise’. Conservation efforts can also be observed in the Azores, Portugal, and in Germany, the United Kingdom, and Norway. However, in Continental Portugal there were no conservation programmes reported for kelp forests (Araújo et al., 2016).

LAMINARIA OCHROLEUCA

ENVIRONMENTAL CONDITIONS

Kelp are regulated by environmental conditions, which determines the extent to which individuals can subsist in a specific area. Macroalgae growth is dependent of essential drivers, such as, light, nutrient, salinity, and temperature (Kain, 1971; Bolton and Luning, 1982; Graham et al., 2016). However, it is also well-documented that water motion is a fundamental parameter that affects its morphology and physiological processes (Hurd, 2017; Hurd et al., 2014; Kregting et al., 2008).

Light properties (e.g., light quantity, photoperiod, and seasonal fluctuations) play an essential role in kelp growth, depth distribution and biomass accumulation. Macroalgae have evolved and are proficient to acclimate and adapt to a high variance of available light. For example, responses can happen as regulation (e.g., dynamic photoinhibition), acclimation (e.g., variation in the concentration of photosynthetic pigments) and adaptation (modifying thallus morphology, increasing surface area to volume ratios) (Ramus et al. 1976; Falkowski and LaRoche 1991; Henley and Dunton 1995; Kirk 2011).

Nutrient concentration can impact metabolic processes such as photosynthesis, influencing productivity and growth of seaweeds (Graham et al., 2007; Johnson et al., 2011). Phosphorus (P) and nitrogen (N) have been considered as a crucial regulating nutrient for macroalgae in several environments (Howarth, 1988; Jackson, 1977; Lapointe et al., 1992). Some perennial Laminariales can have a competitive advantage over ephemeral algal species when the availability of nutrients is high (often in winter), as they are able to store excess nutrient and then subsist over periods of low nutrient availability (i.e., summer) by using these internal nitrogen reserves (Chapman & Craigie, 1977; Chapman & Lindley, 1980; Henley & Dunton, 1997; Mizuta et al., 1992).

Moderate wave exposure may affect positively photosynthesis and growth, through a reduction of the stagnant boundary layer around the blades, increasing the uptake of nutrients and inorganic carbon, and by oscillating kelp so individuals can access light that otherwise would be in shadows (Hurd, 2000; Pedersen et al., 2012). In high flow areas, kelp usually have thicker blades and stipes, are more strap-like or streamlined, less crenulated or undulate, and are more strongly attached to substratum than kelp in slower environments (Gerard & Mann, 1979; Koehl et al., 2008; Miller et al., 2011). However, high wave exposure can have unfavourable effect as may resuspend sediments, increasing turbidity and consequently decrease light availability, damage kelp

blades, holdfasts and stipes, and may also remove individuals from the substrate, and separate them from the population (Pedersen et al., 2012).

Salinity is an important factor that influences the growth, distribution and reproduction of macroalgae (Reed et al., 1985). Kelp are considered stenohaline (i.e., have low tolerance to salinity changes), with a general tolerance to salinity is around 16 to 50 psu (practical salinity unit), and their optimal growth varies between 30 and 35 psu (Lüning et al., 1990). The typical Portuguese seawater is around 35 psu.

Rocky substrate is a requirement for the presence of kelp. Some kelp species appears to prefer more resistant rocks (such as basalt, gneiss, quartz, and schist) (Allorge & Manguin, 1941; Wehr & Stein, 1985). Kelp are evanescent when attached to unstable substrate. Substrate rugosity or surface roughness is an essential factor that boosts zoospore aggregation, allowing kelp population to persevere (Muth, 2012).

As said before, temperature is one of the main determining factors. *L. ochroleuca* optimum temperature for growth was described to be between 10–15 ºC, for spore development at 12–18°C (Izquierdo et al., 2001), their gametophyte development can happen in low temperatures (5°C) (Lüning et al., 1990), and its ecological niche matches thermal physiological responses (10–24°C), which is an important feature to notice climate-driven range shifts (Assis, Tyberghein, et al., 2018; Barbosa et al., 2017; Pereira et al., 2011).

Morphology, Growth, Life Cycle and Seasonality

Kelp sporophytes are composed of a blade, a stipe, and a holdfast. The holdfast is a root-like structure, composed by hapterae, that anchors kelp to the substrate and is the most complex microhabitat offered by kelp (Arnold et al., 2015). A stipe is a strong, yet flexible stalk-like structure, allowing kelp to sway in the currents. The blade is responsible for offering a large surface area for photosynthesis. Between the perennial stipe and the blade, there is the meristematic region, which ensures constant growth, hopefully faster than degradation, and growth extension responsible for thallus elongation. Reproductive tissues, known as sori, are usually found in blade's distal part.

Laminaria species are classified as season anticipators, growing and reproducing in response to environmental triggers (e.g., daylength) instead of responding to favourable conditions (Lüning & tom Dieck, 1989). As season anticipators, kelp grow quicker during winter, when light availability and temperature are lower but nutrient availability is higher,

as some kelp have the capacity to accumulate nitrogen and phosphate, starting to decline when the reserves are depleted (Broch & Slagstad, 2011; Gagne et al., 1982; Nielsen et al., 2014). When summer arrives, the growth is slowed as energy is allocated to sporogenesis (Luning, 1979; Nielsen et al., 2014). Their growth seems to be divided into periods of rapid and slow vegetative growth, that form rings or lines on the stipes, and can be used to determine their age.

Seasonality and biological processes (such as growth and reproduction) are expected to be less influenced by photoperiod, in lower latitudes, and become more affected by the upwelling season and the nutrient availability oscillations connected with it (Franco et al., 2018; Kain, 1989; Pereira et al., 2017).

Dispersal and Recruitment

Dispersal and recruitment are essential to maintain the resilience and guaranteeing the stability of habitat-forming species and for population recovery subsequent instabilities in sessile species, as these processes ensure population connectivity, gene flow and the establishment in new habitats (e.g., Coulson et al., 2001; Lloret et al., 2012; Wernberg et al., 2010).

L. ochroleuca dispersal capability can reach tens of kilometres, as reproductive blades can remain viable for a long period of time, more than previously expected (≈1 day). Still, long effective dispersal (i.e., resulting in settlement and recruitment) is not common in this species (Assis, Serrão, et al., 2018). In catastrophic events this species has an advantage compared to other Laminarian species, as the constant spore production allows a complete recovery without depending on spore dispersal from adjacent areas (Pereira et al., 2017).

Two factors typically influence plant and macroalgal recruitment – propagule/seed and substrate availability (Grubb, 1977; Harper et al., 1961; Harper et al., 1965). Studies suggests that microscopic propagules have lower necessities for survival than other life stages and the capacity of remain dormant until environmental conditions are adequate for recruitment. Another important factor that influences macroalgae recruitment is competition for space and/or light. Competition has been noticed even at microscopic life stages and at larger life stages, influencing macroalgal community structure (Reed, 1990).

DISTRIBUTION

L. ochroleuca can be found in Portugal (continental coast and in the Azores), in the Northwest coasts of Spain, in Morocco and the Strait of Messina, in Brittany (France), the English and Bristol Channels, and more recently, in Ireland (Béal an Mhuirthead) (Figure 3). In *L. ochroleuca*, ocean warming has been associated with range expansions at the poleward edge, and studies shows that in the future may replace other *Laminaria* species (Smale et al., 2015). It usually inhabits deep intertidal pools and the subtidal, from 0–30m deep. Along the Portuguese coast, their depth range distribution was from 3 m depth to 23 m (Assis et al., 2009).

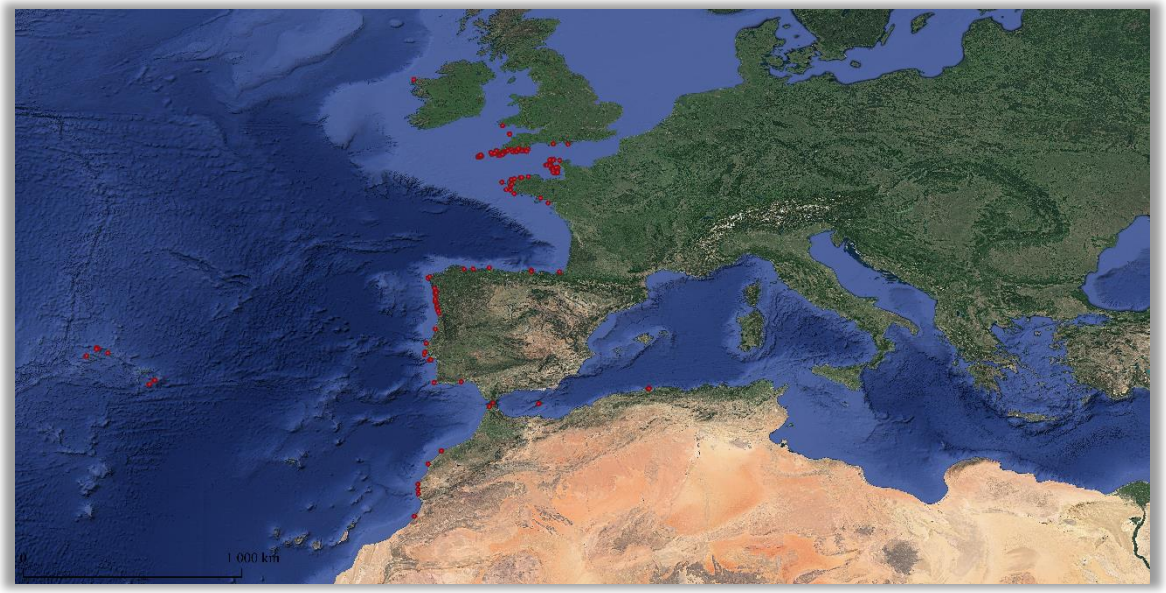

Figure 3- Records of *L. ochroleuca* (red dots) along the European coast (Source: GBIF).

OBJECTIVES

The main purpose of this work was to develop efficient reforestation methods for the Iberian kelp *L. ochroleuca*. To achieve this, it was essential to:

Understand the importance of canopy protection in kelp recruitment, and how their absence can impact reforestation efforts.

Determine the ability of *L. ochroleuca* to attach to different natural (gravel) substrate, as to improve the “green gravel” method.

Test the efficacy of the “green gravel” method in areas affected by strong hydrodynamics.

Test the suitability of natural rope as a substrate for sporophytes do attach and grow.

METHODOLOGY

EXPERIMENT 1

The “green gavel” method consists of sowing small rocks with kelp and cultivate them in the laboratory until they reach 1 cm in length, before out-planting to the field. To improve the “green gravel” method, fertile tissues (sori) from 12 individuals of *L. ochroleuca* were collected in May 2021 (spring) from intertidal pools in Carreço (≈ 41°44'30.3"N 8°52'38.3"W). The fertile tissue was rinsed to rid of epiphytes and protozoa. They were then clean with paper towel, pat dry, and left in paper towel in a fridge for 30 minutes to cause a slight osmotic stress. Afterwards, the sori were put in a beaker with autoclaved seawater and left in a fridge for 1h. The resulting solution, with *L. ochroleuca* sporophytes, was sprayed directly onto small rocks (granite, limestone, quartz, and schist; Figure 4), as they are dominant in intertidal and shallow subtidal of the Portuguese coast. The day after sowing, the tanks were filled. The system included an UV sterilizer and a refrigerator (to maintain temperature at ≈ 15°C).

Figure 4- Indoor trial with various types of green gravel (granite, limestone, quartz, and schist).

The tanks were illuminated with OSRAM L 58 W /965 Biolux fluorescent lights, in constant light (100 µmol $m^{-2}s^{-1}$) with a 12:12h light-dark cycle (Pereira et al., 2019).

Water renewal and the monitoring of environmental conditions (dissolved oxygen, pH, salinity, and temperature) with Hach HQ40D Portable Multi Meter, were done weekly. Monitoring was done on a weekly basis. Ten stones were selected randomly from each tank (5 for each green gravel type) and observed under a Leica EZ4W Stereomicroscope (Figure 5).

Figure 5- Stones observation under a Leica EZ4W Stereomicroscope

From each sample, 15 pictures were captured and analysed with ImageJ (US National Institutes of Health, Bethesda, MD, USA), and used to calculate the kelp size and area.

DATA TREATMENT

Since the samples were chosen randomly and measured over time, growth was analysed with one-way ANOVA and compared between substrata.

The size of individuals of *L. ochroleuca* was analysed independently using 1-way ANOVA, considering Substrate and Time as factors, using IBM SPSS Statistic for Windows version 26.0 (Armonk, NY, USA). When results indicated a significant difference, Post Hoc Tests were performed for pairwise comparisons (with $p < 0.05$ considered as significant).

The density of grazers was determined by calculating the percentage of occupied space within the pools, using ImageJ (US National Institutes of Health, Bethesda, MD, USA).

Densities were estimated by counting the number of fields of view until at least 10 kelp individuals could be observed and dividing by total area.

$$D = \frac{n^{o}\ individuals\ (>10)}{total\ area}$$

EXPERIMENT 2

A field experiment in large lower intertidal pools was initiated applying kelp sowing technique, with mesh bags. The two chosen locations were in Carreço (C, 41°44'30.3"N 8°52'38.3"W) and Viana do Castelo (VC, 41°41'37.9"N 8°50'58.5"W) (Figure 6).

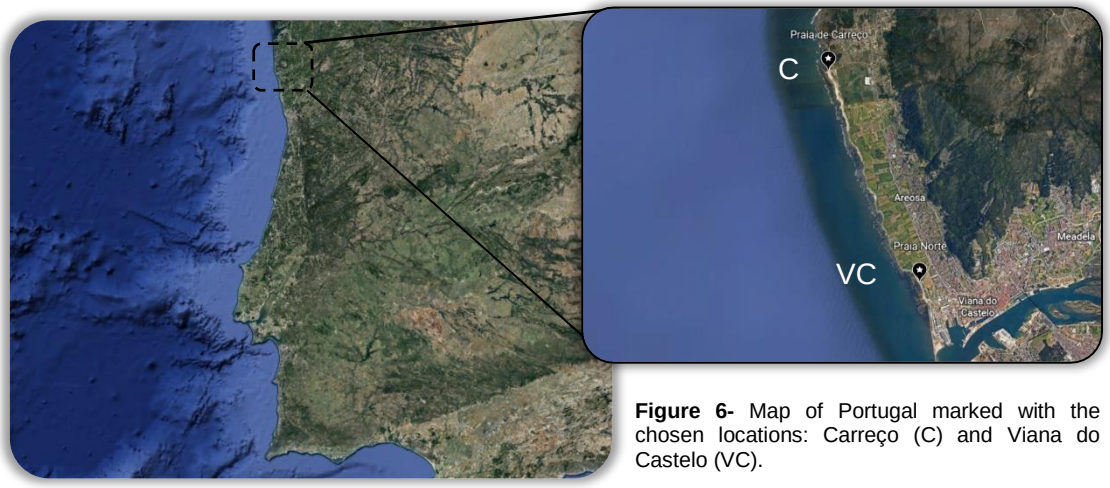

Figure 6- Map of Portugal marked with the chosen locations: Carreço (C) and Viana do Castelo (VC).

Surveys were conducted at two sites less than 7 km apart. Viana do Castelo, with kelp patch, was used as control for Carreço, without kelp in that pool, as protection by canopy is known to decrease mortality. The pools were chosen taken into consideration that had to be deep enough so *L. ochroleuca* was no exposed to air during low tide. This study was set up in March, when no reproductive individuals could be observed in the area, to ensure any recruitment would be the results of our input. Gravel, scrapped surface, and control were considered as a replicate unit (Figure 7).

Figure 7- Replicate unit considered in this field experiment (gravel, scrapped surface, and control).

At each site, six replicates in intertidal pools were assembled (Figure 8). The surveys were done by tagging two opposite corners of the area.

Figure 8- Study area in (A) Viana do Castelo; (B) control and (C) experiment. Arrows pointed to the chosen areas. Photographs by Silvia Chemello.

Reproductive tissue of 5 individuals, collected in Carreço (≈ 41°44'26.8"N 8°52'35.5"W), were put in each mesh bag and each mesh bag was fixed in the middle of a replicate (Figure 9). Near the marks, different natural substrate (gravel) was deployed, to unsure that any recruitments would originate from the material in the mesh bags, and to assess the viability of using the green gravel method, used in Experiment 1, in areas affected by strong hydrodynamics. Along with that, at each pool, turf and associated sediments were removed from a 20x30 cm frame using paint scrapers, to provide clean and available substrate, to test if it would favour the recovery of *L. ochroleuca* forests, and other area was used as control. Three days later, the mesh bags were removed to avoid getting them lost and adding plastic to the ocean. Monitoring was carried out monthly when sea conditions allowed.

Figure 9- Mesh bag (with five *L. ochroleuca* individuals) attached in a replicate unit.

EXPERIMENT 3

To test the suitability of different types of natural rope for sporophyte development, fertile tissues from individuals of *L. ochroleuca* with visible sori were collected from reefs in Carreço (≈ 41°44'30.3"N 8°52'38.3"W). Zoospores were released (see Experiment 1). The resulting spore solution was sprayed directly onto different types of biodegradable ropes (cotton, linen, and hemp), on 100L tanks and were submerged after 1h and temperature as kept at 15°C (Figure 10). After two days, aeration was initiated. The tanks were illuminated with AquaRay LED Lighting System, with a 12:12h light-dark cycle and a photon fluency of 200 µmol m^{-2} s^{-1}.

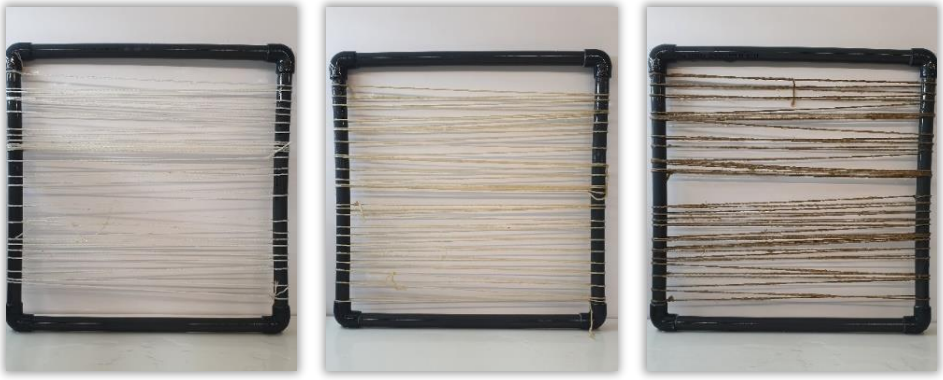

Figure 10- Indoor trial with different types of ropes (cotton, linen, and hemp).

Water exchange and the monitoring of environmental conditions (dissolved oxygen, pH, salinity values and temperature), with Hach HQ40D Portable Multi Meter, were done weekly. Recruitment on the ropes was monitored once a week using Leica EZ4W Stereomicroscope (Figure 11).

Figure 11- Rope fragments observations with Leica EZ4W Stereomicroscope.

From each sample, 30 pictures were captured and analysed with ImageJ (US National Institutes of Health, Bethesda, MD, USA), to calculate kelp size and area.

EXPERIMENT 4

A field experiment was started in June, in Viana do Castelo (41°41'57.2"N 8°51'19.84"W), in an intertidal pool with a large and dense intertidal patch (>30 individuals/m^2) of *L. ochroleuca* canopy (Figure 12). The main purpose of this experiment was to test the viability of kelp transplantation methods (using microscopic, juveniles, and adults stages). This time of the year was chosen to match with peak recruitment of *L. ochroleuca*. Distance to the kelp patch was a factor as canopy is known to offer protection for recruits. Three treatments were considered: away, at the edge and in the middle of the patch, to determine if lack of protection by canopy would impair the efficiency of an otherwise viable method.

Figure 12- Drone footage of the study site (Viana do Castelo). Photography: Silvia Chemello.

Three different life stages of *L. ochroleuca* were transplanted in each experimental plot (n=5): (I) two adult individuals, fastened by the holdfast to a PVC plate using a cotton tie, to allow kelp natural movement and avoid damage to the stipe; (II) three juvenile individuals (total length <20 cm), fastened by the holdfast to PVC plate (Figure 13); and (III) ropes sowed microscopic sporophytes, attached to PVC plates. The plates, which were 16 x 4 cm, were fixed to the substratum. All adult and juvenile individuals were collected in Viana do Castelo and individually numbered. Lamina length of juveniles and adults was measured, and adult lamina was cut at the same length (30 cm) to avoid differences in drag by water movement.

Figure 13- Adult individuals of L. ochroleuca attached to a PVC plate before transplantation.

For the growth assessment, the “hole punch method” described by Parke (1948) was employed, in which two perforations were made above the meristem, 3 cm from the junction between the stipe and blade. Individual’s survival and length was assessed in situ every month after deployment (August and September) by measuring the distance between the two holes and the base of the blade and subtracting the initial distance (3 cm).

RESULTS

EXPERIMENT 1

The lab trial occurred between April and June, for a total of 8 weeks. The first kelp recruitment was observed in the stereomicroscope in the 3rd week (Figure 14).

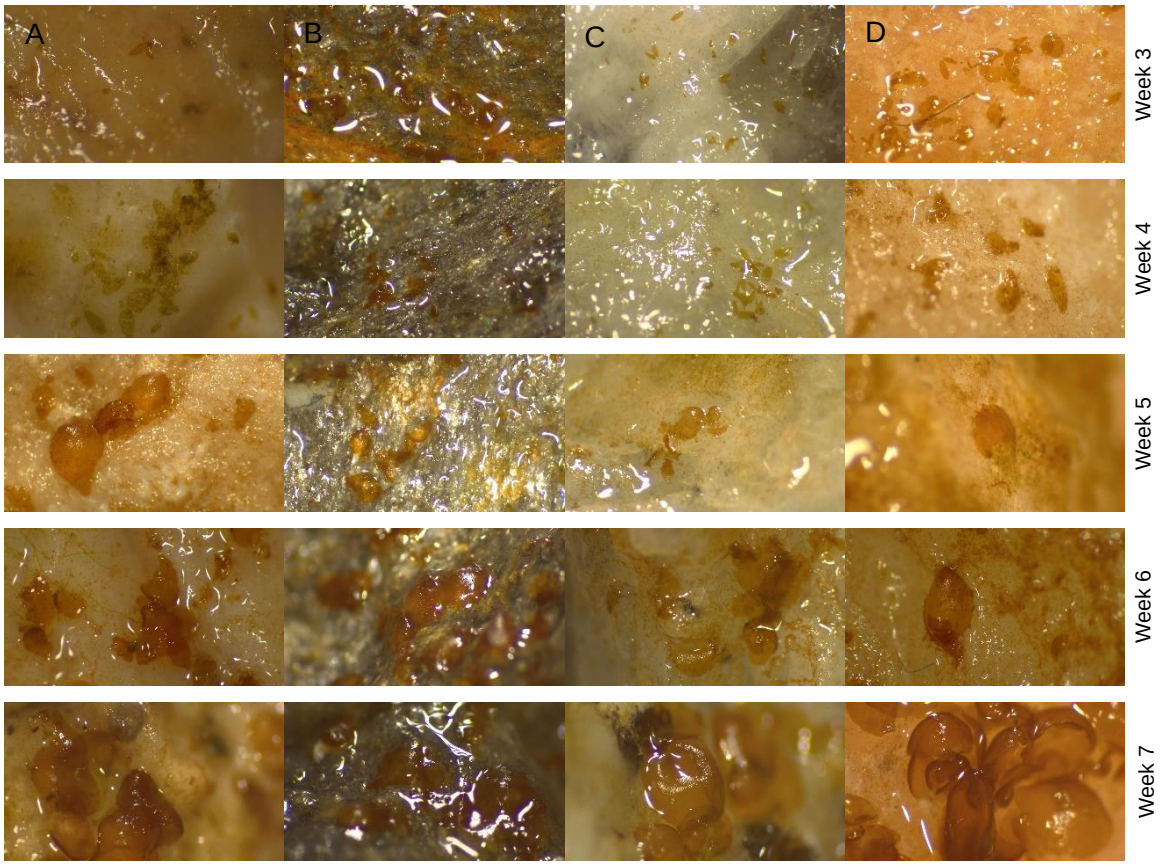

Figure 14- Kelp individuals in distinct substratum [A] limestone, [B] schist, [C] granite, and [D] quartz, during sampling time, since the first observation of kelp individuals. Pictures taken by camera Leica EZ4W.

Throughout week three, kelp size in schist was significantly higher when compared with the other substrata (Figure 15), reaching an average size of 0.05906 ± 0.054813 mm (one-way ANOVA; area, $p < 0.05$). In contrast, limestone had the smallest kelp, only reaching an average size of 0.01277 ± 0.018055 mm, yet not significantly different from the length in other substrata. However, after week three, kelp growth rate in both quartz and limestone was higher than in schist.

Throughout week four, growth was observed in all substrata apart from schist. Overall, kelp were significantly bigger in quartz (0.09873 ± 0.090561 mm) than the other substrata. No significant difference was observed among the other substrates.

Throughout week five, kelp size was significantly bigger in quartz, when compared with granite and schist, reaching an average size of 0.11624 ± 0.156292 mm. Granite presented the smallest kelp, only reaching an average size of 0.07099 ± 0.089949 mm. Kelp size in granite was significantly different from limestone and quartz (one-way ANOVA; area, $p < 0.05$).

Throughout week six, kelp were significantly bigger in quartz (0.30964 ± 0.448317 mm) when compared with the other substrata. Limestone presented the smallest kelp (0.14390 ± 0.203315). Kelp size was significantly different in all substrata, except in granite when compared with schist (one-way ANOVA; area, $p < 0.05$).

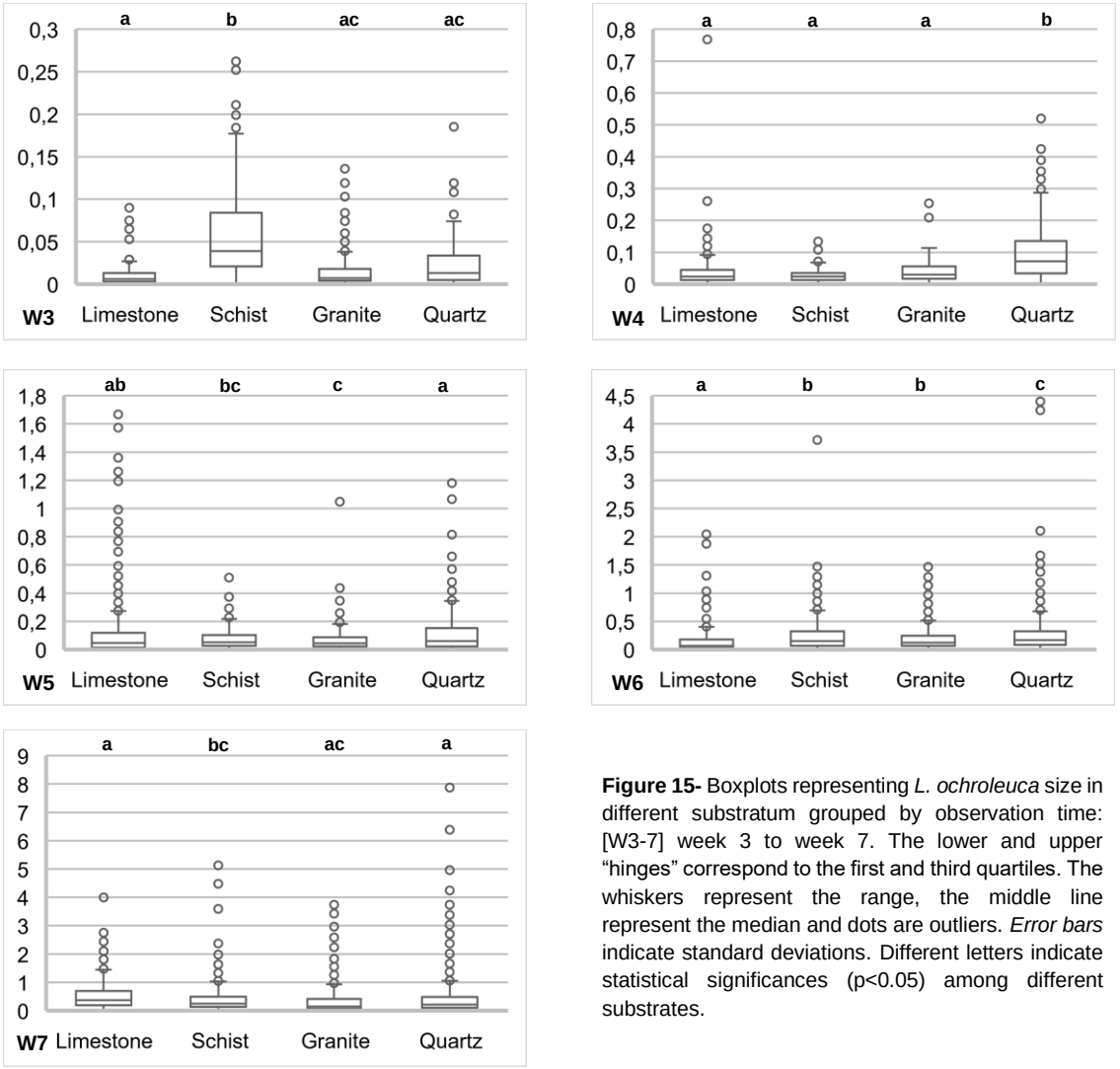

Figure 15- Boxplots representing *L. ochroleuca* size in different substratum grouped by observation time: [W3-7] week 3 to week 7. The lower and upper "hinges" correspond to the first and third quartiles. The whiskers represent the range, the middle line represent the median and dots are outliers. *Error bars* indicate standard deviations. Different letters indicate statistical significances ($p<0.05$) among different substrates.

A maximum size of 7.873 mm was reached, in quartz, during the 7th week. However, the average kelp size was bigger in limestone. The smallest kelp were observed in schist (0.41390 ± 0.518204 mm)**.** Kelp size was only significantly different in schist when compared with limestone and quartz (one-way ANOVA; area, $p < 0.05$).

Overall, the largest kelp individual was observed in quartz, although the highest overall size (0.56805 ± 0.616799 mm) and highest kelp growth (≈ 0,017912 ± 0,001325 mm d^{-1}) was observed in limestone (Figure 16). Kelp growth in granite and quartz was slightly lower than in limestone, with values of ≈ 0,017752 ± 0,001325 mm d^{-1} and ≈ 0,017022 ± 0,000695 mm d^{-1}, respectively. Schist obtained the smaller value of growth, only ≈ 0,011446 ± 0,003247 mm d^{-1}. On average, the seeded kelp grew 0,0160 ± 0,0031SD mm d^{-1} in the laboratory.

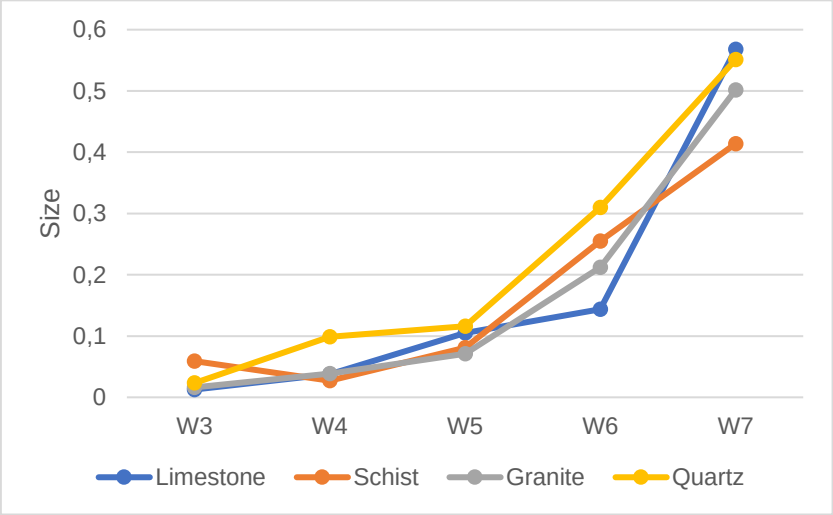

Figure 16- Kelp growth across sampling time.

Regarding the density of kelp in each substratum (Figure 17), at W3, the number of individuals per area was significantly higher in quartz (≈ 8.47) when compared with limestone (≈ 3.70) (one-way ANOVA; number of individuals, $p < 0.05$). At W5, the number of individuals per area in limestone (≈ 9.36) was significantly higher than the other substrata (≈ 6.42 in schist, ≈ 5.43 in granite and ≈ 5.03 in quartz).

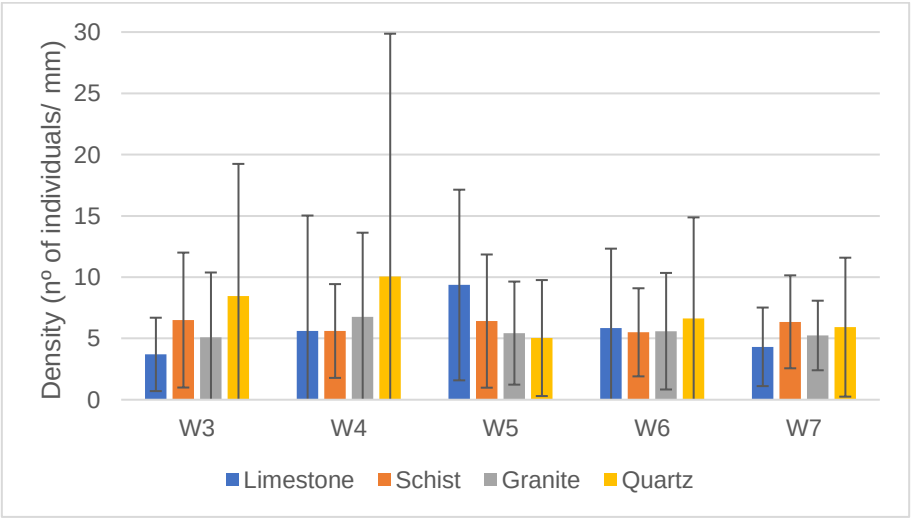

Figure 17- Number of individuals per area (mm) across sampling time. *Error bars* indicate standard deviations.

In the remaining sampling time, there were not found significant differences between substrata. Quartz had the highest mean value for density and granite the lowest.

Limestone presented the smallest variation of density and its maximum only reached 0,0439 in the sixth week. Granite presented the highest mean density value, followed by quartz and then schist. Despite a higher initial recruitment of granite and quartz, it was followed by high mortality (Figure 18).

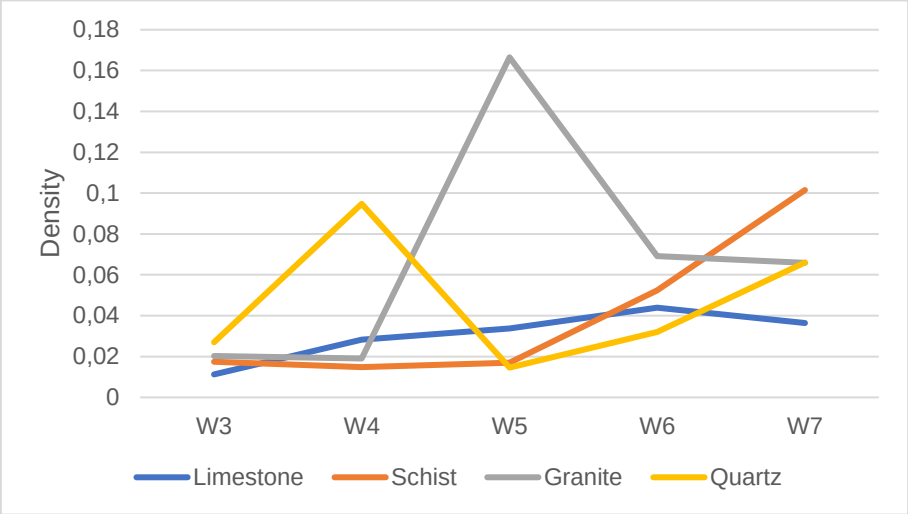

Figure 18- Kelp density (number of fields of view until at least 10 kelp individuals could be observed per total area) across sampling time

EXPERIMENT 2

In Carreço, the removal of turf and associated sediment, to provide available substrata, resulted in higher recruitment of sea lettuce (genus *Ulva*) when compared to the control where turf was not removed (Figure 19).

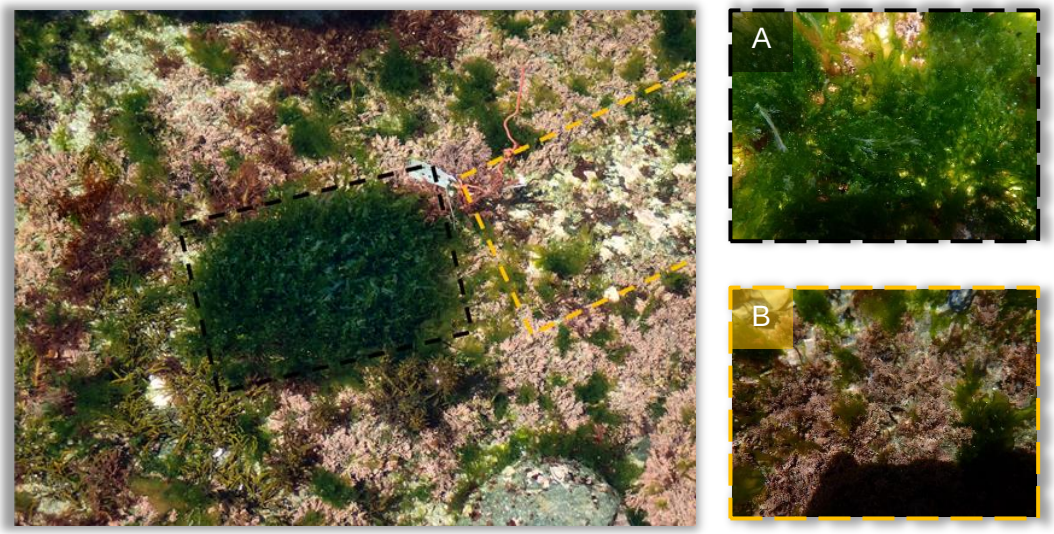

Figure 19- Comparison of canopy recovery between treatments from which turfs were removed (A) and control (B) approximately 1 month after start-up in Carreço.

In Viana do Castelo, the removal of turf and associated sediment also resulted in higher recruitment of sea lettuce when compared to the control (Figure 20). Comparing control and scraped quadrats, in Carreço and Viana do Castelo, it was observed that in the first location both areas had higher abundance of competitors than at the later.

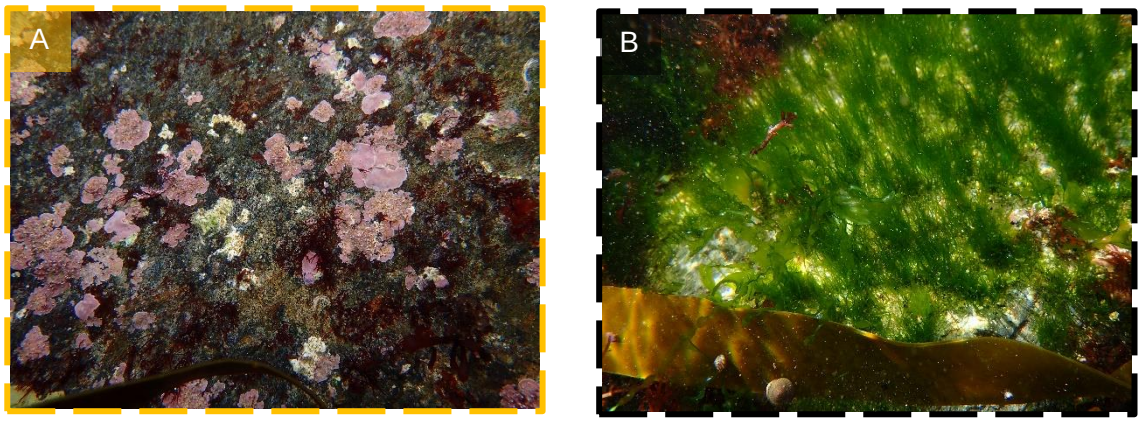

Figure 20- Comparison of canopy recovery between control treatment (A) and where turf were removed (B), approximately 1 month after start-up in Viana do Castelo.

As sea-urchins could have a negative impact, their density was assessed, however, although present, sea urchin density was low (around ≈0,13016 in Carreço and ≈0,151823 in Viana do Castelo; Figure 21).

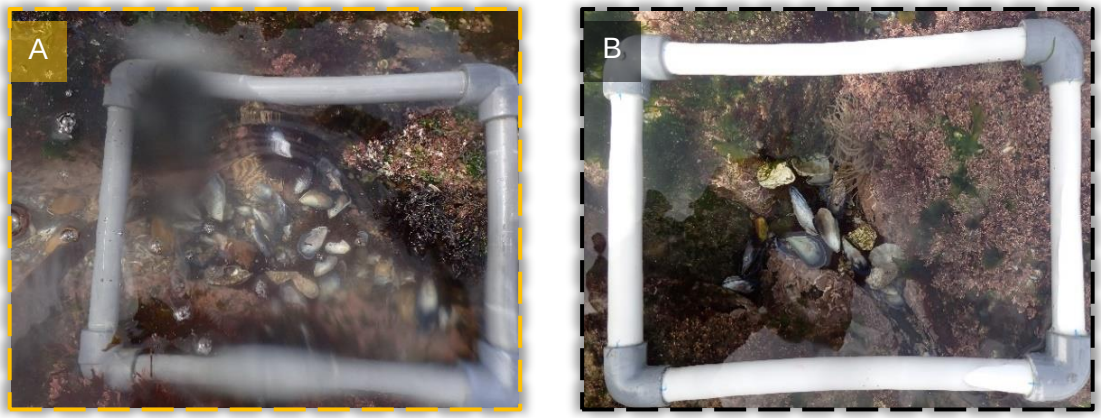

Figure 21- Purple sea urchin, *Paracentrotus lividus*, nearby the marks at (A) Viana do Castelo and (B) Carreço.

Gravel was deployed directly on the seafloor, but approximately one month later few of the rocks could be found, as the majority were dispersed away from the quadrat (Figure 22).

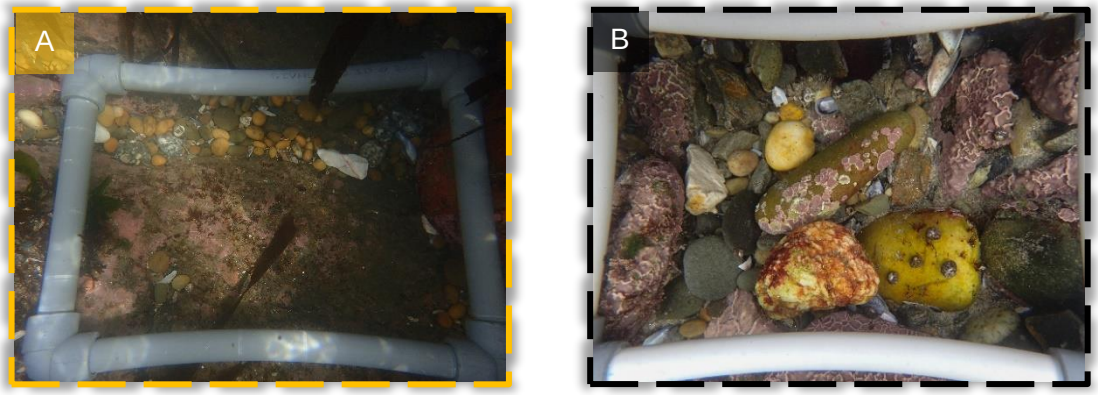

Figure 22- Comparison green-gravel presence between Viana do Castelo (A) and Carreço (B) approximately 1 month after start-up.

The few stones that were found contained green algae (genus *Ulva*) and a few mollusks, but no kelp (Figure 23).

Figure 23- Green gravel collected in the field in Viana do Castelo and Carreço, approximately 1 month after start-up.

EXPERIMENT 3

The lab trial was started in January and ran for 4 weeks. No kelp were found, and after four weeks contaminations were visible in both the tanks and ropes (Figure 24). No differences were seen between ropes and replicates.

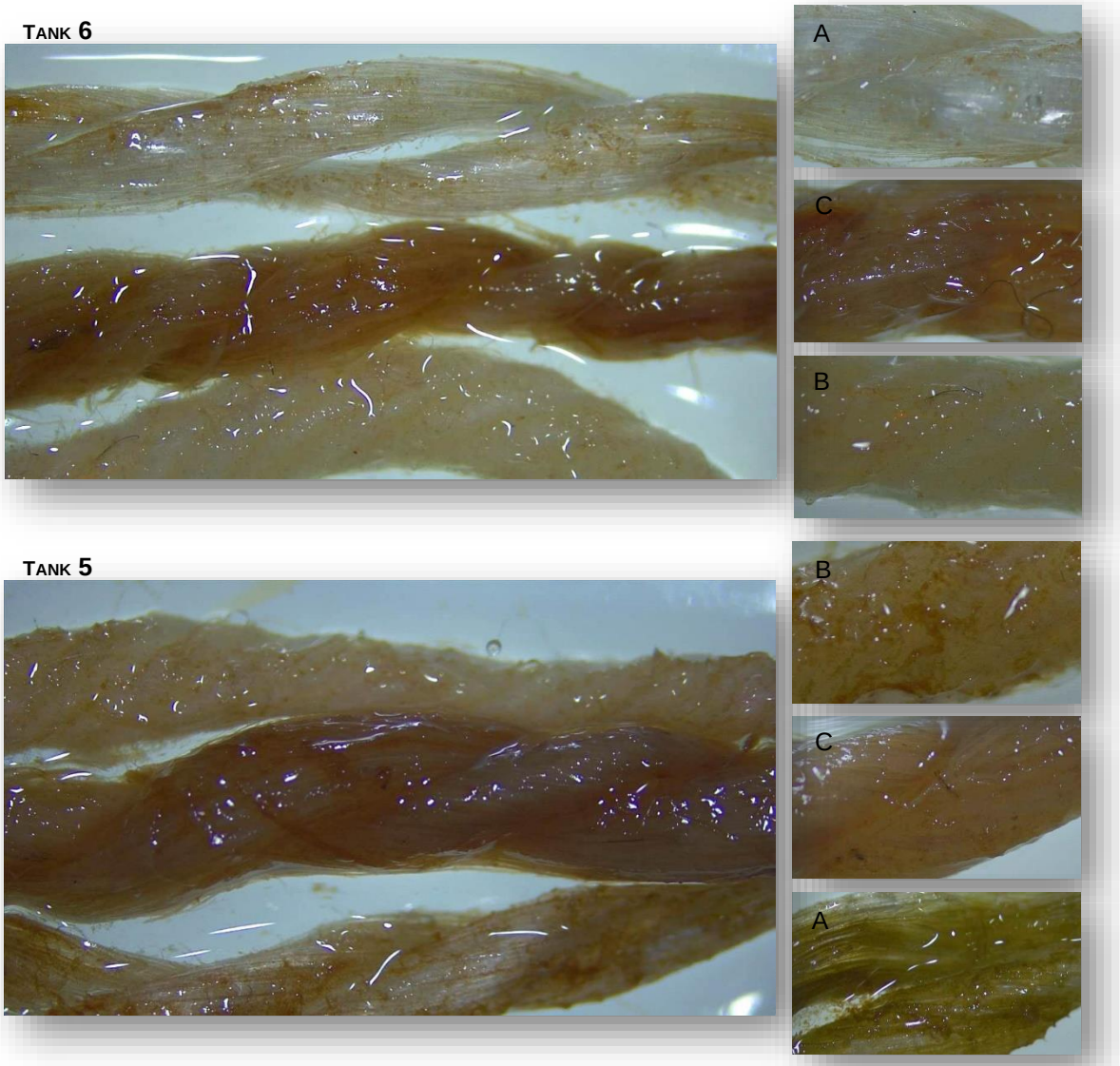

Figure 24- Ropes observations in tank 5 and 6, four weeks after trial initiation. (A) cotton, (B) linen, and (C) hemp. Pictures taken by camera Leica EZ4W.

EXPERIMENT 4

This field experiment started in July and lasted 8 weeks. In the first month, PVC plates (with adults, juveniles, and sporophytes) were monitored. 98% of transplanted juveniles were lost as well as over 50% of adults, with a survival rate of about 41%. The remaining adults showed an increase in length (average linear growth: 18,6 ± 2,64 mm/month). Two months later (September), 95% of transplanted adults were lost, but there was still visible some individuals growing (average linear growth: 50 ± 4,5 mm/month).

Regarding the ropes sown with microscopic sporophytes, in the first month, there was no loss in deployed units, but they were entirely overgrown by turf (green and red algae; Figure 25).

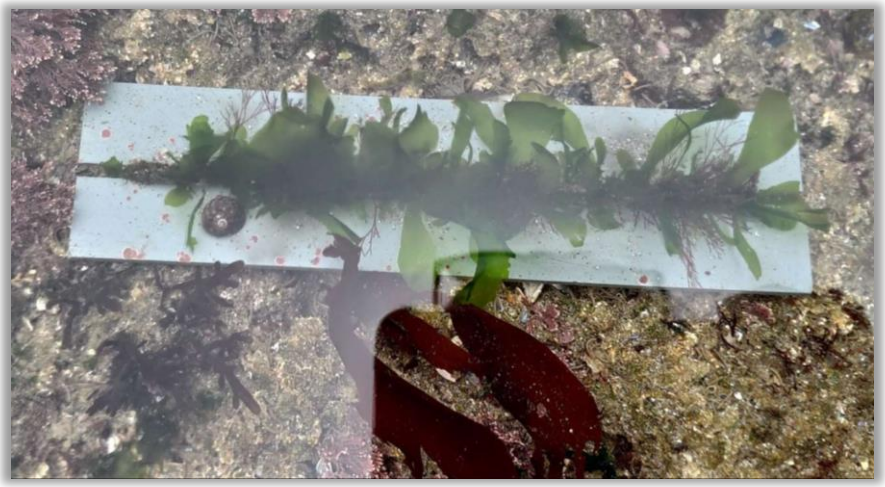

Figure 25- Turf algae growing on out-planting sporophytes experimental units.

The plates were taken to the laboratory, to observe under stereoscope if kelp recruits were still present under the turf. Turf was separately quantified as fresh weight and dry weight. To measure dry weight, red and green algae were dried at 60°C for 48h. Furthermore, total length of each single rope was measured, to assess the quantity of dry weight of turf algae per centimetre of rope. Approximately 62% of turf was green algae (mainly *Ulva* spp.), with an average value of 158,16 ± 7,92 mg DW cm^{-1}, and only 96,89 ± 10,31 mg DW cm^{-1} of red algae species.

A second out-planting attempt was performed in August, using ropes with macroscopic sporophytes (>1mm). The same sporophyte culture was used, obtained by the vegetative growth of gametophytes, which was left to grow for another month. A culture with a higher number of sporophytes per cm was chosen as higher densities of sporophytes could probably benefit kelp when competing for space with turf algae. However, the result was the same as before, it was entirely overgrown by turf (green and red algae).

DISCUSSION

EXPERIMENT 1

Our study provides the first experimental test of the “green gravel” method in *L. ochroleuca* and how different substrata (granite, limestone, quartz, and schist) could affect their recruitment. We found that the type of stone can significantly influence kelp recruitment. These results are in accordance with the outcomes of Jones and Bennett (2017) and Guidetti et al. (2004) where they demonstrate that substrate composition can influence the preferential colonization of some marine species.

The results showed that when kelp recruitment was first observed, kelp where significantly bigger in schist than other substrate. However, from then on, growth rate in both quartz and limestone surpassed that of schist. Quartz presented the highest maximum size, and limestone presented the highest average growth but the lowest density. In contrast, schist presented the lowest overall maximum size and growth. Substrate colour could explain the initial results, impacting initial recruitment, but the substrate surface would soon be obscure with initial colonization. Studies showed that substrate colour, that is related to the amount of energy reflected and absorbed by a substratum, as well as to the temperature of the substratum, influence rates of algal settlement and growth, with higher settlement on darker substrates (e.g., Dahlem et al., 1984; Swain et al., 2006). Overall, quartz and limestone provided the best results in kelp size and density. This could be explained by substrate complexity, as quartz has more rugosity that other substrates, supporting the substrate hardness/complexity hypothesis. In accordance to this results, Randell et al. (2022) suggested that substrate complexity (i.e. surface rugosity) influences regulatory processes in kelp forests. The success of limestone could be explained by their “low pH”, as it is close to the seawater pH (around 7.5), therefore more favourable to macrophytes. Hayek et al. (2020), demonstrated that the pH surface influenced the kinetics and the extent of the development of the bacterial biofilm and could influence the bio-colonization.

This demonstrates there are different physical attributes associated with rock-type differences that can affect marine assemblage and should be taken into consideration in decision-making processes regarding material choice for reforestation.

EXPERIMENT 2

In this study, the removal of turf and associated sediment to provide clean and available substrate was not beneficial to the recovery of *L. ochroleuca* forests. We observed that the presence of the opportunistic *Ulva* spp. was higher in the scraped quadrats than in the control. Comparing control and scraped quadrats from both locations we observed that Carreço, without canopy, had higher abundance of competitors than Viana do Castelo, with canopy, supporting our initial hypothesis that canopy could provide recruits a competitive advantage.

Sea urchins' presence in both locations was in low abundance and therefore considered as not significant. We also observed that the gravel that was dropped directly on the seafloor dispersed away from the quadrat in both locations due to high hydrodynamics. This may also be the explanation for the lack of kelp in the gravel, as it may have helped clean the substrate after settlement, preventing *L. ochroleuca* growth.

In opposition to these results, Gorman and Connell (2009) showed that in *Ecklonia radiata*, the removal of turfs resulted in higher rates of canopy recovery than control plots, where turf was not removed. Light availability may be a crucial factor to explain the distinction between turf removal and control quadrats, and between the two locations, as it can have adverse effects on the microscopic life stages of kelp, leading to an eventual exclusion from the space due to competition with algal turf (Altamirano et al., 2004; Cie & Edwards, 2008). In accordance with our results and initial hypothesis, studies have demonstrated that the removal of kelp canopy, in general, results in an increase in the abundance of opportunistic species (e.g., Benes & Carpenter, 2015; Dayton et al., 1984; Reed & Foster, 1984). Turf can swiftly take over primary substrate, trapping and accumulating significant amounts of sediments, and change the chemical environment (e.g., lowering oxygen availability or concentrating contaminants), contributing to an unfavourable ecosystem degradation with associated losses (e.g., food, habitat, and productivity) (Airoldi & Beck, 2007; Connell et al., 2014; Gorgula & Connell, 2004). Kelp forests are gradually being replaced by turf reefs, with less biodiversity and provide less ecosystem services, and this is more worrying since there have been no records of kelp forests recovering after shifting to turfs (Filbee-Dexter & Wernberg, 2018). Fredriksen et al. (2020), demonstrated the success of this technique with gravel sown and cultivated in the laboratory until small sporophytes are visible, proving competitive advantage, and then deployed in the subtidal. "Green gravel" method may not be appropriate for areas affected by strong hydrodynamic. That having been said, in this region, reforestation methods should take hydrodynamic into consideration.

EXPERIMENT 3

Our results demonstrated that cotton, linen, and hemp rope were not adequate for sporophytes to recruitment, as no kelps were observed growing on the ropes. Rope industrial treatments may be an explanation for these results since some ropes have anti-mold, -rodent, and -insect treatment, and this could prevent kelp recruitment.

In contrast to our results, our team and many studies have shown that out-planting kelp sporophytes onto ropes are a viable cultivation method (e.g., Devinny & Leventhal, 1979; Forbord et al., 2012; Peteiro et al., 2014). However, this has been achieved with artificial rope (polyethylene and nylon) (Forbord et al., 2012; Peteiro et al., 2014). While natural fibers were chosen for being and eco-friendly alternative, they present some obstacles and previous studies have resorted to chemical treatments to improving fiber strength, fiber fitness and fiber–matrix adhesion (e.g., Li et al., 2004; Paul et al., 1997; Sreekala, 2003).

Alternative rope materials without chemical treatments should be explored as lower impact alternatives particularly as these would biodegrade and not contribute to microplastic pollution.

EXPERIMENT 4

Presence of canopy of conspecific individuals is reported to have contrasting effects: it can either mitigate environmental condition promoting individuals' growth and survival (Schiel & Foster, 2006) or inhibit their growth by competitive shading (Reed & Foster, 1984). In our case, we considered distance to the kelp patch as essential factor but there were no differences in the survival of adult and juvenile individuals since all transplanted kelps were subjected to dislodgment and stipe breakage due to intense hydrodynamics. This could be explained by the resistance offered by their fronds, due to the excessive strain on the stipe, just above the tying point.

In out-planting sporophyte technique, recruitment was observed on the seeded ropes, but they ended up being outcompeted by turf (such as *Ulva* spp.). Presence of turf algae, and competition for light and substratum can affect juvenile individuals' survival (Altamirano et al., 2004; Cie & Edwards, 2008). Turf algae are mainly composed by fast-growing opportunistic species, which seem to be able to outgrow kelps (Filbee-Dexter & Wernberg, 2018). Out-planting techniques should be in specific periods of time to avoid when competitive algae are at their highest densities and recruitment. In our case, to limit the colonization of turf a second out-planting was attempted, with visible recruits (>1mm), as larger sporophytes may be better able to compete for space. Moreover, the small sporophytes seem to be equally affected by turf overgrowth, without difference based on their distance to kelp patch.

These results highlight some of the difficulties already stumble upon by previous studies (Carney et al., 2005; Hernández-Carmona et al., 2000), such as the choice of a suitable transplantation method and restoration site. For example, Carney et al. (2005) used transplantation method, with juvenile sporophytes (<15 cm stipe length) from natural populations, ignoring the culturing phase. However, stipe breakage posed the largest limiting factor on transplant survival, and they recognized that this method was not suitable, supporting the present findings.

CONCLUSION

Currently, conservation actions, even though important, are not enough to preserve our ocean's health, and need to be accompanied by restorative efforts. The present study provides useful information concerning *Laminaria ochroleuca* reforestation, more specifically in developing efficient methods for future efforts.

In the laboratory, testing different natural (gravel) substrate, quartz demonstrated, overall, the most promising results. Substrate complexity seems to be the main reason, as quartz surface has more rugosity that granite, schist, or limestone.

In the field, results contrasted with expectations, as the removal of turfs and associated sediment did not improve the recovery of kelp forests. The main explanation was light availability since *L. ochroleuca* in microscopic stages was outcompeted by algal turf species. That having been said, we can assume the inefficiency of transplanted reproductive tissue of adult *L. ochroleuca* in areas affected by strong hydrodynamics. In this case, out-planting juveniles or adults might be an efficient option instead of microscopic sporophytes, that can be easily scattered and buried by sediment, providing higher success.

The green gravel technique used in the intertidal area was not a suitable technique as stones are dragged which might results in scrapping of their surface. Other solution may be out-planting in specific periods of time to avoid when competitive algae are at their highest densities and recruitment. However, in this technique kelp individuals ought to be smaller (≈1 cm) to facilitate transportation and to prevent breakage. Out-plant green gravel (e.g., quartz) could be a solution, yet some changes need to be done to surpass previous obstacles. Kelp reared in the laboratory must have at least 2 cm and used in subtidal. A solution for the mesh bags technique may be seed an organic net and transplant in the field, allowing to fixate in several points and trying to reach a larger coverage area.

We observed that the main obstacle to reforestation efforts in the intertidal is the competition by fast growing, opportunistic turf species, followed by the strong hydrodynamics and sedimentation which need to be considered when choosing the reforestation method.

This study provides insightful information for further reforestation efforts that can be used for other perennial kelp species and along other coastal regions. It is still worth, however, testing these techniques in less exposed areas and in the subtidal.

REFERENCES

Airoldi, L. (2003). The effects of sedimentation on rocky coast assemblages. *Oceanography and Marine Biology*, *41*, 169-171.

Airoldi, L., & Beck, M. (2007). Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology*, *45*, 345-405.

Allorge, P., & Manguin, E. (1941). Algues d'eau douce des Pyrénées basques.

Altamirano, M., Murakami, A., & Kawai, H. (2004). High light stress in the kelp *Ecklonia cava*. *Aquatic Botany*, *79*, 125-135.

Andersen, G. S., Pedersen, M., & Nielsen, S. (2013). Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *Journal of Phycology*, *49*.

Araújo, R., Assis, J., Aguillar, R., Airoldi, L., Bárbara, I., Bartsch, I., Bekkby, T., Christie, H., Davoult, D., Derrien-Courtel, S., Fernandez, C., Fredriksen, S., Gevaert, F., Gundersen, H., Gál, A., Lévêque, L., Mieszkowska, N., Norderhaug, K., Oliveira, P., . . . Sousa-Pinto, I. (2016). Status, trends and drivers of kelp forests in Europe: an expert assessment. *Biodiversity and Conservation*, *25*, 1319-1348.

Arnold, M. J., Teagle, H., Brown, M. P., & Smale, D. (2015). The structure of biogenic habitat and epibiotic assemblages associated with the global invasive kelp *Undaria pinnatifida* in comparison to native macroalgae. *Biological Invasions*, *18*, 661-676.

Assis, J., Serrão, E. A., Coelho, N. C., Tempera, F., Valero, M., & Alberto, F. (2018). Past climate changes and strong oceanographic barriers structured lo— latitude genetic relics for the golden kelp *Laminaria ochroleuca*. *Journal of Biogeography*.

Assis, J., Tavares, D., Tavares, J., Cunha, A., Alberto, F., & Serrão, E. A. (2009). Findkelp, a GIS-Based Community Participation Project to Assess Portuguese Kelp Conservation Status. *Journal of Coastal Research*, 1469-1473.

Assis, J., Tyberghein, L., Bosch, S., Verbruggen, H., Serrão, E. A., & Clerck, O. D. (2018). Bi• ORACLE v2.0: Extending marine data layers for bioclimatic modelling. *Global Ecology and Biogeography*, *27*, 277-284.

Balasse, M., Tresset, A., Dobney, K., & Ambrose, S. (2005). The use of isotope ratios to test for seaweed eating in sheep. *Journal of Zoology*, *266*, 283-291.

Barbosa, M., Fernandes, F., Pereira, D. M., Azevedo, I. C., Sousa-Pinto, I., Andrade, P. B., & Valentão, P. (2017). Fatty acid patterns of the kelps *Saccharina latissima*, *Saccorhiza polyschides* and *Laminaria ochroleuca*: Influence of changing environmental conditions. *Arabian Journal of Chemistry*, *13*, 45-58.

Bearham, D., Vanderklift, M., & Gunson, J. (2013). Temperature and light explain spatial variation in growth and productivity of the kelp *Ecklonia radiata*. *Marine Ecology Progress Series*, *476*, 59-70.

Belkin, I. (2009). Rapid warming of Large Marine Ecosystems. *Progress in Oceanography*, *81*, 207-213.

Benes, K. M., & Carpenter, R. C. (2015). Kelp canopy facilitates understory algal assemblage via competitive release during early stages of secondary succession. *Ecology*, *96*(1), 241-251.

Bennett, S., Wernberg, T., Connell, S., Hobday, A. J., Johnson, C. R., & Poloczanska, E. S. (2016). The 'Great Southern Reef': social, ecological and economic value of Australia's neglected kelp forests. *Marine and Freshwater Research*, *67*, 47-56.

Bertocci, I., Araújo, R., Oliveira, P. J., & Sousa-Pinto, I. (2015). Review: Potential effects of kelp species on local fisheries. *Journal of Applied Ecology*, *52*, 1216-1226.

Bettignies, T. d., Wernberg, T., Lavery, P., Vanderklift, M., & Mohring, M. B. (2013). Contrasting mechanisms of dislodgement and erosion contribute to production of kelp detritus. *Limnology and Oceanography*, *58*, 1680-1688.

Blamey, L., & Bolton, J. (2017). The economic value of South African kelp forests and temperate reefs: Past, present and future. *Journal of Marine Systems*, *188*, 172-181.

Blight, A. J., & Thompson, R. (2008). Epibiont species richness varies between holdfasts of a northern and a southerly distributed kelp species. *Journal of the Marine Biological Association of the United Kingdom*, *88*, 469-475.

Britton-Simmons, K., Rhoades, A. L., Pacunski, R. E., Galloway, A. E., Lowe, A., Sosik, E. A., Dethier, M., & Duggins, D. O. (2012). Habitat and bathymetry influence the landscap… scale distribution and abundance of drift macrophytes and associated invertebrates. *Limnology and Oceanography*, *57*, 176-184.

Broch, O. J., & Slagstad, D. (2011). Modelling seasonal growth and composition of the kelp *Saccharina latissima*. *Journal of Applied Phycology*, *24*, 759-776.

Carnell, P., & Keough, M. (2019). Reconstructing Historical Marine Populations Reveals Major Decline of a Kelp Forest Ecosystem in Australia. *Estuaries and Coasts*, *42*, 765-778.

Carney, L. T., Waaland, J. R., Klinger, T., & Ewing, K. (2005). Restoration of the bull kelp *Nereocystis luetkeana* in nearshore rocky habitats. *Marine Ecology Progress Series*, *302*, 49-61.

Casado-Amezúa, P., Araújo, R., Bárbara, I., Bermejo, R., Borja, A., Díez, I., Fernández, C., Gorostiaga, J. M., Guinda, X., Hernández, I., Juanes, J., Peña, V., Peteiro, C., Puente, A., Quintana, I., Tuya, F., Viejo, R., Altamirano, M., Gallardo, T., & Martinez, B. (2019). Distributional shifts of canopy-forming seaweeds from the Atlantic coast of Southern Europe. *Biodiversity and Conservation*, *28*, 1151-1172.

Chapman, A., & Craigie, J. (1977). Seasonal growth in Laminaria longicruris: Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology*, *40*, 197-205.

Chapman, A., & Lindley, J. (1980). Seasonal growth of Laminaria solidungula in the Canadian High Arctic in relation to irradiance and dissolved nutrient concentrations. *Marine Biology*, *57*, 1-5.

Chapman, V. J. (1970). Seaweeds as animal fodder and manure. In *Seaweeds and their uses* (2nd ed., pp. 63–85). Camelot.

Christie, H., Jørgensen, N., & Norderhaug, K. (2007). Bushy or smooth, high or low; importance of habitat architecture and vertical position for distribution of fauna on kelp. *Journal of Sea Research*, *58*, 198-208.

Christie, H., Jørgensen, N., Norderhaug, K., & Waage-Nielsen, E. (2003). Species distribution and habitat exploitation of fauna associated with kelp (*Laminaria Hyperborea*) along the Norwegian Coast. *Journal of the Marine Biological Association of the United Kingdom*, *83*, 687-699.

Christie, H., Norderhaug, K., & Fredriksen, S. (2009). Macrophytes as habitat for fauna. *Marine Ecology Progress Series*, *396*, 221-233.

Cie, D. K., & Edwards, M. S. (2008). The effects of high irradiance on the settlement competency and viability of kelp zoospores. *Journal of Phycology*, *44*.

Cole, S. (1991). Women of the Praia: Work and Lives in a Portuguese Coastal Community.

Connell, S., Russell, B., Turner, D., Shepherd, S., Kildea, T., Miller, D., Airoldi, L., & Cheshire, A. (2008). Recovering a lost baseline: missing kelp forests from a metropolitan coast. *Marine Ecology Progress Series*, *360*, 63-72.

Connell, S. D., Foster, M. S., & Airoldi, L. (2014). What are algal turfs? Towards a better description of turfs. *Marine Ecology Progress Series*, *495*, 299-307.

Coulson, S. J., Bullock, J. M., Stevenson, M. J., & Pywell, R. F. (2001). Colonization of grassland by sown species: dispersal versus microsite limitation in responses to management. *Journal of Applied Ecology*, *38*, 204-216.

Dahlem, C., Moran, P. J., & Grant, T. (1984). Larval settlement of marine sessile invertebrates on surfaces of different colour and position.

Dayton, P. (1985). Ecology of Kelp Communities. *Annual Review of Ecology, Evolution, and Systematics*, *16*, 215-245.

Dayton, P. K., Currie, V., Gerrodette, T., Keller, B. D., Rosenthal, R., & Tresca, D. V. (1984). Patch Dynamics and Stability of Some California Kelp Communities. *Ecological Monographs*, *54*(3), 254-289.

Devinny, J. S., & Leventhal, J. (1979). New methods for mass culture of *Macrocystis pyrifera* sporophytes. *Aquaculture*, *17*(3), 241-250.

Duggins, D. O., Simenstad, C., & Estes, J. (1989). Magnification of Secondary Production by Kelp Detritus in Coastal Marine Ecosystems. *Science*, *245*, 170 - 173.

Erlandson, J., Graham, M., Bourque, B., Corbett, D., Estes, J., & Steneck, R. (2007). The Kelp Highway Hypothesis: Marine Ecology, the Coastal Migration Theory, and the Peopling of the Americas. *The Journal of Island and Coastal Archaeology*, *2*, 161 - 174.

Filbee-Dexter, K., Feehan, C. J., & Scheibling, R. E. (2016). Large-scale degradation of a kelp ecosystem in an ocean warming hotspot. *Marine Ecology Progress Series*, *543*, 141-152.

Filbee-Dexter, K., & Scheibling, R. (2012). Hurricane-mediated defoliation of kelp beds and pulsed delivery of kelp detritus to offshore sedimentary habitats. *Marine Ecology Progress Series*, *455*, 51-64.

Filbee-Dexter, K., & Scheibling, R. E. (2014). Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Marine Ecology Progress Series*, *495*, 1-25.

Filbee-Dexter, K., & Wernberg, T. (2018). Rise of Turfs: A New Battlefront for Globally Declining Kelp Forests. *BioScience*, *68*, 64 76.

Forbord, S., Skjermo, J., Arff, J., Handå, A., Reitan, K. I., Bjerregaard, R., & Lüning, K. (2012). Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. *Journal of Applied Phycology*, *24*(3), 393-399.

Franco, J. N., Tuya, F., Bertocci, I., Rodríguez, L., Martínez, B., Sousa-Pinto, I., & Arenas, F. (2018). The 'golden kelp' *Laminaria ochroleuca* under global change: Integrating multiple eco-physiological responses with species distribution models. *Journal of Ecology*, *106*, 47-58.

Fredriksen, S., Filbe… Dexter, K., Norderhaug, K. M., Steen, H., Bodvin, T., Coleman, M. A., Moy, F. E., & Wernberg, T. (2020). Green gravel: a novel restoration tool to combat kelp forest decline. *Scientific Reports*, *10*.

Gachon, C., Sime-Ngando, T., Strittmatter, M., Chambouvet, A., & Kim, G. (2010). Algal diseases: spotlight on a black box. *Trends in plant science*, *15 11*, 633-640.

Gagne, J. A., Mann, K. H., & Chapman, A. (1982). Seasonal patterns of growth and storage in *Laminaria longicruris* in relation to differing patterns of availability of nitrogen in the water. *Marine Biology*, *69*, 91-101.

Gao, X., Endo, H., Yamana, M., Taniguchi, K., & Agatsuma, Y. (2013). Compensatory abilities depending on seasonal timing of thallus excision of the kelp *Undaria pinnatifida* cultivated in Matsushima Bay, northern Japan. *Journal of Applied Phycology*, *25*, 1331-1340.

Gaspar, R., Pereira, L., & Sousa-Pinto, I. (2019). The seaweed resources of Portugal. *Botanica Marina*, *62*, 499 - 525.

GBIF.org (2022), GBIF Home Page. Available from: https://www.gbif.org [19 June 2022].

Gerard, V. A., & Mann, K. (1979). Growth and production of *Laminaria longicruris* (Phaeophyta) populations exposed to different intensities of water movement. *Journal of Phycology*, *15*.

Gorgula, S. K., & Connell, S. D. (2004). Expansive covers of turf-forming algae on human-dominated coast: the relative effects of increasing nutrient and sediment loads. *Marine Biology*, *145*, 613-619.

Gorman, D., & Connell, S. (2009). Recovering subtidal forests in human-dominated landscapes. *Journal of Applied Ecology*, *46*, 1258-1265.

Gorman, D., Russell, B., & Connell, S. (2009). Land-to-sea connectivity: linking human-derived terrestrial subsidies to subtidal habitat change on open rocky coasts. *Ecological applications : a publication of the Ecological Society of America*, *19 5*, 1114-1126.

Graham, M. (2004). Effects of Local Deforestation on the Diversity and Structure of Southern California Giant Kelp Forest Food Webs. *Ecosystems*, *7*, 341-357.

Graham, M., Kinlan, B., Druehl, L., Garske, L. E., & Banks, S. (2007). Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. *Proceedings of the National Academy of Sciences*, *104*, 16576 - 16580.

Grubb, P. J. (1977). The Maintenance of Species-Richness in Plant Communities: The Importance of the Regeneration Niche. *Biological Reviews*, *52*.

Guidetti, P., Bianchi, C. N., Chiantore, M., Schiaparelli, S., Morri, C., & Cattaneo-Vietti, R. (2004). Living on the rocks: substrate mineralogy and the structure of subtidal rocky substrate communities in the Mediterranean Sea. *Marine Ecology Progress Series*, *274*, 57-68.

Harper, J. L., Clatworthy, J., Mcnaughton, I. H., & Sagar, G. R. (1961). The evolution and ecology of closely related species living in the same area. *Evolution*, *15*.

Harper, J. L., Williams, J. T., & Sagar, G. R. (1965). The Behaviour of Seeds in Soil: I. The Heterogeneity of Soil surfaces and its Role in Determining the Establishment of Plants from Seed. *Journal of Ecology*, *53*, 273.

Hayek, M., Salgues, M., Habouzit, F., Bayle, S., Souche, J.-C., De Weerdt, K., & Pioch, S. (2020). In vitro and in situ tests to evaluate the bacterial colonization of cementitious materials in the marine environment. *Cement and Concrete Composites*.

Henley, W. J., & Dunton, K. (1997). Effects of nitrogen supply and continuous darkness on growth and photosynthesis of the arctic kelp *Laminaria solidungula*. *Limnology and Oceanography*, *42*, 209-216.

Hernández-Carmona, G., García, O. E. M., Robledo, D., & Foster, M. A. (2000). Restoration Techniques for *Macrocystis pyrifera* (Phaeophyceae) Populations at the Southern Limit of Their Distribution in México.

Howarth, R. (1988). Nutrient Limitation of Net Primary Production in Marine Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *19*, 89-110.

Hughes, S., Holliday, N. P., Kennedy, J., Berry, D., Kent, E. C., Sherwin, T., Dye, S., Inall, M., Shammon, T., & Smyth, T. (2010). Temperature (air and sea).

Hurd, C. L. (2000). Water motion, marine macroalgal physiology and production. *Journal of Phycology*, *36*.

Hurd, C. L. (2017). Shaken and stirred: the fundamental role of water motion in resource acquisition and seaweed productivity.

Hurd, C. L., Harrison, P. J., Bischof, K., & Lobban, C. S. (2014). *Seaweed Ecology and Physiology*. Cambridge University Press.

Irving, A. D., & Connell, S. (2006). Physical disturbance by kelp abrades erect algae from the understorey. *Marine Ecology Progress Series*, *324*, 127-137.

Izquierdo, J., Pérez-ruzafa, I., & Gallardo, T. (2001). Effect of temperature and photon fluence rate on gametophytes and young sporophytes of *Laminaria ochroleuca* Pylaie. *Helgoland Marine Research*, *55*, 285-292.

Jackson, G. (1977). Nutrients and production of giant kelp, *Macrocystis pyrifera*, off southern California1. *Limnology and Oceanography*, *22*, 979-995.

Jiang, Z., Liu, J., Li, S., Chen, Y., Du, P., Zhu, Y., Liao, Y., Chen, Q., Shou, L., Yan, X., Zeng, J., & Chen, J. (2020). Kelp cultivation effectively improves water quality

and regulates phytoplankton community in a turbid, highly eutrophic bay. *Science of the Total Environment*, *707*.

Johnson, C., Banks, S., Barrett, N., Cazassus, F., Dunstan, P., Edgar, G., Frusher, S., Gardner, C., Haddon, M., Helidoniotis, F., Hill, K., Holbrook, N., Hosie, G., Ling, S., Melbourne-Thomas, J., Miller, K., Pecl, G., Richardson, A., Ridgway, K., . . . Taw, N. (2011). Climate change cascades: Shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania. *Journal of Experimental Marine Biology and Ecology*, *400*, 17-32.

Jones, A. A., & Bennett, P. C. (2017). Mineral Ecology: Surface Specific Colonization and Geochemical Drivers of Biofilm Accumulation, Composition, and Phylogeny. *Frontiers in microbiology*, *8*, 491.

Jones, C., Lawton, J., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, *69*, 130-147.

Kain, J. M. (1989). The seasons in the subtidal. *European Journal of Phycology*, *24*, 203-215.

Koehl, M., Silk, W., Liang, H., & Mahadevan, L. (2008). How kelp produce blade shapes suited to different flow regimes: A new wrinkle. *Integrative and comparative biology*, *48 6*, 834-851.

Kregting, L., Hurd, C., Pilditch, C., & Stevens, C. (2008). The Relative Importance of water motion on nitrogen uptake by the subtidal macroalga *Adamsiella chauvinii* (Rhodophyta) in winter and summer. *Journal of Phycology*, *44*.

Krumhansl, K. A., Bergman, J. N., & Salomon, A. (2017). Assessing the ecosystem-level consequences of a small-scale artisanal kelp fishery within the context of climate-change. *Ecological applications : a publication of the Ecological Society of America*, *27 3*, 799-813.

Krumhansl, K. A., & Scheibling, R. (2011). Detrital production in Nova Scotian kelp beds: patterns and processes. *Marine Ecology Progress Series*, *421*, 67-82.

Krumhansl, K. A., & Scheibling, R. (2012). Production and fate of kelp detritus. *Marine Ecology Progress Series*, *467*, 281-302.

Lapointe, B., Littler, M., & Littler, D. S. (1992). Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters. *Estuaries*, *15*, 75-82.

Li, X., Panigrahi, S. T., L.G., & Crerar, W. J. (2004, September). 2004 CSAE/ASAE Annual Intersectional Meeting. Winnipeg, Canada.

Lima, F. P., & Wethey, D. S. (2012). Three decades of high-resolution coastal sea surface temperatures reveal more than warming. *Nature communications*, *3*, 704.

Lloret, F., Escudero, A., Iriondo, J. M., Martínez Vilalta, J., & Valladares, F. (2012). Extreme climatic events and vegetation: the role of stabilizing processes. *Global Change Biology*, *18*, 797-805.

Los, D., & Murata, N. (2004). Membrane fluidity and its roles in the perception of environmental signals. *Biochimica et biophysica acta*, *1666 1-2*, 142-157.

Løvås, S. M., & Tørum, A. (2001). Effect of the kelp *Laminaria hyperborea* upon sand dune erosion and water particle velocities. *Coastal Engineering*, *44*, 37-63.

Luning, K. (1979). Growth Strategies of Three Laminaria Species (Phaeophyceae) Inhabiting Different Depth Zones in the Sublittoral Region of Helgoland (North Sea). *Marine Ecology Progress Series*, *1*, 195-207.

Lüning, K., & tom Dieck, I. (1989). Environmental Triggers in Algal Seasonality.

Lüning, K., Yarish, C., & Kirkman, H. (1990). Seaweeds: Their Environment, Biogeography, and Ecophysiology.

Mabin, C. J. T., Johnson, C. R., & Wright, J. T. (2019). Physiological response to temperature, light, and nitrates in the giant kelp *Macrocystis pyrifera* from Tasmania, Australia. *Marine Ecology Progress Series*, *614*, 1-19.

Machalek, K. M., Davison, I., & Falkowski, P. (1996). Thermal acclimation and photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*. *Plant Cell and Environment*, *19*, 1005-1016.

Middelboe, A., & Sand-Jensen, K. (2000). Long-term changes in macroalgal communities in a Danish estuary. *Phycologia*, *39*, 245 - 257.

Miller, S. M., Hurd, C., & Wing, S. (2011). Variations in growth, erosion, productivity, and morphology of *Ecklonia radiata* (Alariaceae; Laminariales) along a fjord in Southern New Zealand. *Journal of Phycology*, *47*.

Mizuta, H., Maita, Y., & Yanada, M. (1992). Seasonal changes of nitrogen metabolism in the sporophyte of *Laminaria japonica* (Phaeophyceae). *Nippon Suisan Gakkaishi*, *58*, 2345-2350.

Moore, P. G. (1973). The kelp fauna of northeast Britain. I. Introduction and the physical environment. *Journal of Experimental Marine Biology and Ecology*, *13*, 97-125.

Mork, M. (1996). The effect of kelp in wave damping. *Sarsia*, *80*, 323-327.

Moy, F., & Christie, H. (2012). Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. *Marine Biology Research*, *8*, 309 - 321.

Muth, A. F. (2012). Effects of Zoospore Aggregation and Substrate Rugosity on Kelp Recruitment Success. *Journal of Phycology*, *48*.

Nielsen, M. M., Kraus… Jensen, D., Olesen, B., Thinggaard, R., Christensen, P. B., & Bruhn, A. (2014). Growth dynamics of *Saccharina latissima* (Laminariales, Phaeophyceae) in Aarhus Bay, Denmark, and along the specie“ distribution range. *Marine Biology*, *161*, 2011-2022.

Norderhaug, K., & Christie, H. (2009). Sea urchin grazing and kelp re-vegetation in the NE Atlantic. *Marine Biology Research*, *5*, 515 - 528.

o Brien, J., & Scheibling, R. (2016). Nipped in the bud: mesograzer feeding preference contributes to kelp decline. *Ecology*, *97 7*, 1873-1886.

Palminha, F. (1971). *Exploração e utilização de algas marinhas na plataforma portuguesa e nas ilhas do Arquipélago dos Açores* (Vol. 7). Junta Nacional do Fomento das Pescas.

Parke, M. (1948). Studies on British Laminariaceae. I. Growth in *Laminaria Saccharina* (L.) Lamour. *Journal of the Marine Biological Association of the United Kingdom*, *27*(3), 651-709.

Paul, A., Joseph, K., & Thomas, S. (1997).

Pedersen, M., Nejrup, L. B., Fredriksen, S., Christie, H., & Norderhaug, K. (2012). Effects of wave exposure on population structure, demography, biomass and productivity of the kelp *Laminaria hyperborea*. *Marine Ecology Progress Series*, *451*, 45-60.

Pereira, T. R., Azevedo, I. C., Oliveira, P., Silva, D. M., & Sousa-Pinto, I. (2019). Life history traits of *Laminaria ochroleuca* in Portugal: The range-center of its geographical distribution. *Aquatic Botany*.

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

Pereira, T. R., Engelen, A. H., Pearson, G. A., Valero, M., & Serrão, E. A. (2017). Population dynamics of temperate kelp forests near their low-latitude limit. *Aquatic Botany*, *139*, 8-18.

Peteiro, C. s., Sánchez, N., Dueñas-Liaño, C., & Martínez, B. (2014). Open-sea cultivation by transplanting young fronds of the kelp *Saccharina latissima*. *Journal of Applied Phycology*, *26*(1), 519-528.

Pfister, C., Berry, H., & Mumford, T. (2018). The dynamics of Kelp Forests in the Northeast Pacific Ocean and the relationship with environmental drivers. *Journal of Ecology*, *106*, 1520-1533.

Piñeiro-Corbeira, C., Barreiro, R., & Cremades, J. (2016). Decadal changes in the distribution of common intertidal seaweeds in Galicia (NW Iberia). *Marine environmental research*, *113*, 106-115.

Pinsky, M., Eikeset, A. M., McCauley, D., Payne, J., & Sunday, J. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, *569*, 108-111.

Pörtner, H., & Farrell, A. (2008). Physiology and Climate Change. *Science*, *322*, 690 - 692.

Randell, Z., Kenner, M. C., Tomoleoni, J. A., Yee, J. L., & Novak, M. (2022). Kelp-forest dynamics controlled by substrate complexity. *Proceedings of the National Academy of Sciences of the United States of America*, *119*.

Reed, D. C. (1990). The Effects of Variable Settlement and Early Competition on Patterns of Kelp Recruitment. *Ecology*, *71*, 776-787.

Reed, D. C., & Foster, M. S. (1984). The Effects of Canopy Shadings on Algal Recruitment and Growth in a Giant Kelp Forest. *Ecology*, *65*(3), 937-948.

Reed, R., Lobban, C., Harrison, P., & Duncan, M. (1985). The physiological ecology of seaweeds.

Santos, R., & Duarte, P. (1996). Fecundity, spore recruitment and size in *Gelidium sesquipedale* (Gelidiales, Rhodophyta). *Hydrobiologia*, *326-327*, 223-228.

Schiel, D. R., & Foster, M. S. (2006). The Population Biology of Large Brown Seaweeds: Ecological Consequences of Multiphase Life Histories in Dynamic Coastal. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 343-372.

Silberfeld, T., Rousseau, F., & Reviers, B. d. (2014). An Updated Classification of Brown Algae (Ochrophyta, Phaeophyceae). *Cryptogamie, Algologie*, *35*(2), 117-156, 140.

Smale, D. (2019). Impacts of ocean warming on kelp forest ecosystems. *The New phytologist*.

Smale, D., Burrows, M., Moore, P., O'Connor, N. E., & Hawkins, S. (2013). Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. *Ecology and Evolution*, *3*, 4016 - 4038.

Smale, D., Wernberg, T., Peck, L., & Barnes, D. (2011). Turning on the Heat: Ecological Response to Simulated Warming in the Sea. *PLoS ONE*, *6*.

Smale, D., Wernberg, T., Yunnie, A. L. E., & Vance, T. (2015). The rise of Laminaria ochroleuca in the Western English Channel (UK) and comparisons with its competitor and assemblage dominant *Laminaria hyperborea*. *Marine Ecology*, *36*, 1033-1044.

Sreekala, M. S. T., S. (2003).

Starko, S., Bailey, L., Creviston, E., James, K. A., Warren, A., Brophy, M. K., Danasel, A., Fass, M. P., Townsend, J. A., & Neufeld, C. (2019). Environmental heterogeneity mediates scale-dependent declines in kelp diversity on intertidal rocky shores. *PLoS ONE*, *14*.

Steneck, R., Graham, M., Bourque, B., Corbett, D., Erlandson, J., Estes, J., & Tegner, M. (2002). Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, *29*, 436 - 459.

Steneck, R., & Johnson, C. R. (2014). Kelp forests: dynamic patterns, processes, and feedbacks.

Stewart, H. L., Fram, J. P., Reed, D. C., Williams, S., Brzezinski, M., Macintyre, S., & Gaylord, B. (2009). Differences in growth, morphology and tissue carbon and nitrogen of *Macrocystis pyrifera* within and at the outer edge of a giant kelp forest in California, USA. *Marine Ecology Progress Series*, *375*, 101-112.

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

Swain, G. W., Herpe, S., Ralston, E., & Tribou, M. (2006). Short-term testing of antifouling surfaces: the importance of colour. *Biofouling*, *22*, 425 - 429.

Teagle, H., Hawkins, S. J., Moore, P., & Smale, D. (2017). The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology*, *492*, 81-98.

Thiel, M., & Vásquez, J. A. (2000). Are kelp holdfasts islands on the ocean floor? indication for temporarily closed aggregations of peracarid crustaceans. *Hydrobiologia*, *440*, 45-54.

Thomsen, M., Wernberg, T., Altieri, A., Tuya, F., Gulbransen, D., McGlathery, K., Holmer, M., & Silliman, B. (2010). Habitat cascades: the conceptual context and global relevance of facilitation cascades via habitat formation and modification. *Integrative and comparative biology*, *50 2*, 158-175.

Vásquez, J. (2007). Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery. *Journal of Applied Phycology*, *20*, 457-467.

Vásquez, J., Zúñiga, S., Tala, F., Piaget, N., Rodriguez, D., & Vega, J. M. A. (2013). Economic valuation of kelp forests in northern Chile: values of goods and services of the ecosystem. *Journal of Applied Phycology*, *26*, 1081-1088.

Vetter, E. W. (1995). Detritus-based patches of high secondary production in the nearshore benthos. *Marine Ecology Progress Series*, *120*, 251-262.

Voerman, S. E., Llera, E., & Rico, J. M. (2013). Climate driven changes in subtidal kelp forest communities in NW Spain. *Marine environmental research*, *90*, 119-127.

Wahl, M., Molis, M., Hobday, A., Dudgeon, S., Neumann, R., Steinberg, P., Campbell, A. H., Marzinelli, E., & Connell, S. (2015). The responses of brown macroalgae to environmental change from local to global scales: direct versus ecologically mediated effects.

Wehr, J., & Stein, J. (1985). Studies on the biogeography and ecology of the freshwater phaeophycean alga Heribaudiella fluviatilis. *Journal of Phycology*, *21*.

Wernberg, T., Bennett, S., Babcock, R., Bettignies, T. d., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C., Hovey, R., Harvey, E., Holmes, T., Kendrick, G., Radford, B., Santana-Garcon, J., Saunders, B. J., Smale, D., Thomsen, M., Tuckett, C. A., . . . Wilson, S. (2016a). Climate-driven regime shift of a temperate marine ecosystem. *Science*, *353*, 169 - 172.

Wernberg, T., Bennett, S., Babcock, R., Bettignies, T. d., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C. J., Hovey, R., Harvey, E., Holmes, T. H., Kendrick, G., Radford, B., Santana-Garcon, J., Saunders, B. J., Smale, D., Thomsen, M., Tuckett, C. A., . . . Wilson, S. (2016b). Climate-driven regime shift of a temperate marine ecosystem. *Science*, *353*, 169 - 172.

Wernberg, T., Kendrick, G., & Toohey, B. D. (2005). Modification of the physical environment by an *Ecklonia radiata* (Laminariales) canopy and implications for associated foliose algae. *Aquatic Ecology*, *39*, 419-430.

Wernberg, T., Krumhansl, K. A., Filbee-Dexter, K., & Pedersen, M. (2019). Status and Trends for the World's Kelp Forests.

Wernberg, T., Thomsen, M. S., Tuya, F., Kendrick, G. A., Staehr, P. A., & Toohey, B. D. (2010). Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. *Ecology Letters*, *13 6*, 685-694.

Xiao, X., Bettignies, T. d., Olsen, Y., Agustí, S., Duarte, C., & Wernberg, T. (2015). Sensitivity and Acclimation of Three Canopy-Forming Seaweeds to UVB Radiation and Warming. *PLoS ONE*, *10*.