Irish Seagrass Ecology and Habitat Mapping in the Context of Climate Change

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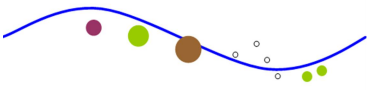

Algal BioSciences

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Acknowledgements

Before I begin thanking all the people who have helped me to have this great opportunity and to live this unique experience, I want to describe what this period has meant to me. I arrived at Galway in November 2013 from the Balearic Islands, with my baggage, without a single friend, without an idea about the city, not even a hostel booking and with my girlfriend (Silvia) in Spain. But something was telling me that it will be a good decision. After 5 years, I can absolutely ensure that this was one of the best decisions I have ever made in my life. I arrived here with two main aims, one learning English, the second, to get a PhD in any part of the world. I was not expecting to do it in Galway, I even did not know that there was an university in the city. After 3 emails to my current supervisor Dagmar Stengel, we met, wrote a proposal and the rest, as they say, is history.

During this time, I discovered a land of legends, songs, poets, writers and beautiful people. With Celtic ruins everywhere, medieval castles and outstanding landscapes. It was like being in "The Lord of the Rings". In fact, I always said that Ireland is like Hobbiton, with people talking, enjoying, drinking and laughing. But my story in this beautiful island is written with big adventures in the west coast "Searching for Seagrasses" with Silvia, Tomasso, Marc, Alvarito, Freddy, Ricardo, Tom... Discovering new seagrass meadows in the outermost areas of Ireland, snorkelling, diving and fishing in indescribable seascapes, with the company of dogfishes, spider crabs and sometimes seals. Several times I felt like an adventurer, going into these cold and savage waters and discovering unknown seagrass habitats. But also, great nights in pubs in the middle of nowhere, drinking Guinness and listening to this magical Irish music, surrounding by granddaddies, speaking and joking with them. I just can smile remembering all these great experiences. This country has given to me one of the most beautiful experiences ever, and I tried, at least in some way, to return my gratitude providing some knowledge that can be use for good purposes.

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General abstract

Seagrasses play an important ecological role worldwide, providing numerous ecosystems services. *Zostera marina* is a dominant meadow-forming seagrass in temperate regions in the northern hemisphere, including Irish coasts. This study primarily aimed at providing new ecological and spatial information on this species in Ireland and evaluated the potential of fatty acids as a physiological indicator of different environmental scenarios. Firstly, we assessed growth and population responses alongside with the fatty acid and photosynthesis pigment production in Irish *Z. marina* populations across seasonal and depth gradients. Our results revealed that Irish eelgrass populations displayed shoot and population dynamics similar to other shallow and deep-adapted perennial populations inhabiting similar latitudes and exposed to comparable climate regimes. Plants under colder and darker environmental conditions accumulated more total fatty acids (TFA) and also exhibited larger concentration of polyunsaturated fatty acids (PUFA) relative to saturated fatty acids (SFA). Additionally, the comparison of FA composition of *Z. marina* across its latitudinal distribution range (from southern Spain to Greenland) showed that southern populations adapted to warm *in-situ* seawater temperatures had significantly lower PUFA/SFA ratios than northern, cold-adapted populations. Furthermore, we studied both morphological and biochemical responses of *Halophila stipulacea* populations from Gulf of Aqava (Red Sea) across an irradiance gradient. Also, we performed two warming experiments; one with Irish *Z. marina* populations and a second with the Mediterranean seagrass species *Posidonia oceanica* and *Cymodocea nodosa.* Moreover, experimental and *in-situ* analysis of FA suggested that future warming may negatively affect the lipid nutritional value of *Z. marina* and the Mediterranean seagrass species; this may have implications for higher trophic levels. In combination, these results highlight the capacity of seagrasses to adjust their lipid composition to achieve optimal membrane fluidity under variable environmental conditions. Finally, we mapped large areas of previously undocumented seagrass meadows in the Irish coast by developing a new mapping approach, integrating species distribution models (SDM), satellite-derived images and field surveys.

This project is particularly relevant due to (i) the previous scarcity of knowledge available on seagrass ecology and spatial information in Ireland, (ii) the undisturbed status of the eelgrass meadows described, and (iii) the potential application of these baseline data in assessing impacts of anthropogenic disturbances or future climate change effects on these valuables ecosystems.

Abbreviations and Keywords

Abbreviations

Transect 1 (T1); Transect 2 (T2); Lettemore (LM); Tír an Fhia (TI); Finavarra (FV); Meadow 1 (M1); Meadow 2 (M2); Sea surface temperature (SST); Maximum (Max); Minimum (Min); Days (d); Minutes (min); Meters (m); Gas chromatography (GC); Fatty acids (FA); Essential fatty acids (EFA); Polyunsaturated fatty acids (PUFA); Saturated fatty acids (SFA); Mono unsaturated fatty acids (MUFA); Total fatty acids (TFA); Chlorophyll *a* (Chl. *a*); Chlorophyll *b* (Chl. *b*); Geographic information system (GIS); Species distribution model (SDM); Maximum Entropy (MAXENT); Gulf of Aqaba (GoA); Kapissillit (KA); Kobbejord (KB); Vouzas (VZ); Sada (SA); Difference (Dif); Community Climate System Model (CCSM);

Keywords

Seagrasses; *Zostera marina*; Temporal monitoring; Depth-acclimation; Plasticity; Field survey; Morphometric descriptors; Population structure; Annual productivity; Latitudinal comparison; Biochemical plasticity; Latitudinal responses; Fatty acids (FA); Essential fatty acids (EFA); Polyunsaturated fatty acids (PUFA); Saturated fatty acids (SFA); Mono unsaturated fatty acids (MUFA); Total fatty acids (TFA); Chlorophyll *a*; Chlorophyll *b*; Photosynthetic pigments; Lipid composition; Nutritional value; *Zostera noltii;* Maerl; Geographic Information System; Mapping; Species distribution models; Remote sensing; MAXENT; Ireland; North Atlantic; Temperate seagrasses; Mediterranean Sea; *Posidonia oceanica*; *Cymodocea nodosa*; Climate change; Heat wave; Warming; Fatty acid plasticity; Laboratory experiment; Acclimatization; Temperature; *Halophila stipulacea*; Gulf of Aqaba; Red Sea; Invasive species.

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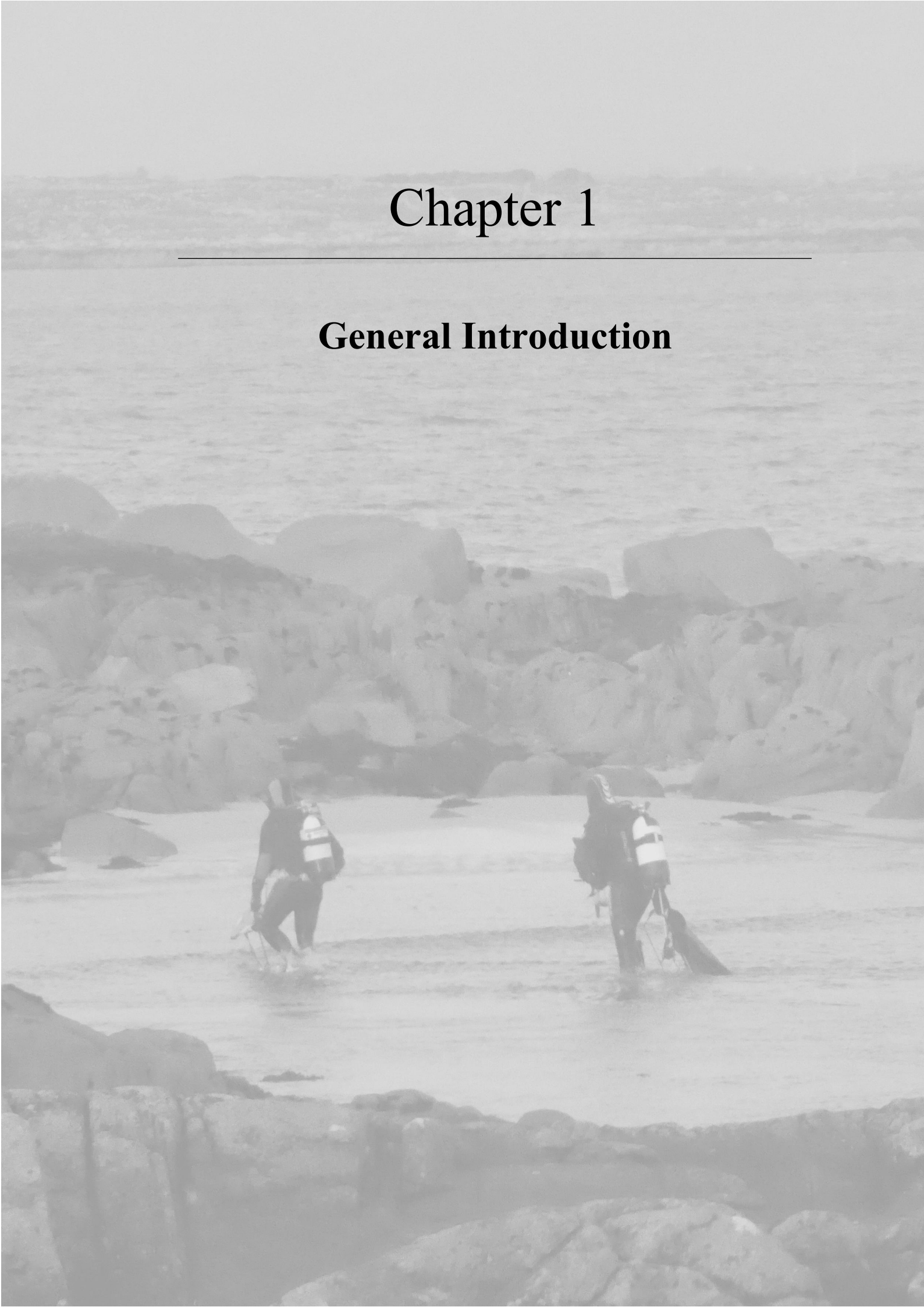
Chapter 1

General Introduction



1.1. Research questions of this PhD

The aims of this thesis were to address to the following questions:

- Which are the main ecological characteristic of Irish seagrasses regarding temporal growth and population dynamics?

- How do seagrasses adjust their fatty acid content and composition in response to variations in climate and other environmental conditions?

- What is the actual extent of seagrass meadows in Ireland?

- How do projected increases in temperature may affect the lipid nutritional value of seagrasses?

1.2. General aspects of seagrasses

Seagrasses are marine angiosperm inhabiting shallow and sheltered marine coastal environments (Duarte et al., 2002). These plants evolved from terrestrial plants in the Cretaceous period approximately 100 million years ago (Les et al., 1997). Genetic studies confirmed that seagrass lineages derived from monocotyledonous flowering freshwater plants and suggested an intricate evolution which may explain the low diversity seagrass species (Les et al., 1997). Seagrasses developed different adaptive mechanisms to cope with marine and salty environmental conditions (Olsen et al., 2016). For instance, marine angiosperms lost their stomata as they do not need to transport the water; they removed the info-chemical mechanisms to communicate with other plants and also eliminated their main defensive functions against grazers. On the other hand, marine plants developed sodium pumps to remove the excess salt in their tissues and created cell walls to favour gas exchange (Sand-Jensen et al., 2016).

Marine plants are monocots characterized by the presence of similar morphological above-ground and below-ground structures to terrestrial plants, such as, leaves, roots, rhizomes and flowers (Hemminga & Duarte 2000) (Fig. 1.1, 1.2, 1.3). Leaves perform the photosynthesis and are characterized by the presence of veins which

allow seagrasses to transport elemental compounds associated with the photosynthetic activity such as nutrients, minerals or gases to specific tissues (Alcoverro et al., 2001; Duarte 2002). Also, the roots enable seagrass nutrient uptake from the sediment, whereas some species can also obtain these compounds through their leaves (Larkum et al., 2006). Seagrasses accumulate different resources such as carbohydrates, fatty acids or nitrogen in their rhizomes, which can be utilized depending on physiological demands (Hemminga & Duarte 2000: Marba et al., 2002, 2006). The presence of roots and rhizomes in seagrasses allowed them to colonize and expand in soft sediments, which is generally an unsuitable substratum for seaweeds (Short et al., 2007). Through the rhizomes, seagrasses can extent both horizontally and vertically; they thus represent a mechanism to occupy new areas (Hemminga & Duarte 2000). Marine angiosperms can create mono to multi-specific meadows and can also develop from small patchy meadows to extensive beds of several km^2 (Short et al., 2007). Generally, seagrasses are distributed in shallow depths and sheltered sites, from intertidal to subtidal areas, being able to survive in depths higher than 65 meters, such as *Halophila stipulacea* in the Red Sea (Winters et al., 2017).

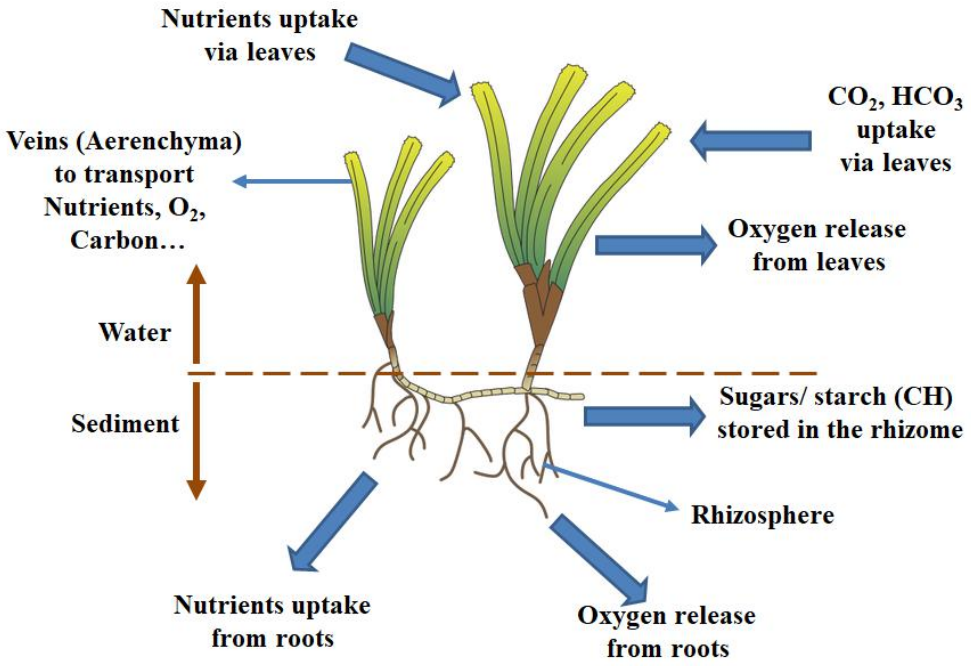


Fig. 1.1 Diagram of a seagrass shoot describing the different parts of the seagrass and indicating some of their main functions.

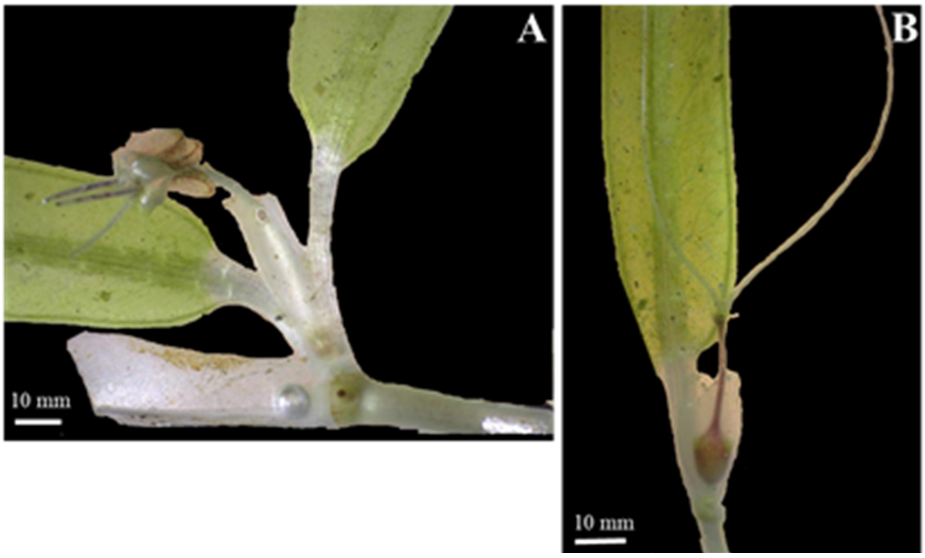


Fig. 1.2 *Halophila stipulacea* - examples of male (A) and female (B) flowers from Gulf of Aqava (GoA), Israel. Provided by Gidon Winters.

Commonly, seagrasses can spread to new locations by fragmentation or by developing new horizontal rhizomes, thus increasing the meadow size; this mechanism is known as clonal reproduction (den Hartog 1970; Duarte et al., 2006). Clonal growth and expansion reduces energetic demands and allows for longer survival (Ackerman 2006). Marine plants can also colonize new areas throug sexual reproduction, hence producing seeds which can later germinate in newsites (den Hartog,1970; Duarte et al., 2006). The main limitation of their sexual reproduction is the flower pollination due to the scarcity of pollinators in marine waters (van Tussenbroek et al., 2012, 2016). The reproductive effort varies depending on climate responses and amongst species. For example, *Zostera marina* is characterized by a high presence of reproductive shoots, therefore producing large quantities of seeds and seedlings (Kuo et al., 1991). Seeds can travel several km from their formation site to the location of germination, and thus representing an effective mechanism for seagrass dispersion (Olesen et al., 1994).

1.3. Seagrass families

Seagrasses are not a taxonomic entity; in fact, "seagrass" is an ecological term, and the many seagrass families are not necessarily genetically linked (Kuo & den Hartog 2006; Green & Short 2003). To be classified as a "seagrass", the angiosperm must live in submerged marine environments, anchored to the sediment through their rhizomes and roots, and must have a hydrophilous pollination strategy (Kuo & den Hartog 2006). There are only four families of seagrass, and only three of them are considered true seagrasses (Posidoniaceae, Zosteraceae and Cymodoceaceae); the family Hydrocharitaceae contains some species that live in marine ecosystems and others in freshwater systems (Kuo & den Hartog 2006). These four families encompass 12 genera including 55 - 60 species (Larkum et al., 2006). The incorporation of another two families, Ruppiaceae and Zannichelliaceae to the seagrass group is still up for debate as some species (*Ruppia chirrosa* and *maritima* and *Lepilaena marina*) do not occur exclusively in marine ecosystems but also in freshwater (Kuo & den Hartog 2006).

The family Zosteracea comprises the two major temperate seagrasses in the northern hemisphere, *Zostera marina* and *Z. noltii*, which are the two most representative species in Ireland (Short et al., 2006). These species are commonly observed from intertidal to subtidal areas, at maximum depths of 10 meters, inhabiting sheltered areas such as estuaries or bays. *Z. marina* is a medium-sized seagrass while *Z. noltii* is a small-sized species (den Hartog 1970).

1.4. Species of interest in this study

1.4.1. *Zostera marina*

Zostera marina (Linnaeus) 1753, commonly known as eelgrass, is distributed from subtropical to Arctic regions, and is distributed in the Mediterranean Sea, the Temperate North Atlantic and Temperate North Pacific (Short et al., 2007). This species is characterized by creating shoots from 10 cm to more than 1 meter long

(Green & Short 2003). Due to its broad distribution, *Z. marina* is able to adapt to a wide range of environmental conditions, rendering this species an exceptionally resilient character and a great plasticity in terms of growth, productivity, population dynamics or reproductive effort (Marsh et al., 1986). Its conservation status by the International Union for Conservation of Nature (IUCN) is considered as "the least concern" (LC) (http://www.iucnredlist.org). LC are species which has been categorized by the IUCN as evaluated but not classified in other category, such as, threatened, near threatened, or conservation dependent.

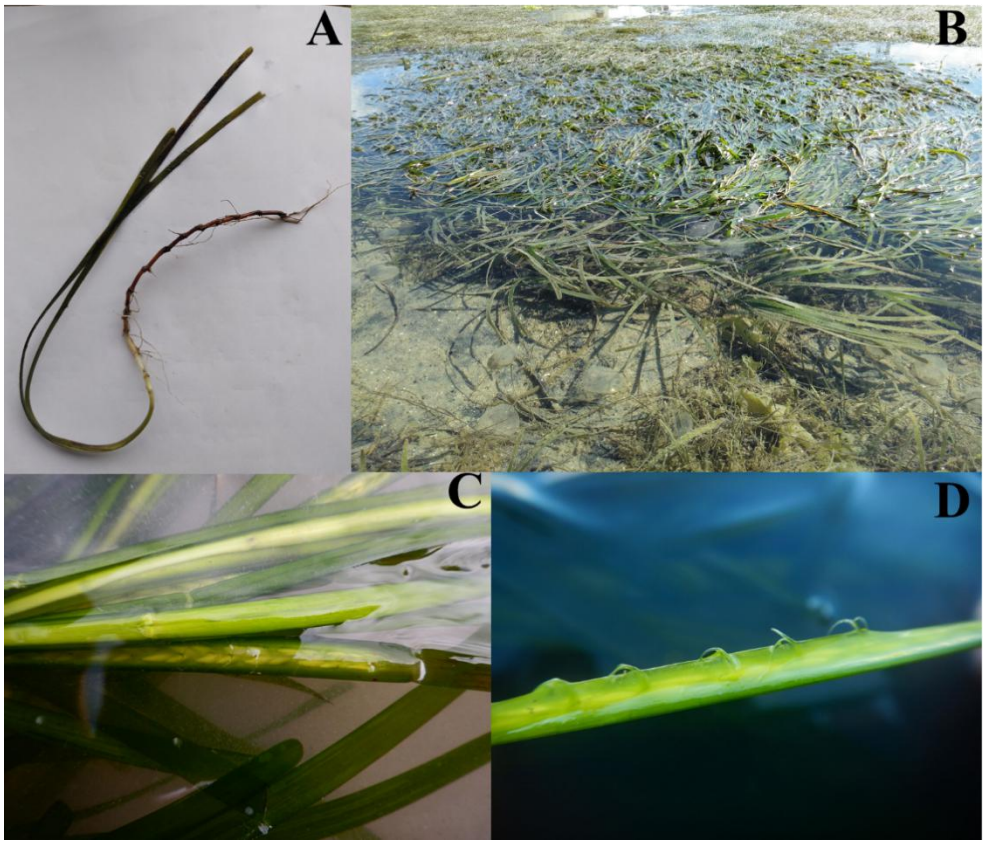


Fig. 1.3 Photograph of Irish *Zostera marina* (A and B) and reproductive structures (C and D).

1.4.2. *Zostera noltii*

Zostera noltii (Hornemann) 1832, is mainly found in intertidal or shallow subtidal coastal waters, mainly located in sheltered sites such as estuaries or lagoons (Peralta et al., 2005). This species is distributed from subtropical regions such as Cape Verde and desert areas on the coast of Mauritania, to the Baltic Sea in Sweden or Norway in North Europe. This species grows up to 18-22 cm long, characterised by having 3 to 6 leaves per shoot with a leaf width of 1-3 mm. *Z. noltii* grows preferentially in soft sediments such as mud or fine sand, and can tolerate a wide range of salinity (Sousa et al., 2017). Sometimes this species forms monospecific meadows or can cohabitate with *Z. marina* or *Ruppia* species. Its conservation status by the International Union for Conservation of Nature is considered as "the least concern".

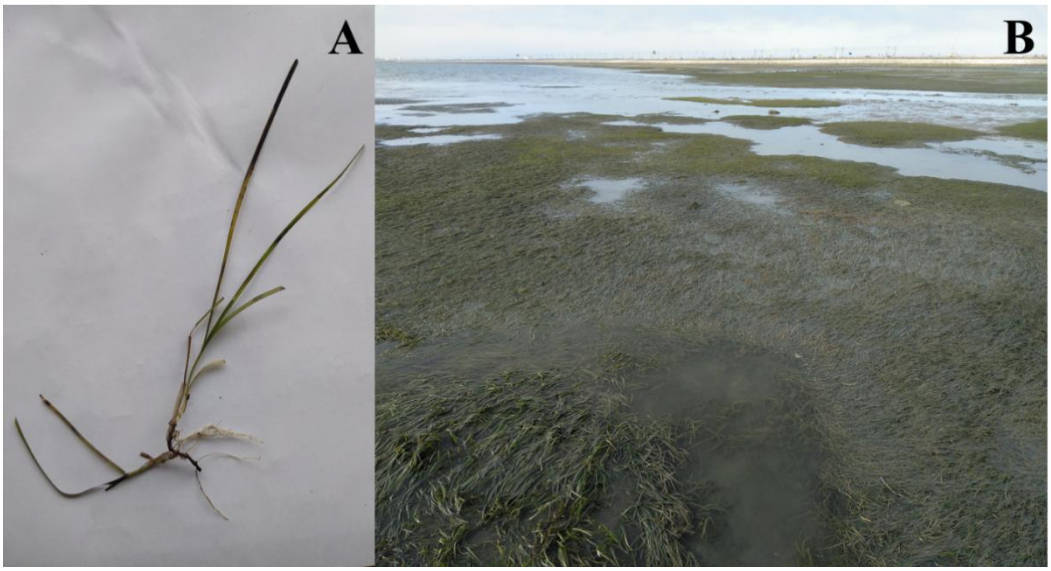


Fig. 1.4 Photograph (A) and (B) of Irish *Zostera noltii*.

1.4.3. *Halophila stipulacea*

Halophila stipulacea (Forsskål) Ascherson 1867, belongs to the family Hydrocharitaceae. This species is a small tropical dioecious marine angiosperm native to the Indian Ocean and Red Sea, distributed in subtidal marine areas. This species is characterized by a linear oblong and membranous blade (up to 60 mm long). *H. stipulacea* is capable of acclimatising to a wide range of environmental gradients including temperature, salinity, irradiance and nutrient concentration (Sharon et al., 2011; Lipkin et al., 2003). *H. stipulacea* is currently found in non-native locations in the Mediterranean for the last 150 years (a Lessesspian migrant) (El Shaffi 2011). In 2002, this seagrass species was also observed in the Caribbean Sea and even on the South American continent. It is currently considered one of the most successful invasive seagrass species worldwide (Winter et al., 2017). Its conservation status by the International Union for Conservation of Nature is considered as "the least concern (LC)" (http://www.iucnredlist.org).

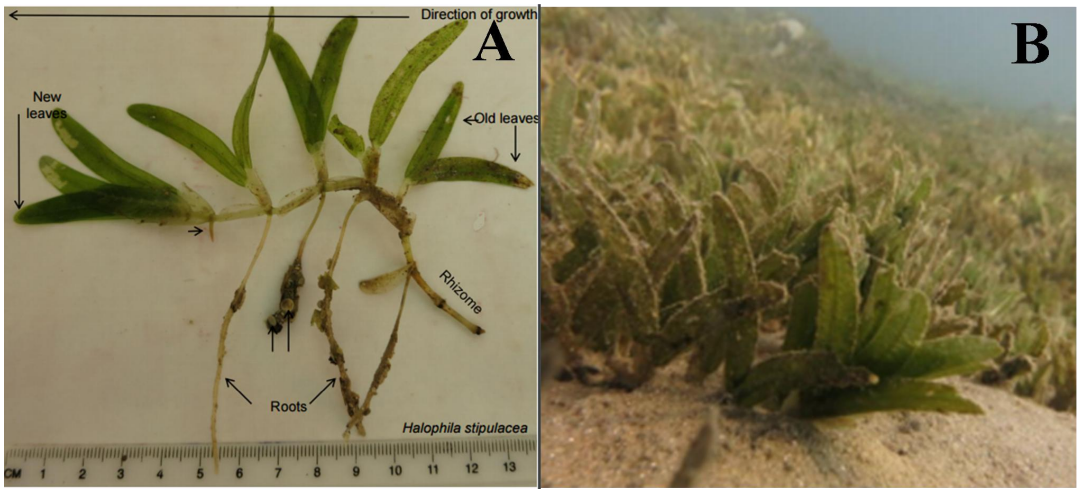


Fig. 1.5 Photograph (A) of *Halophila stipulacea* provided by Gidon Winters

1.4.4. *Posidonia oceanica*

Posidonia oceanica (Linnaeus) Delile 1813, belongs to the family Posidoniaceae. *P. oceanica* is an endemic species to the Mediterranean Sea and, there is the most dominant species in infralittoral seafloors to a maximum depth of 25–40 m (Procaccini et al., 2003). It is a large, long-lived (Arnaud-Haond 2012) seagrass with slow growth and recovery rates (Duarte et al., 2006), making it particularly vulnerable to environmental disturbances, such as eutrophication or heat waves (Marbá et al., 2005). Their leaves are ribbon-like and can grow up to 1.5 metres with an average leaf width of 10 mm. Declines in the extent of *P. oceanica* meadows have been widely reported mainly caused by the anthropogenic impacts of coastal development (Boudouresque et al., 2000). Its conservation status by the International Union for Conservation of Nature is considered as "the least concern".

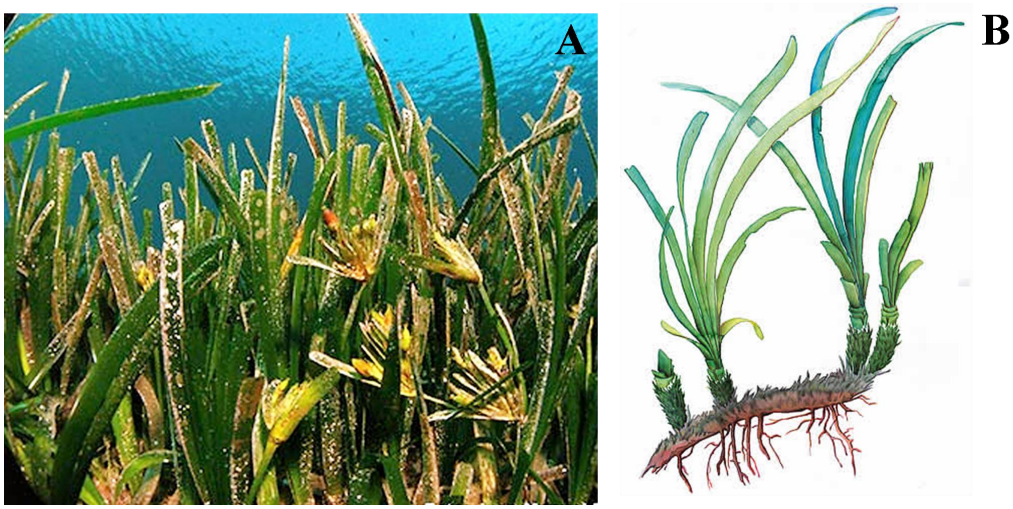


Fig. 1.6 Photograph (A) and drawing (B) of *Posidonia oceanica* provided by Juan Manuel Ruiz

1.4.5. *Cymodocea nodosa*

Cymodocea nodosa (Ucria) Ascherson 1870, belongs to the family Cymodoceaceae. This plant is the second most abundant seagrass species in the Mediterranean Sea, and its distribution ranges from the Atlantic coast in the southern Iberian Peninsula to subtropical areas such as Senegal and Cape Verde (Short et al., 2007). This is a small seagrass in size, adapted to a wide range of coastal habitats with contrasting regimes of salinity, temperature and nutrients. It is found from semi-exposed areas to enclosed coastal lagoons. It is characterized by high growth rates, high phenotypic plasticity, high resilience and rapid recovery from disturbances (Olesen et al., 2002; Duarte et al., 2006; Sandoval-Gil et al., 2014). Its conservation status by the International Union for Conservation of Nature is considered as "the least concern".

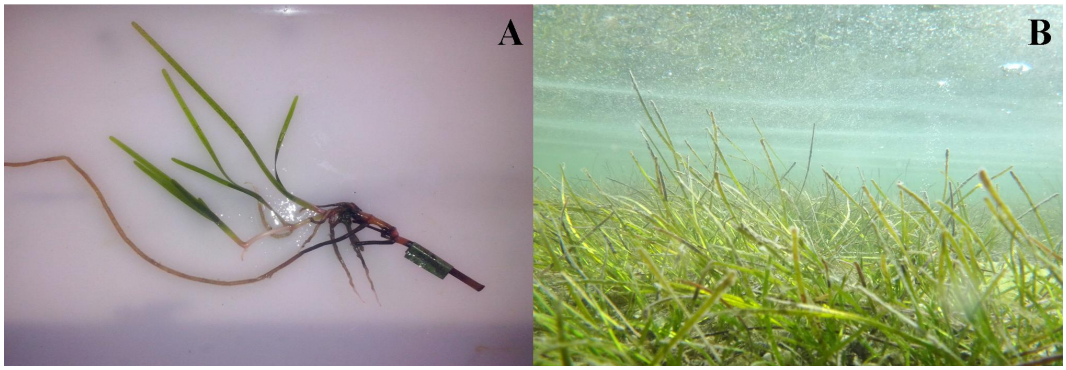


Fig. 1.7 Photograph (A) and (B) of *Cymodocea nodosa* from the Bay of Cadiz (Spain).

1.5. Ecological functions and services of seagrasses

Seagrasses are engineering species that can modify the physical environments, such as altering the current velocity or sediment characteristics, thus modifying their habitat structure (Hemminga & Duarte 2000). This capacity renders seagrasses to have a key role in structuring marine habitats and creating ecologically important three-dimensional systems (Costanza et al., 1997). Marine angiosperms are recognized as vital ecosystems because of the wide variety of ecological services that

they provide compared to other charismatic ecosystems such as tropical rainforests and coral reefs (Duarte & Chiscano 1999; Nordlund et al., 2016, 2017). Marine plant meadows are considered a hot-spot of biodiversity supporting high productivity (Dale et al., 2007). For instance, they provide habitat and shelter for marine species; it is reported that around 50 % of commercial species live in seagrass meadows at some stage of their lives, with seagrass representing and important food source and providing a nursery habitat for these species (Green & Short 2003; Heck et al., 1984). Also, seagrasses are the most important food source for some charismatic animals such as turtles, dugongs (*Dugong dugon*) or manatees (*Trichechus manatus*) (Short et al.,2007).

Marine plants are recognized as one of the most important carbon sinks worldwide being capable of capturing great amounts of inorganic carbon and storing it in the sediment as organic matter, through to be accumulating around 10-12 % of the global CO_2 emissions (Fourqurean et al., 2012). Annual seagrass carbon accumulation is estimated to vary between 48 and 112 Tg C yr^{-1} (Mcleod et al., 2011), and they may sequester up to 3,000 tonnes every year (Duarte et al., 2013). As a result, these marine angiosperms are considered one of the most effective ecosystems worldwide mitigating the effects of climate change (Duarte et al., 2013). Also, seagrasses improve water conditions by removing the excess of nutrients, sediments and suspended debris from the water column (Chen et al., 2007; Orth et al., 2006; Duarte, 2002). Particularly, reducing the excess of nutrients in the environment is highly relevant to decrease the risk of eutrophication (Cloern 2001). Moreover, seagrasses protect the shoreline by creating a physical barrier against hydrodynamic forces such as erosion or wave action (Chen et al., 2007; Granata et al., 2001). In this sense, previous studies reported it effectiveness to protect neighbouring habitats during storms or hurricanes (Guannel et al., 2016).

Interestingly, a recent study proved the capacity of marine plants to reduce the bacterial pathogens for different marine animals and humans (Lamb et al., 2017). Due to its responsivity to depletions in water quality related to eutrophication events or reductions in water quality, seagrasses represent an important ecological indicator (Krause-Jensen et al., 2005). Based on the ecological services and goods that marine

plants provide, their annual economic value has been quantified as $ 34,000 per hc^2 (Costanza et al., 1997). It is noteworthy that different seagrass species provide different ecological services. For instance, in temperate seagrasses these ecological functions may differ from one season to another as their morphological structure changes significantly during the annual seasons; thus, there are still several gaps that may need to be addressed regarding their ecological services (Nordlund et al., 2017). Unfortunately, in spite of the relevant role that seagrass ecosystems play worldwide, they have not received the international recognition that they deserve; thus, it is crucial for researches to spread to message of their ecological value to a wider audience (Nordlund et al., 2017).

1.6. Global seagrass distribution

Seagrasses represent 0.02 % of phanerogam species worldwide (Hemminga & Duarte 2000). They are present in all the marine coastal areas worldwide with the exception of the Antarctic (den Hartog 1970). Its distribution covers approximately 600,000 km^2 which corresponds to 0.1 - 0.2 % of the global ocean seafloor, however, different studies predicted that its distribution may cover more than 1,000,000 km^2 (Short et al., 2007). The lack of this spatial distribution can be attributed to (i) the difficulty of mapping subtidal deep meadows and (ii) the incapacity to access remote areas, mainly in undeveloped countries (Hemminga & Duarte 2000). The largest density of seagrasses was found in tropical and subtropical areas; the highest density globally being reported in Shark Bay, western Australia, with 13 different species present (Short et al., 2007). In temperate and colder regions, in both northern and southern hemispheres the seagrass density is markedly lower than at warmer latitudes.

1.7. Environmental drivers controlling the seagrass performance

Marine plants are highly sensitive to fluctuations in environmental conditions adjusting their metabolism, growth and productivity (Olesen et al., 2013). Their

growth and productivity is mainly environmentally-regulated, with temperature and irradiance representing the most important environmental factors controlling their physiological and growth performance (Diaz- Almela & Marba 2007). Under optimal temperatures, growth rates and productivity are enhanced, maximizing carbon uptake and releasing large amounts of oxygen (Greve & Binzer 2004). Optimal temperatures for seagrasses differed among species and populations, resulting in local adaptation to *in-situ* conditions (Duarte 2002). For instance, *Z. marina* is the species capable of surviving at the coldest temperatures (-2 ˚C) in Arctic regions (Olesen et al., 2015), whereas some subtropical and tropical species can healthily live at temperatures higher than 28 ˚C, such as *H. stipulacea* in the Red Sea (Greve & Binzer 2004). Moreover, recent studies have showed that populations of *Z. marina* living at different temperature regimes respond differently to projected warming scenarios, with plants living at *in-situ* warmer conditions being less vulnerable than colder adapted populations (Winters et al., 2011). When temperature for growth and production exceeds optimal conditions, seagrasses respiration rates increase, and oxygen production is reduced. If unfavourable thermal conditions persist over time (days or weeks), marine plants may limit their growth rates and start consuming their energetic reserves (carbohydrates) which can result in local die-backs and population collapse (Alcoverro et al., 2001). Furthermore, light energy is considered the most important factor determining the seagrass vertical growth as irradiance controls photosynthetic processes. Marine plants are reported to live in depths receiving approximately 3 to 11 % of total surface irradiance (Duarte 1991; Winters et al., 2017). In fact, these requirements greatly differ between species; for instance, in the Indian River Lagoon (USA), *Halodule wrightii* required a minimum of 24 - 37 % of surface irradiance to survive (Kenworthy & Fonseca 1996). Other factors, such as, nutrients or substratum types, can also play a relevant role explaining the presence of seagrasses. They have a higher affinity for soft and medium sized sediments that allow and effective anchor, such as mud or sand (Peralta et al., 2005, 2006), whereas they can settle only exceptionally in coarse or hard substrata.

1.8. Seasonal variability of temperate *Zostera marina*

Seagrass growth, production and distribution are controlled by the chemical, physical and biological processes of the habitats they occupy (Greve & Binzer 2004). Therefore, seasonal climate gradients control the life cycle of seagrasses such as *Z. marina* or *Z. noltii*. Generally, temperate *Z. marina* populations exhibit marked temporal growth with higher productivity and growth rates, mainly associated with increases in their active leaf structures observed in warmer months, with higher day light hours (i.e. Olesen et al., 2002). During these seasons photosynthetic processes are favoured and seagrasses can store energy compounds such as carbohydrates (Bay et al., 1996; Alcoverro et al., 2001). In spring and summer, the largest shoot density was commonly reported mainly related to the seed germination and seedlings of new shoots (Boström et al., 2003). By contrast, in colder and darker periods, seagrasses readjust their shoot size by decreasing their productivity and reducing photosynthetic tissues, thus maintaining low metabolic levels and utilising existing energy reserves (Olesen et al., 2002; Lee et al., 2005). Also, a depletion in the shoot density was observed during less favourable environmental conditions (Boström et al., 2003). Accompanied by these temporal fluctuations in shoot and population level, biochemical adjustments in seagrass structures also occur in response climate changes. For instance, it was reported that seagrasses reduce pigment production under higher irradiance, whereas they increase their synthesis in darker periods to ensure optimal light absorption (e.g. Cummings et al., 2003; Olive et al., 2013). Other studies addressed peripherally seasonal responses of fatty acid (FA) production and composition under different climate conditions in marine plants (e.g. Viso et al. 1993; Sanina et al., 2004). These studies reported increases in polyunsaturated fatty acids (PUFA) and reductions in saturated fatty acids (SFA) which were linked to decreases in temperature and reductions in irradiance.

1.9. Depth-adaptation of *Zostera marina*

Light energy controls the vertical distribution of seagrasses, generating morphological and biochemical changes and influencing their population structure (Ralph et al., 2007). In temperate regions, light energy limitation for eelgrasses is most pronounced in deep-adapted populations where carbon budget is only just positive on an annual basis (Dennison 1987). At high latitudes, deep-adapted populations are generally characterized by low densities but greater shoot size than shallow populations (Olesen et al., 2017). This adaptive strategy allows plants to reduce shelf-shading and absorb more light, thus favouring a deeper colonization (Olesen et al., 2017). Alongside these morphological and population acclimatizations, marine plants adjust their biochemical composition, such as in pigments and carbohydrates (Alcoverro et al., 1995). For instance, increments in pigments were positively correlated with increases in depths (Cummings et al., 2003; Zimmerman et al., 1995). It is noteworthy, that temperate *Z. marina* populations inhabiting the deepest edges of the meadows, maintained leaf structures in colder and darker periods, suggesting that they continue performing some photosynthetic activity (Evans et al., 1986; Olesen & Sand-Jensen 1993).

1.10. Current status and main threats

Current estimations suggest that 30-50 % of global seagrass distribution has disappeared over the last few decades, with an annual rate of decline of 7 % since 1990 (Orth et al., 2006; Mcleod et al., 2011; Waycott et al., 2009). Seagrasses are mainly distributed from intertidal to shallow areas, thus, they are particularly subject to anthropogenic disturbances and climate change effects (den Hartog 1970). Coastal construction, industrialisation development or water euthropication are some the main anthropogenic activities causing losses in marine plant habitats, whereas its decline is a result of multiple stressors (Duarte 2002; Orth et al., 2006; Waycott et al., 2009). For seagrasses inhabiting temperate regions, depletions in water quality related to eutrophication processes are reported to be the main cause generating seagrass losses (Orth et al., 2006).

One of the species which has experienced the largest habitat lossglobally is *Z. marina.* For instance, in 1930 a fungus (*Labyrinthula zostera*)infection generated its highest documented decline in the North Atlantic (den Hartog1987; Muehlstein, Porter & Short 1991). This problem was also linked to the seagrass disappearance in other regions in the North Atlantic (den Hartog 1987). To date, many of these areas have been recovered and there are also current projects following this process of habitat restoration.

The effects of climate change are currently observed among different species of primary producers worldwide. Specifically, increases in water temperature are driving the metabolic and physiological responses of marine primary producers with implications for their growth, productivity and distribution (Marsh et al., 1986; Sang & Park 2005). Recent observations highlighted that variations in thermal regimes are leading to changes in the seagrass distribution (Hogarth 2007). For instance, in the Arctic, the sea-ice is thinning and melting due to the reported increases in sea surface temperature (SST), allowing light to penetrate to deeper areas, therefore improving the environmental conditions for macrophytes such as seagrasses and kelps (Krause-Jensen & Duarte 2014; Olesen et al., 2015). Similarly, recent studies observed that vegetation in colder habitats is expanding towards the poles, such as in kelp species (Müller et al., 2009). On the contrary, southern populations of *Z. marina* are experiencing thermal stress conditions due to anomalously high summer temperatures exceeding 40 °C, thus generating die-backs with occasional local population extinctions (Moore et al., 1996; Reusch et al., 2005). In temperate regions, experimental evidence suggested that cold-adapted Danish populations may be negatively affected by projected increases in temperature by the end of this century (Beca-Carretero et al., 2018*b*). Therefore, further investigations are necessary to understand how projected climate change will may affect seagrass species on local or global scales.

1.11. Conservation, monitoring and restoration

1.11.1. Conservation

The international growing awareness of the importance of seagrass and the decline of their habitats have generated their inclusion in government programmes, thus developing legislative, protective and conservational policies (i.e. Duarte 2002; Orth et al., 2006). For instance, several countries have implemented measures to mitigate seagrass habitat destruction with some nations introducing zero-loss environmental policies (i.e. Duarte 2002; Orth et al., 2006). In the European Union (EU), *Zostera* habitats are protected by the Annex I of the European Habitat Directive, 1992. Also, *Z. marina* beds were added in 2004 to the List of Threatened and/or Declining Species and Habitats produced by OSPAR. Moreover, within the EU, seagrasses are used as a Biological Quality Element (BQE) under the Water Framework Directive (WFD) (Foden 2007; EC 2000). In Ireland, since 2006, the Environmental Protective Agency (EPA) initiated monitoring programs in intertidal seagrass beds as part of the European WFD and the River Basin Management Plan (RBMP) (Foden & Brazier 2007). Strategies for seagrass monitoring in the United Kingdom and Ireland (UK-IE) were also developed by the Marine Plant Task Team (MPTT) (Foden & Brazier 2007; Uktag 2014).

The mapping of target species has been identified as a key aspect in monitoring potential changes in habitat distribution included seagrasses. Novel geographic information tools have emerged that allow the mapping of new seagrass habitats and evaluating temporal spatial variations in seagrass. For instance, species distribution models (SDM) have been implemented to assess optimal habitat conditions for seagrass distribution and restoration (Valle et al., 2013, 2014). These models allow for an understanding of the environmental parameters which better define the presence, and also create maps of the potential habitat suitability for the target species. SDM techniques are more commonly used to localize deep-adapted populations which cannot be located by applying satellite or drone images (Valle et al., 2014). Potentially, the integration of diverse map techniques may allow

researchers to create new strategies to discover new seagrass meadows and potentially reduce project costs, as was previously addressed by Winters et al., (2017).

1.11.2. Monitoring

The monitoring of seagrasses has been identified as a key step to assess the health status of marine coastal areas due their rapid response to reductions in their environmental status, such as nutrient increases or irradiance reductions (Mckenzie et al., 2012). Different monitoring methods and strategies can provide a picture of habitat quality, but it is important to define the adequate methodology in relation to the particular aims of the study (Krause-Jensen et al., 2004). A large quantity of monitoring methods are used to evaluate the habitat status; from changes in biochemical composition, to variations in landscape patterns using satellite derived images. General monitoring strategies have been focussed on assessing key aspects at both shoot and population levels, such as changes in morphometry and productivity or meadow dynamics of seagrasses (Borum et al., 2004). Over the last number of decades, different biological and physiological approaches have been widely applied to marine plants to assess their ecological status including, isotope, nutrient or phenol content (e.g. Rottini et al., 2017). As seagrasses are considered important indicator species, it is crucial for the research community to identify those key responsive aspects which may help to recognize early signs of stress or vulnerability (Krause-Jensen et al., 2007).

1.11.3. Restoration

Several seagrass monitoring and restoration projects have been conducted globally since the 1970s in response to dramatic losses in seagrasses experienced in the last century (Paling et al., 2009). The disappearing of these ecosystems is accompanied by the loss of all the associated ecological services, and biodiversity, generating relevant economic losses (Waycott et al., 2009). To date, more than 450 seagrass

restoration programmes have been performed with different rates of success. Commonly, restoration projects are conducted at small scales (< 1 ha^2) as these initiatives require a great economic effort (Paling et al., 2009). A restoration trial is considered to be successful when the seagrasses are established in the target location two years after the beginning of the programme (van Katwijk et al., 2009). To date, 26 seagrass species have been employed in these programmes, with *Z. marina* utilized in more than 50 % of these restoration projects (Short et al., 2002). Different approaches and strategies have been commonly used with different levels of success; the type of transplantation method or the number of samples transplanted explain this variability. To date, it is estimated that 30 % of restoration projects have succeeded (Fonseca et al., 2001; van Katwijk et al., 2009). A recent systematic review (van Katwijk et al., 2016) reported that best performances were related to anchoring techniques and application of weights such as seedlings, rhizome fragments or sand bags to shoots. However, worst results were obtained using no anchoring approaches. The same review also found that there is a correlated relationship between the number of specimens planted (plants or seeds) and the trial success. The authors indicated that a threshold of 1000 specimen is necessary to guarantee a positive population survival. Overall, despite the growing efforts to mitigate seagrass losses worldwide and the social awareness of their ecological relevance, their decline is continuing (van Katwijk et al., 2009). In Ireland there are no records of seagrass habitat destruction, which can be attributed to the healthiness of the Irish populations or to the lack of continuous monitoring programs to identify habitat loss. However, initiating monitoring and management projects in Ireland is crucial to assess potential effects of anthropogenic activities and climate change, and also due to the current scarcity of available seagrass ecological information.

1.12. Fatty acids as an eco-physiological tool

Primary producers biosynthesise the majority of fatty acids (FA) which are later transferred to higher trophic levels (Behrens et al., 1996; Dalsgaard et al., 2003). FA are part of the membrane structure and also represent an important energetic source

in plants and algae (Rabbani et al., 1998; Mendoza et al., 1999; Klyachko-Gurvich et al., 1999). FA are formed with a carboxylic acid group and with an aliphatic chain of carbon atoms which can be either unsaturated or saturated. Monounsaturated fatty acids (MUFA) (Fig. 1.8) are characterised by the presence of one double bond in the fatty acid chain. MUFA are mainly portioned into structural lipids, forming the membranes of the cellular organelles, such as Golgi apparatus or endoplasmic reticulum. Most common MUFA in many primary producers are palmitoleic acid (16:1 n-7), cis-vaccenic acid (18:1 n-7) and oleic acid (18:1 n-9). Polyunsaturated fatty acids (PUFA) are characterised by the presence of more than one double bond in the fatty acid chain (Fig. 1.8). More common PUFA in primary producers are linoleic acid (18:2 n-6) or α-Linolenic acid (18:3 n-3) (Schmid et al., 2014). PUFA are mostly partitioned into structural lipids (glycolipids and phospholipids), particularly forming part of the thylakoid membranes of chloroplasts, promoting their optimal fluidity and favouring electron transport among photosystems (Gombos et al., 1994; Sanina et al., 2008). By contrast, saturated fatty acids (SFA) have no double bonds and are mainly partitioned into triacylglycerols (TAG) as storage compounds (Fig. 1.8). The most abundant SFA in primary producers, including seagrasses are myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0) (Sanina et al., 2008).

Essential FA (EFA) are those FA that consumers cannot biosynthesise and need to obtain from food sources (Charpy-Roubaud & Sournia 1990; Iken et al., 1998; Dalsgaard et al., 2003). FA play different key physiological and structural roles in the life stages of consumers such as in reproduction or fertility (Rodríguez et al., 2004; Bell et al., 1994; Sorbera et al., 1998). FA production and its biological importance have been extensively investigated in microalgae, seaweeds or terrestrial plants (Khotimchenko et al., 2002; Sanina et al., 2004). The use of FA synthesis to assess the eco-physiological status of primary producers has been widely applied in agricultural species with commercial interest (e.g. Murakami et al., 2000; Vigh et al., 1998). Also, variations in FA composition (i.e. n-6/n-3 and PUFA/SFA ratios) have been used as a health indicator in terrestrial and marine organisms (French et al., 2000; Pommier et al., 2012; Parrish 2009).

To date, FA in marine plants have been primarily used as qualitative markers to investigate trophic relationships between species (Auel et al., 2002). Some studies have investigated the responses of lipid metabolism and FA synthesis in response to environmental variations, such as, irradiance, temperature or salinity (e.g. Beca-Carretero et al., 2018*a* and *c*; Viso et al., 1993; Sanina et al., 2004; Sousa et al., 2016). In aquatic plants, FA metabolism is strongly regulated by environmental factors (Viso et al., 1993); and therefore, varies in accordance to lipid and fatty acid composition to climate gradients (Guschina & Harwood 2006; Gerasimenko et al., 2011). Previous studies on marine primary producers, including seaweeds and marine plants, observed an increase of PUFA levels with a decrease of temperature and irradiance, whereas an increase in SFA was observed with increases in temperature (Gosch et al., 2015; Schmid et al., 2017*a*; Beca-Carretero et al., 2018*b*). Recent studies suggest that future warming could markedly affect the nutritional value of seagrasses, a reduction in the synthesis EFA (i.e. ALA and LA) pigment concentration and carbohydrates, may generate changes in the herbivore diet preferences (Hernán et al., 2017; Beca-Carretero et al., 2018*a* and *b*).

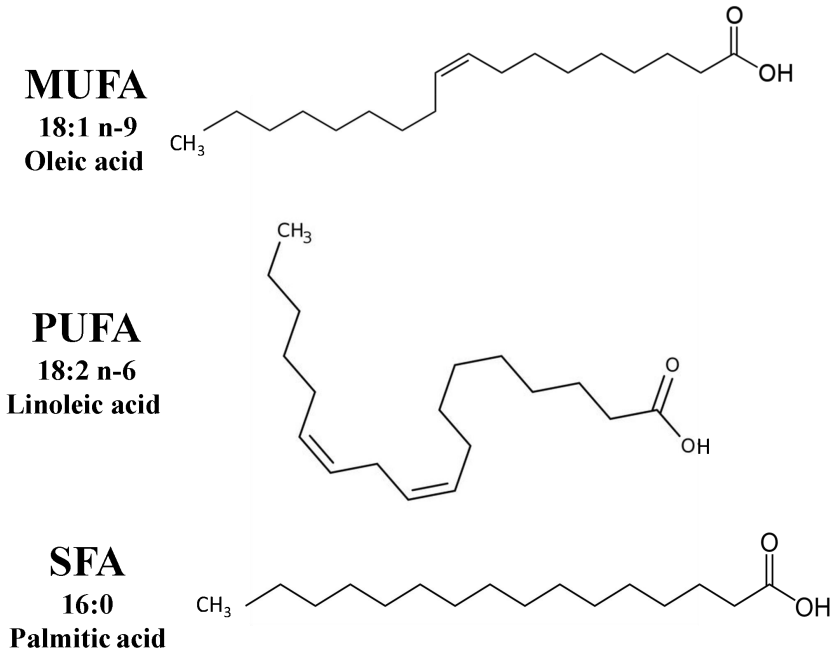


Fig. 1.8 Drawing of monounsaturated fatty acid (MUFA), polyunsaturated fatty acid and saturated fatty acid (SFA).

1.13. Seagrasses in Ireland

Current biological, ecological and spatial information on Irish seagrasses is scarce and sometimes outdated. Early studies of Irish seagrass biology and ecology were conducted in the 1980s and 1990s (Cullinane et al., 1985; Whelan & Cullinane 1985; Whelan 1986; Dawes & Guiry 1992; Madden et al., 1993) which reported on seagrass density and associated algae communities in Co. Cork and Co. Kerry. Some recent studies addressed key biological and ecological aspects of these ecosystems (Robinson 2003; Dale et al., 2007; Jones & Unsworth 2016; Wilkes et al., 2017). Of these studies, only Dale et al (2007) provided information regarding shoot growth and population structures and dynamics and associated macrofaunal communities.

Regarding the spatial information in Ireland, previous studies mapped their distribution of both intertidal and subtidal populations as part of general seabed mapping efforts over the last few years; such as, the MESH Atlantic project (http://www.emodnet-seabedhabitats.eu/map), the Site-Specific Conservation Objectives Marine Community Types project (ttps://data.gov.ie), and the OSPAR Habitats project (http://www.ospar.org/). These studies commenced in 1997 as part of the BioMar program and were later continued by the Marine Institute survey in 2010-2011 (Tully & Clarke 2012). However, a large distribution of seagrass meadows may remain undiscovered due to their relative inaccessibility and the generally low anthropogenic impacts in remote coastal areas in western Ireland where large virgin and unspoiled seagrass meadows may have persisted until the present day.

There are some reports from different environmental organizations which provide information on Irish seagrasses (i.e. MERC, 2005; NPWS, 2014; EPA, 2015). However, there is a lack of current depth-based knowledge of Irish seagrass ecology which is required to develop a baseline for the future management of seagrasses

1.14. Hypotheses and objectives of this study

In this PhD we have formulated the following hypotheses:

Hypothesis 1. The Irish climate is defined as a temperate oceanic climate, characterized by seasonal environmental periods with a lack of extreme cold or warm thermal gradients. Therefore, Irish eelgrasses may show a discernible temporal pattern regarding shoot growth and population dynamics compared to other perennial seagrass populations inhabiting similar latitudes and exposed to similar environmental conditions. Moreover, we propose that plants living in their deepest distribution limit may show different temporal morphological properties and population dynamics which are different from plants in shallow sites.

- We investigated these assumptions by (i) characterizing the temporal changes of plant descriptors at shoot and population levels in three populations from western Ireland (Chapter 2) and also by (ii) studying the adaptations to depth-related environmental conditions by comparing temporal performance of plants from different depths (Chapter 2).

Hypothesis 2. Biochemical adjustments in seagrasses, in particular the synthesis of fatty acids (FA) and photosynthetic pigments occur in response to varying environmental conditions, such as temperature and irradiance, and may allow us to understand the eco-physiological status of eelgrasses.

- To test this, we conducted two different studies. Firstly, we assessed temporal changes in total FA production and composition and shifts in photosynthetic pigment levels in Irish *Zostera marina* populations (Chapter 3). Secondly, we studied depth-induced adjustments of FA synthesis in relation to pigment production and biometric responses of *Halophila stipulacea* in Gulf of Aqava in the Red Sea (Israel) (Chapter 8).

Hypothesis 3. We hypothesized that the actual distribution of seagrass in Ireland may be larger than reported as Irish coastal areas are remote and sometimes inaccessible, because of the low research attention that Irish seagrass has received in

the last number of decades and also the associated difficulties in mapping subtidal seagrass regions.

- To achieve this, we conducted two different studies, one at a local scale (Kilkieran Bay) (Chapter 4) and another at a regional scale (Ireland) (Chapter 5). We applied different geographic information system (GIS) approaches, such as, species distribution models (SDM) or satellite-derived images to assess the significance of different environmental descriptors outlining the presence of eelgrass meadows and to forecast the potential habitat suitability. Also, we establish a volunteer-based study to gather the spatial information concerning the seagrass presence in Ireland from local collaborators (Chapter 5). Later, we validated the predictive ability of our methods and the supplied information from the collaborators by undertaking field surveys in areas where the data suggested eelgrass presence. Finally, we constructed a new, and more complete, map of seagrass distribution (Chapter 4 and 5).

Hypothesis 4. Previous studies of micro-and macroalgae and or terrestrial primary producers widely reported structural adjustments in FA in response to thermal regimes. Therefore, we propose that warming may induce a depletion in TFA and in PUFA/SFA ratio, thus reducing the lipid nutritional value of seagrasses at high temperatures. Also, we suggested that changes in FA production and composition can be implemented as an effective eco-physiological tool to assess thermal stressed states of seagrasses. Moreover, we propose that future projected warming (> 2-3 °C) in Ireland may positively affect Irish seagrass populations as they are currently exposed to summer temperatures ranging between 15 and 17 °C and optimal temperatures for eelgrass growth and productivity at similar latitudes were reported to be between 18 and 24 °C.

- To demonstrate this, we studied the effects of simulated warming events in mesocosm systems in key biochemical indicators, carrying out two different experiments; one experiment was conducted with Irish *Z. marina* populations (Chapter 6), and another with two Mediterranean seagrass species, *Posidonia oceanica* and *Cymodocea nodosa* (Chapter 7). Finally, as a part of this study, we also investigated the variations in PUFA and PUFA/SFA ratios in different *Z. marina* populations across its latitudinal geographical range (Chapter 3).

Chapter 2

Temporal and spatial changes in the structure, morphometry and productivity of pristine *Zostera marina* meadows in western Ireland

Pedro Beca-Carretero, Clara S. Stanschewski, Marc Julia-Miralles, Alvaro Sanchez-Gallego, Dagmar B. Stengel. Temporal changes in the structure, morphometry and productivity of pristine *Zostera marina* meadows in western Ireland. Aquatic Botany. (Under review).

Author contribution

Pedro Beca-Carretero developed the experimental design and the statistical analysis; Pedro Beca-Carretero, Marc Julia-Miralles, Clara S. Stanschewski, Alvaro Sanchez-Gallego collected the data in the field and conducted the review; Dagmar B. Stengel resources and funding for the project; all the authors contributed to the interpretation of the data and the writing of the manuscript.

Abstract from original article

The eelgrass *Zostera marina* is a dominant, subtidal meadow-forming seagrass in temperate regions in the northern hemisphere. Due to the numerous ecological services they provide, seagrass systems rank amongst the most valuable ecosystems worldwide, although their conservation status in Ireland is currently not clear, and there are no detailed baselines available to contrast ecological monitoring information. For the first time this investigation assessed temporal changes and depth acclimations at shoot and population level of different unpolluted and pristine *Z. marina* meadows in three areas located in protected nature reserves in western Ireland. Furthermore, annual leaf formation rates and above-ground biomass were estimated and compared to those of eelgrass populations from different latitudes (28˚-66˚) reported in the literature. Most parameters revealed a marked temporal pattern, displaying clearunimodal responses, with peaks attained in July, intermediate values in April and November, and lowest values observed in January. Moreover, plants inhabiting deeper areas (> 5 meters), in cohabitation with maerl, displayed a larger morphological plasticity with higher coefficients of variation (CV) than plants at the shallowest site. Eelgrass cover and density decreased from 76.1 ± 6.5 % in shallow meadows to 34 ± 4.5 % at greater depths; inversely, other parameters such as shoot biomass or shoot length increased 2-3 fold with depth. Furthermore, a latitudinal comparison revealed a consistent correlation between leaf production and *in-situ* sea surface temperature (SST) in eelgrass populations, within an increment of 1.0 leaf production with every 1°C-rise in annual SST. This study is particularly relevant due to (i) the current scarcity of information available on seagrass ecology in

Ireland, (ii) the non-disturbed status of the eelgrass meadows described, and (iii) the potential to assess effects of future climate change in these indicator systems.

Keywords: *Zostera marina*; temporal monitoring; depth-acclimation; plasticity; morphometric descriptors; population structure; annual productivity; latitudinal comparison; Ireland.

2.1. Introduction

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The study of keystone species is vital for biological management and conservation, in particular, in view of currently observed impacts of global change, including anthropogenic disturbances and climatic environmental shifts (Thomas et al., 2004). Relevant changes at community structure and ecosystem level have been increasing dramatically over the last few decades, so much so that there is a critical requirement for species monitoring, and preservation of key environmental indicators (Hoegh-Guldberg et al., 2010). Non-polluted and undisturbed areas have been widely used as a baseline to monitor habitat disturbances and degradation; however, nowadays there is a lack of pristine environments, particularly in marine coastal ecosystems due to exposure to direct and indirect human pressures (Jackson et al., 2001).

Seagrasses are angiosperms that complete their life cycle within marine environments and are typically associated with sheltered and shallow habitats (Hemminga & Duarte 2000). These macrophyte communities rank among the most valuable ecosystems worldwide due to the numerous ecological services they provide, such as shoreline protection, habitat and nursery grounds, high oxygen productivity and carbon sequestration (e.g. Costanza et al., 1997; Fourqurean et al., 2012). However, they do not enjoy the international and social recognition as other charismatic ecosystems such as coral reefs or tropical forests (e.g. Costanza et al., 1997; Nordlund et al., 2017; Fourqurean et al., 2012). In temperate regions of the northern hemisphere, one of the main meadow-forming seagrasses is the eelgrass, *Zostera marina* Linnaeus. Displaying a broad distribution from subtropical regions to the Arctic Circle, between 27° and 70° N, the species is adapted to a wide range of temperature regimes, from -1 °C in Arctic regions, to 30 °C in subtropical areas (Ibarra-Obando et al., 1999; Olesen et al., 2015; Ruiz et al., 2016). This adaptability renders *Z. marina* an exceptionally plastic character with regard to vegetative growth, life cycle and reproductive effort (Duarte et al., 1989). *Z. marina* is commonly used as an indicator species because of its high sensitivity and responsivity to environmental shifts, including reduction in water quality or other anthropogenic

pressures (e.g. Fourqurean et al., 2016; Krause-Jensen et al., 2005; Marbá et al., 2013).

Site-specific environmental conditions reportedly affect the performance of seagrasses in terms of abundance and productivity, resulting in local morphological and physiological adaptations (e.g. Hauxwell et al., 2006; Kaldy & Lee 2007). Temperature and irradiance have been identified as the most relevant factors driving temporal variations of temperate seagrasses; they control metabolic processes such as photosynthesis and respiration (e.g. Zimmerman et al., 1995; Marsh et al., 1986; Sang and Park, 2005*),* but also affect the population structure, flowering and seed germination (i.e. Durako & Moffler 1987; Diaz-Almela et al., 2007). Cold-temperate eelgrasses typically exhibit marked temporal growth, with optimal productivity rates observed during warmer periods with higher daylight hours in which they increase their active leaf structures thus enhancing their photosynthetic performance and their ability to store energy-rich compounds such as carbohydrates, mainly located in below-ground structures (Bay et al., 1996; Alcoverro et al., 2001). On the contrary, in colder and darker periods, seagrasses readjust their shoot size by reducing photosynthetic tissues, thus maintaining low metabolic levels and utilising existing energy reserves (e.g. Duarte 1989; Olesen & Sand-Jensen 1993; Lee et al., 2005; Laugier et al., 1999; Chabot and Hicks, 1982). Irradiance is the main factor limiting seagrass vertical distribution, affecting the shoot growth and productivity and inducing changes at population structure (e.g. Duarte 1991; Olesen et al., 2002; Ralph et al., 2007). In temperate regions, light energy limitation of eelgrass growth is most pronounced for deep adapted populations where irradiance allows seagrasses just one positive carbon budget on an annual basis (Dennison 1987). At intermediate latitudes in the deep limit distribution, eelgrass meadows are generally characterized by low densities with greater shoot size than shallow populations (Olesen et al., 2017). This adaptive strategy allows plants to reduce the shelf shading and to reach higher light intensities, thus favouring a deeper colonization. Distributed in the middle of its latitudinal range, *Z. marina* is the most dominant subtidal seagrass in Ireland and can colonize maximum depths of 10 meters (NPWS, 2014; MERC, 2005). Although only few studies have, even peripherally, addressed Irish eelgrass population ecology (Dale et al., 2007; Jones & Unsworth 2016; Wilkes et al., 2017).

Ireland's climate is defined as a temperate oceanic climate, characterized by clearly defined climatic periods with a lack of extreme cold or warm temperatures (https://www.met.ie; Peel et al., 2007). Due to their relative inaccessibility and the generally low anthropogenic impacts in remote coastal areas in western Ireland, large virgin and unspoiled seagrass meadows may have persisted until the present day. These can be used as a baseline for vital comparison from local to global regions. Hence, starting to monitor their temporal dynamics represents a vital contribution to current efforts in seagrass management worldwide.

We hypothesize that Irish eelgrasses may show a discernible temporal pattern regarding shoot growth and population dynamics as Ireland is characterized by distinct environmental seasons. In particular, we propose, that populations living in their deepest distribution limit may display a higher temporal morphological plasticity and also may show different morphological and population properties than plants from ashallow site, in response to different irradiance conditions. In addition, we propose that Irish eelgrasses may have similar annual leaf formation rates and above-ground biomass production to populations adapted to equal temperatures and distributed atsimilar latitudes. Therefore, the objectives of this study were to characterize the temporal changes of plant descriptors at shoot and population level in three meadows from western Ireland. Adaptations to depth-related environmental conditions were assessed by comparing temporal performance of plants from different depths. Finally, we evaluated the annual below-ground productivity and annual leaf formation rate across their geographical distribution range (27˚- 66˚ N) in relation to in-situ summer and annual seawater surface temperatures (SST).

2.2. Materials and methods

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2.2.1. Study sites

Of the three *Z. marina* meadows studied in western Ireland, two were located in Kilkieran Bay, northern Galway Bay, i.e. Lettermore (LM) (53°17'58" N, -9°42'43"

W) and Tír an Fhia (TI) (53°15'37.5" N, 9°38'28.8"W) which is classified as a Special Area of Conservation (SAC) (NPWS, 2014). The third population was situated at Finavarra (FV) (53°8'50" N, -9°07'43"W), southern Galway Bay, also characterized as a SAC. These meadows were chosen due to their current protected status, their remoteness and their low exposure to anthropogenic disturbances.

The western Irish coastline is characterised by intertidal ranges of 4-5 meters; *Z. marina* extends typically to depths of 5-6 meters below mean water level (http://www.sailing.ie/). Co-occurrences of *Z. marina* and maerl were previously recorded in Kilkieran Bay (NPWS, 2014). The eelgrass meadow at TI was located along a channel, with an estimated length of 2.5 km and a maximum width of 0.43 km. The meadow at LM extended over 3.2 km x 0.8 km and was located close the mouth of a channel, yet it was a sheltered site protected by a group of islands which reduced hydrodynamic forces such as current velocity and wave action (NPWS, 2014). The meadow at FV was located in a shallow and sheltered bay which contained several smaller, patchy meadows; the meadow chosen for this study had an estimated size of 0.5 km x 0.2 km (Fig. 2.1).

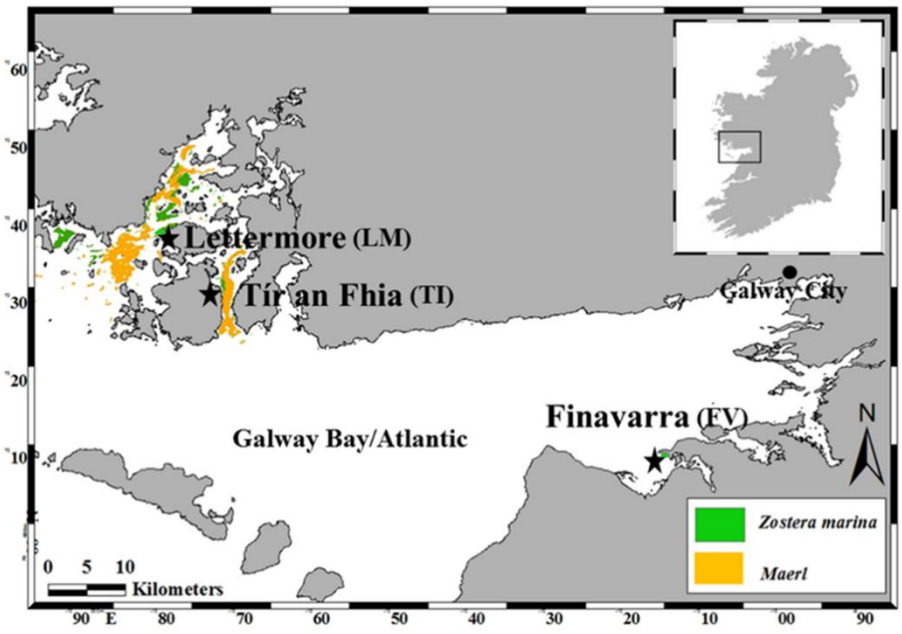

Fig. 2.1 Map of study area in western Ireland with locations of the three eelgrass meadows at Lettermore (LM), Tír an Fhia (TI) and Finavarra (FV). Eelgrass (*Zostera marina)* distribution is represented in green and maerl presence in orange.

2.2.2. Sampling procedure

Two 50 m-long permanent transects (T1 and T2) were placed in each meadow. At TI and FV, transect one (T1) was placed closer to the shoreline, while transect two (T2) was located 20 meters from T1. At these sites, both transects were situated at a depth of 2-2.5 m within the same meadow, representing similar environmental conditions. By contrast, at LM, T1 was placed at 2-2.5 m, while T2 was located 30 meters from T1 at 4.5-5 m, thus representing the upper and lower limits of this meadow, respectively. Eelgrass monitoring was carried over 4 seasons, April, July and November 2015, and January 2016, either on foot during exceptional low tides, or by snorkelling or SCUBA diving during normal tides.

2.2.3. Environmental variables and sedimentological analysis

Temperature (˚C) data for Kilkieran Bay (TI and LM) were provided by the Marine Institute (http://www.marine.ie/Home/). Seawater temperatures for FV were obtained by using a submersible HOBO temperature logger (U22-001, Onset) which was placed just beside the meadow at ca. 2 m depth. This logger recorded data over two seasons (spring and summer 2015). Measurements of daily light were obtained using a light sensor (Li-1400, Li-Cor, USA) located on the roof of the Ryan Institute, NUI Galway (53°16’43" N 9°03’40" W, Galway City, about 25 km from FV and 40 km from LM and TI, Fig. 2.1). Total daily irradiance was calculated as mol photons m^{-2} d^{-1}. Additionally, at each site during sampling days, one Odyssey integrating PAR sensor (Dataflow Systems PTY Limited) was deployed in the sediment at a 90° angle, Loggers recorded underwater *in-situ* instant irradiance (µmol photon m^{-2} s^{-1}) every 15 m for 1-2 h on each sampling occasion. Sediments for grain size analysis were collected from the *Z. marina* beds in July 2015 along the two permanent transects (T1 and T2) at each location.

Grain size was determined according to the protocol described by Folk (1974); samples were taken with a shovel from the sediment surface to a depth of 10 cm (n = 5) and transported to the laboratory in plastic bags. The granulometry was determined following dry-sieving after repeated (n=3) washing of samples with

distilled water to remove sea salts and subsequently oven-drying at 70 °C. Data for grain size were analyzed using GRADISTA software (Blott et al., 2001; Folk and Ward, 1957).

2.2.4. Seagrass meadow characteristics: above-ground and below-ground biomass, vegetative and reproductive shoot density and percentage cover

Over the four months studied (April, July, November and January), above-ground (AG) biomass (leaves and sheath) was assessed by collecting four random samples (n = 4) along the transect using a quadrat of 0.33 m x 0.33 m. Data was calculated in the four replicates and normalized per m^2. Below-ground (BG) biomass (roots and rhizomes) was only collected on two occasions during the study period (July-2015 and January-2016) to minimise the disturbance of the meadows, in three random sites (n = 3) along the transect. BG data was normalized per m^2. For each quadrat, biomass (plant fragments with leaves, rhizomes and roots) was collected and placed into a mesh bag; once in the laboratory, remaining sediment was removed by washing with freshwater, and organisms (including epiphytes) were also removed. Plant parts were then divided into AG and BG compartments. AG material was kept for subsequent analysis. All samples were oven-dried at 60 °C for 48 h and then weighed (Short et al., 2001).

Vegetative and reproductive shoot density were also determined at each site and during each month. To determine density, the number of shoots presented from within a 0.5 m x 0.5 m quadrat (n = 4) were counted and then normalized per m^2. In parallel, flowering frequency was calculated as the number of reproductive shoots relative to total shoot density (Olesen et al., 2017).

Then, eelgrass and maerl percentage cover was assessed by taking 10 - 12 random underwater photographs of the 0.5 m x 0.5 m quadrat throughout the permanent transects (T1 and T2) at each location, using a water-proof camera SJCAM sj4000. To estimate the percentage cover (%) of *Z. marina* and maerl, a grid of 10 x 10 (=100) points was overlaid onto each picture, and points enclosed in the grid with the

presence of macrophytes were counted visually on the computer screen (Winters et al., 2017).

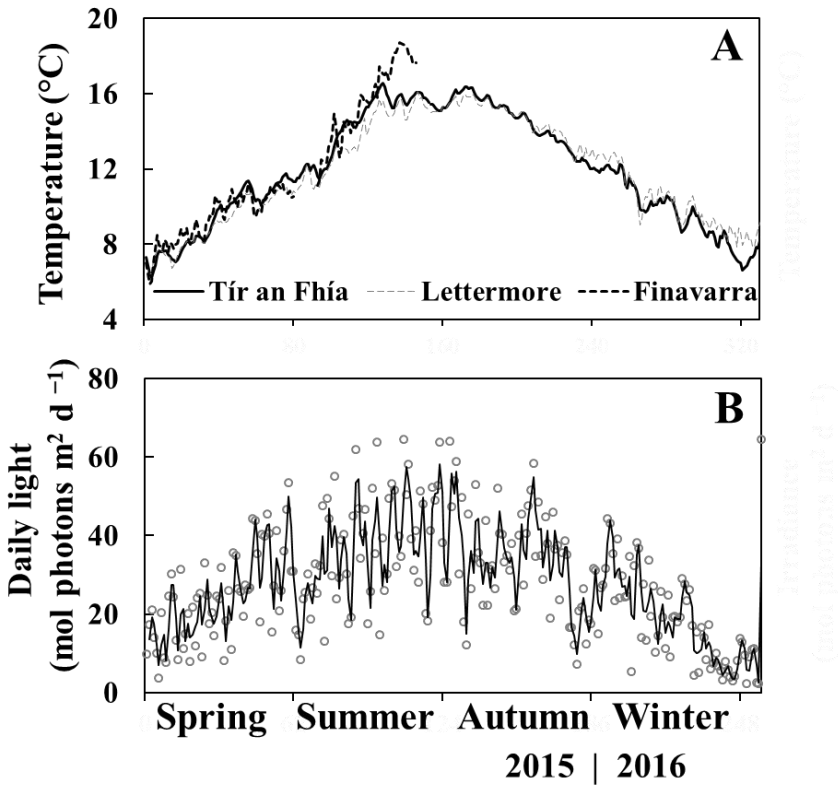

Fig. 2.2 Seasonal data of daily seawater temperature in the shallow eelgrass meadow at Lettermore (LM), Tír an Fhia (TI) and Finavarra (FV) (A). Measurements of daily light (mol photons m^{-2} d^{-1}) measured on the roof of the Martin Ryan Building (B), NUI Galway, Galway City (see Fig.2.1).

Table 2.1 Geographic coordinates of the permanent transects (T1 and T2) at each location (LM, TI and FV) (water depth of each transect relative to the mean water level). Seawater temperature measured at times when *Z. marina* biomass was collected. *In-situ* irradiance (PAR, µmol photon m^{-2} s^{-1}) was measured at each location during sampling.

	LM-T1	LM-T2	TI-T1	TI-T2	FV-T1	FV-T2
Latitude	53.299	53.299	53.260	53.260	53.147	53.147
Longitude	-9.712	-9.713	-9.641	-9.641	-9.128	-9.128
Depth (m)	[2-2.5]	[4.5-5]	[2-2.5]	[2-2.5]	[2-2.5]	[2-2.5]
Temperature (°C)						
April	11	11	10	10	11	11
July	16	15	15	16	17	17
November	12	11	11	11	11	11
January	7	7	7	7	6	6
Irradiance (µmol photons m^{-2} s^{-1})						
April	66.8 ± 19	15 ± 0.6	93.7 ± 48.0	93.7 ± 48.0	71.7 ± 6.8	71.7 ± 6.8
July	43.5 ± 15.10	14.8 ± 4.2	60.0 ± 11.3	60.0 ± 11.3	63.0 ± 15.7	63.0 ± 15.7
November	38.8 ± 20.8	7.1 ± 2.7	35.0 ± 5.8	35.0 ± 5.8	18.4 ± 5.5	18.4 ± 5.5
January	19.3 ± 8.1	4.3 ± 0.6	37.5.0 ± 16.0	37.5.0 ± 16.0	45.3 ± 9.9	45.3 ± 9.9

2.2.5. Morphometric descriptors at shoot level

Shoot characteristics were assessed using all the shoots collected to calculate the AG biomass (n = 4) (see above for details). To assess the characteristics of individuals, the shoots were collected and transported to the laboratory where we calculated (i) mean shoot biomass (leaf and sheath biomass) (g DW), (ii) mean shoot length (cm), (iii) mean number of leaves per shoot, and (iv) mean leaf width per shoot (mm). Leaf width was calculated midway between the base and the tip of the second youngest leaf. In addition, canopy height was assessed by measuring the 10 longest shoots per quadrant (n = 4) (Longstaff et al., 1999). From these shoots we also calculated (i) shoot biomass (g DW), (ii) mean shoot length (cm) and (iii) mean leaf width per shoot (mm).

2.2.6. Annual productivity and latitudinal comparison

The annual leaf formation rate was determined by recreating the seasonal growth cycle of the number of rhizome internodes following the methodology described by

Olesen et al (2015, 2017). To determine the annual recent past rhizome formation, in September 2015, 8-10 healthy apical shoots were collected over a distance of 5 to 10 meters per transect to avoid pseudo replication. We carefully collected the green part of the shoots and the longest possible below-ground biomass (rhizome and roots). Lateral shoots (rhizome branches) were removed (when present) from the apical shoot and they were excluded from subsequent analysis. In cold- temperate regions, during warmer periods, *Z. marina* developed longer rhizome internodes and in a larger number than during the winter period, which was characterized by shorter rhizomes but smaller number of internodes. Thus, by measuring changes in the length of the rhizome segments of the collected shoots we reconstructed the annual seasonal cycle produced in the last year (from autumn 2014 to autumn 2015). Annual leaf formation rate was assumed as a 1:1 relationship between rhizome segment formation and leaf formation (Sand-Jensen, 1975; Duarte, 1994; Olesen et al., 2015). In addition, with the selected BG biomass (rhizome and roots) used to assess the number of internodes produced in one year (internodes $shoot^{-1}$ yr^{-1}), we measured (i) the total length of the rhizome produced in one year from the apex to the oldest internode (cm $shoot^{-1}$ yr^{-1}) and (ii) the weight of the selected annual BG biomass (rhizome and roots) produced in one year (g DW $shoot^{-1}$ yr^{-1}).

These data were used to compare eelgrass above-ground biomass and annual leaf formation rate with previous findings of *Z. marina* across its latitudinal distribution range and at site-specific summer (June to August) and annual seawater surface temperatures (SST). Results were compared with those reported by Olesen (2015) and other recent publications. SST data for the latitudinal comparison were derived from the Bio-ORACLE database (http:// http://www.bio-oracle.org) (Tyberghein et al., 2012) with a resolution of 0.5 km^2.

2.2.7. Statistical Analysis

Firstly, we compared differences between T-1 and T-2 applying *t*-test ($p < 0.05$) in the meadows studied (LM, TI and FV). Subsequently, prior to conducting statistical analyses, homogeneity and normality of data were tested using Levene's test and the Kolmogorov–Smirnov test, respectively (Sokal & Rohlf, 1995). Data that did not

pass the tests were then ln transformed. As data did still not meet these criteria, to study temporal and location patterns and acclimations to different depth gradients at shoot and meadow levels, the non-parametric test PERMANOVA was performed. When possible, data were log transformed and resembled. We developed 2 PERMANOVA models; the first model was run to assess temporal and location effects at population and shoot level, comparing all the shallow meadows (2 - 2.5 m) which included: LM-T1, TI (T1 and T2) and FV (T1 and T2). We used "month" as fixed factor and "location" as random factor. The second model was performed to assess temporal and depth effects at shoot and population level, comparing shallow (LM-T1) versus deep populations (LM-T2). Here, we used both "month" and "depth" as fixed factors. *T*-tests were applied to assess significant effects ($p < 0.05$) between specific months and locations. Additionally, to explore and visualize temporal differences in the morphological variability (shoot biomass, shoot length and leaf width) between shallow and deep-adapted populations, we used (i) the coefficient of variation (CV), (ii) alongside the flexible ordination technique principal coordinate analysis (PCO) and (iii) the distance-based test for homogeneity of multivariate dispersion (PERMADISP). Both statistical techniques, PCO and PERMADISP, were based on a Euclidean similarity matrix. Before performing PCO and PERMADISP analyses, the samples were standardized to ensure the same contribution of each morphological variable. Significant differences regarding the annual productivity parameters and BG biomass between locations (LM-T1, LM-T2, TI and FV) were examined using *t*-test. All the statistical analysis was carried out using the statistical package IBM SPSS Inc., v.13 and PRIMER & PERMANOVA 6.

Table 2.2 Temporal changes in *Zostera marina* shoots morphometric parameters: mean shoot biomass, mean shoot length and mean leaf width at the three locations. *Mean shoot* represent values of all the shoots collected in the 0.5 m x 0.5 m quadrat; *Canopy height shoot* represent values of the shoots collected to assess the height canopy (10 largest shoots) in the 0.5 m x 0.5 m quadrat. Data represented as mean ± standard deviation (n = 4).

Shoot morphometries	**Population**	**CV (%)**	**April**	**July**	**November**	**January**
Mean shoot						
Shoot biomass	LM-T1	**23.0**	0.28 ± 0.03	0.41 ± 0.07	0.36 ± 0.5	0.22 ± 0.03
(g DW shoot^{-1})	LM-T2	**41.5**	0.43 ± 0.10	0.88 ± 0.18	0.78 ± 0.11	0.28 ± 0.03
	TI	**39.3**	0.24 ± 0.13	0.51 ± 0.16	0.41 ± 0.07	0.18 ± 0.03
	FV	**28.8**	0.24 ± 0.03	0.36 ± 0.06	0.36 ± 0.06	0.17 ± 0.04
Shoot length (cm shoot^{-1})	LM-T1	**18.5**	41.7 ± 5.6	65.3 ± 4.8	56.2 ± 8.1	43.8 ± 0.9
(cm shoot^{-1})	LM-T2	**28.4**	43.2 ± 6.7	80.9 ± 13.2	73.1 ± 8.7	43.5 ± 3.5
	TI	**28.2**	39.3 ± 6.8	71.4 ± 14.6	63.8 ± 7.8	37.3 ± 6.2
	FV	**21.8**	38.7 ± 2.8	58.6 ± 11.0	42.9 ± 2.3	33.2 ± 7.1
Leaf width	LM-T1	**10.1**	0.46 ± 0.02	0.52 ± 0.02	0.47 ± 0.01	0.39 ± 0.02
(mm shoot^{-1})	LM-T2	**10.6**	0.53 ± 0.03	0.65 ± 0.05	0.55 ± 0.02	0.49 ± 0.02
	TI	**10.0**	0.41 ± 0.01	0.48 ± 0.05	0.46 ± 0.01	0.37 ± 0.02
	FV	**16.2**	0.49 ± 0.02	0.63 ± 0.06	0.54 ± 0.07	0.40 ± 0.04
Canopy height shoot						
Shoot biomass	LM-T1	**18.4**	0.53 ± 0.11	0.68 ± 0.12	0.57 ± 0.18	0.40 ± 0.03
(g DW shoot^{-1})	LM-T2	**48.8**	0.61 ± 0.13	1.44 ± 0.38	0.94 ± 0.13	0.35 ± 0.07
	TI	**36.7**	0.58 ± 0.10	0.95 ± 0.21	0.82 ± 0.11	0.31 ± 0.05
	FV	**33.9**	0.38 ± 0.07	0.74 ± 0.1	0.61 ± 0.07	0.31 ± 0.06
Shoot length	LM-T1	**13.4**	59.4 ± 5.1	82.4 ± 6.0	71.5 ± 4.2	61.2 ± 6.5
(cm shoot^{-1})	LM-T2	**28.7**	62.1 ± 7.2	103.5 ± 15.1	90.4 ± 14.3	48.5 ± 5.9
	TI	**28.8**	53.3 ± 4.6	96.1 ± 16.2	82.3 ± 8.5	47.5 ± 7.8
	FV	**20.2**	50.4 ± 5.1	74.4 ± 5.1	53.9 ± 3.4	44.5 ± 5.5
Leaf width	LM-T1	**11.5**	0.59 ± 0.09	0.63 ± 0.02	0.51 ± 0.02	0.47 ± 0.01
(mm shoot^{-1})	LM-T2	**11.7**	0.72 ± 0.03	0.81 ± 0.06	0.69 ± 0.03	0.58 ± 0.01
	TI	**12.6**	0.48 ± 0.04	0.60 ± 0.05	0.48 ± 0.04	0.43 ± 0.03
	FV	**13.3**	0.58 ± 0.08	0.72 ± 0.05	0.65 ± 0.06	0.50 ± 0.04

Table 2.3 *Zostera marina.* Vegetative and reproductive shoot densities (n = 4 – 8), percentage cover (n = 10 – 24) an above-ground biomass (n = 4 – 8) at each month at the three locations. Data represented as mean ± standard deviation.

Population descriptors	Population	CV (%)	April	July	November	January
Vegetative shoots	LM-T1	**19.0**	165.3 ± 23.2	269.3 ± 15.4	184 ± 34.4	206.8 ± 68.2
(no. m^{-2})	LM-T2	**9.3**	62 ± 24.1	69.2 ± 24	78.6 ± 20	64.1 ± 28.1
	TI	**18.5**	185.4 ± 17.6	248.2 ± 62.1	148.6 ± 23.6	188.1 ± 34.4
	FV	**9.2**	193.2 ± 29.1	236.1 ± 46.2	194.8 ± 43.3	229.9 ± 32.2
Reproductive shoots	LM-T1	**115.1**	2.0 ± 0.4	5.2 ± 0.4	0.09 ± 0.04	0.0
(no. m^{-2})	LM-T2	**144.4**	0.3 ± 0.1	2.0 ± 0.1	0.0	0.0
	TI	**146.2**	0.8 ± 0.5	5.8 ± 1.7	0.0	0.0
	FV	**80.7**	0.5 ± 0.2	0.7 ± 0.8	0.16 ± 0.06	0.0
Cover (%)	LM-T1	**17.3**	65.9 ± 20.5	97.2 ± 5.1	77.1 ± 18.6	64.1 ± 8.5
	LM-T2	**30.8**	18.4 ± 17.5	44.4 ± 23.0	35.0 ± 19.1	27.2 ± 10.5
	TI	**15.7**	77.9 ± 13.2	91.1 ± 10.2	64.4 ± 17.7	62.3 ± 19.4
	FV	**11.4**	81.6 ± 17	81.4 ± 50.9	72.9 ± 28.8	60.9 ± 15.7
Above-ground biomass	LM-T1	**37.3**	49.7 ± 4.7	116.8 ± 6.0	67.2 ± 5.2	53.4 ± 7.5
(g DW m^{-2})	LM-T2	**41.1**	33.4 ± 8.3	64.3 ± 8.7	63.5 ± 4.5	21.3 ± 5.5
	TI	**51.4**	46.7 ± 9.1	128.5 ± 6.0	61.9 ± 7.5	38.5 ± 10.2
	FV	**32.1**	48.6 ± 11.4	92.1 ± 9.2	70.8 ± 7.5	40.2 ± 3.9
Below-ground biomass	LM-T1	**16.8**	nd	244.1 ± 22.6	nd	202.04 ± 32.8
(g DW m^{-2})	LM-T2	**3.7**	nd	38.4 ± 18.8	nd	41.31 ± 9.3
	TI	**5.3**	nd	168.8 ± 41.8	nd	187.8 ± 43.6
	FV	**23.8**	nd	209.6 ± 62.6	nd	129. 1 ± 42.8
A/B ratio	LM-T1	**29.7**	nd	0.48 ± 0.01	nd	0.26 ± 0.02
	LM-T2	**52.5**	nd	1.67 ± 0.8	nd	0.52 ± 0.1
	TI	**58.3**	nd	0.76 ± 0.2	nd	0.20 ± 0.01
	FV	**17.3**	nd	0.44 ± 0.3	nd	0.31 ± 0.1

2.3. Results

This section has been modified from the original article

2.3.1. Environmental variables and sedimentological analysis

At all locations, maximum seawater temperature peaked in summer (July), with average values of 15.6 ± 0.4 °C; however, in summer maxima at FV (17.6 ± 0.9 °C) were two degrees warmer than at TI and LM (Fig. 2.2, Table 2.1). For the rest of the year, temperatures at the three locations were similar. Lowest (6 - 7 °C) temperatures were attained in winter, and intermediate temperatures (10 - 12 °C) in spring and autumn and Maximum (surface) irradiance coincided with temperature peaks, reaching highest values of 40.9 ± 14.2 mol photons m^{-2} d^{-1} in summer, and minimum values of 11.1 ± 7.4 mol photons m^{-2} d^{-1} occurred in winter. Moreover, annual underwater in-situ instant irradiances measured during sampling in shallow

populations LM-T2 (10.3 µmol photon m^{-2} s^{-1}) was 75.5 % lower than in populations at the shallowest area, LM-T1 (42.1 µmol photon m^{-2} s^{-1}) (Table 2.1).

The three locations were characterized by intermediate sediment grain size, ranging from 0.56 mm to 0.75 mm. Sediments of both transects LM (LM-T1 and LM-T2) contained the highest percentage of mud, with an average value of 5.0 ± 0.3% (Table 2.2). Also, LM-T2, was characterized by the highest percentage of gravel (1.69 mm), which was related to the occurrence of maerl.

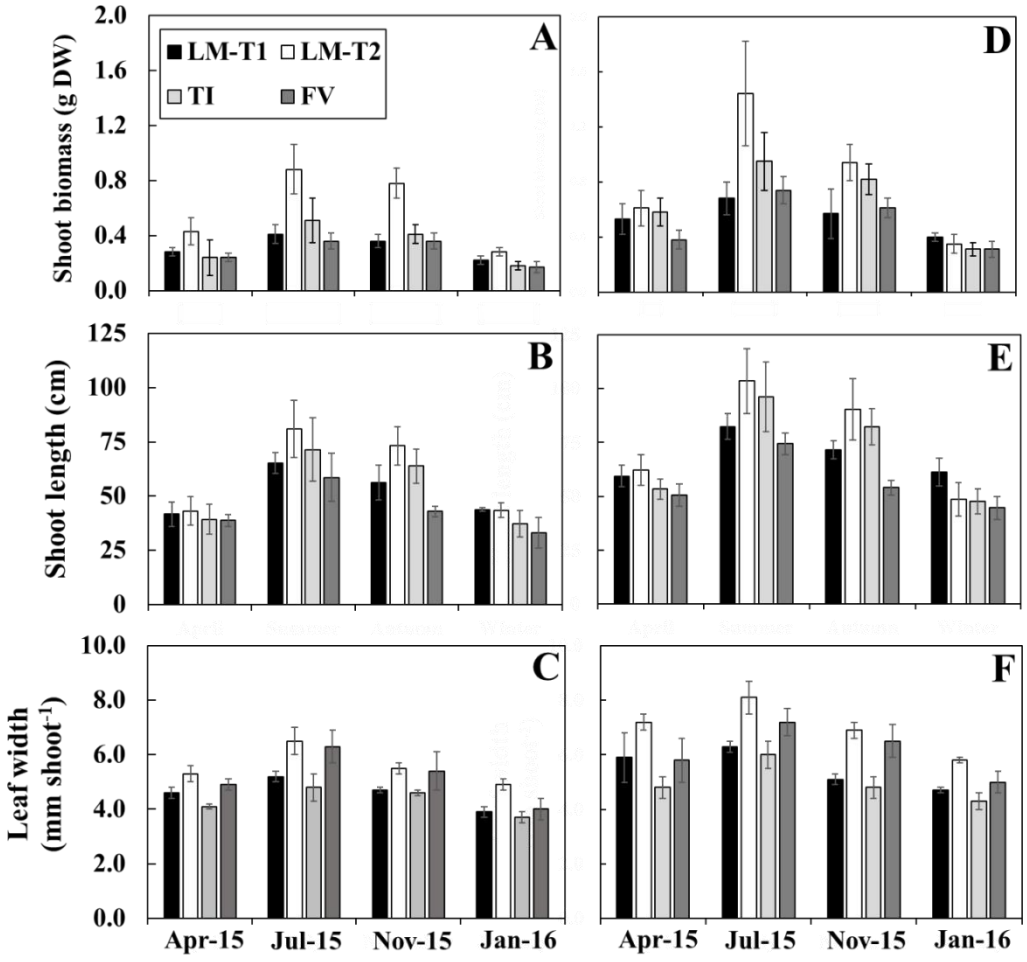

Fig. 2.3 Temporal changes in *Zostera marina* shoots morphometric parameters: mean shoot biomass (A and D), mean shoot length (B and E), mean leaf width (C and F) at the three locations. Left panels (A, B and C) represent values of all the shoots collected in the 0.5 m x 0.5 m quadrat; Right panels (D, E and F) represent values of the shoots collected to assess the height canopy (10 largest shoots) in the 0.5 m x 0.5 m quadrat. Data represent mean ± standard deviation (n = 4).

2.3.2. Morphometric descriptors at shoot level

Overall, results showed that in the majority of cases there were no significant differences between TI-T1 and TI-T2; neither between FV-T1 and FV-T2 over the studied period (t-test, $p < 0.05$), hence, results from both transect (T1 and T2) in these locations were combined (Table 2.2 and 2.3, Fig. 2.3). Morphological results highlighted a significant temporal variability, in terms of shoot biomass, shoot length and leaf width in all the studied populations (Fig. 2.3 A-F) (PERMANOVA, $p < 0.001$, Table 2.2). Also, there was a significant interaction between month and location, indicating that the morphological descriptors in the studied populations were diversely affected by temporal changes (PERMANOVA, $p < 0.05$, Table 2.5). Shoot biomass, length and leaf width displayed clear responses, with higher values attained in July, followed by November and April, and lower values observed in January, at all locations (Table 2.2, Fig. 2.3 A-F). For instance, in TI, FV and LM-T1, shoot biomass increased by a significant 111.8 %, 81.4 % and 183.3 % respectively, from January to July, while shoot length increased by 76.5 % in TI, 91.4 % in FV and 49.1 % in LM-T1 (Fig. 2.3 A-F). Average data for leaf width from all shallow locations (TI, FV and LM-T1) indicated slight but significant temporal variabilities, with generally average widest leaves observed in summer (4.5 ± 0.03 mm), followed by spring (5.4 ± 0.1 mm), autumn (4.9 ± 0.04 mm), and finally most narrow leaves in winter (0.39 ± 0.01 mm) (Table 2.2). The shoots collected to assess the canopy height also displayed temporal and spatially significant differences regarding the biomass, length and leaf width (Fig. 2.3 D-F) (PERMANOVA $p < 0.05$, Table 2.5). For instance, the longest annual average canopy height was observed in TI (69.8 ± 20.1 cm shoot^{-1}), followed by LM-T1 (68.6 ± 9.2 cm shoot^{-1}) and lowest in FV (55.8 ± 11.3 cm shoot^{-1}) (Table 2.2).

In general, results highlighted significant morphological differences between plants living at different depths over the study period (PERMANOVA, $p < 0.05$). All descriptors showed significantly higher annual average values in plants at the deepest area (LM-T2) than those from the shallowest depth (LM-T1) (Table 2.2, Fig. 2.3 A-F). Greatest differences in the morphological descriptors between LM-T1 and LM-T2 where usually observed in July, whereas lowest differences were reported in

January (Fig. 2.3 A-F). For instance, in July, the canopy height from LM-T2 (103.5 ± 15.1 cm shoot^{-1}) was 25.6 % higher than in LM-T1 (82.4 ± 6.0 cm shoot^{-1}), while in January there were no significant differences (Table 2.2, Fig. 2.3 E). Leaves from plants receiving lower irradiance (Table 2.1) were 20.1 % wider than those from shallower parts over the studied period (Fig. 2.3 C). Interestingly, populations from deeper areas (LM-T2) displayed a higher temporal morphological variability than those acclimated to shallower parts (LM-T1) (CPO, Fig. 2.5). Results of shoot biomass and shoot length from LM-T2 displayed a markedly higher temporal dispersion than LM-T1 (PERMADISP, $p < 0.05$, Table 2.7), and also showed a larger temporal coefficient of variation (CV) (Table 2.2). Throughout the study period, temporal variability in shoot biomass (Fig. 2.4) was significantly correlated with changes in shoot length and leave width; For instance, shoot biomass was correlated with shoot length ($r^2 = 0.77$, $p < 0.001$, Fig. 2.4 A and C) and leaf width ($r^2 = 0.46$, $p < 0.05$, Fig. 2.4 B and D).

2.3.3. Seagrass meadow characteristics: above-ground biomass, vegetative and reproductive shoot density, and percentage cover

Above-ground biomass differed temporally at all locations, with significantly higher values observed in summer, followed by intermediate values in spring and autumn and lowest in winter (PERMANOVA, $p < 0.01$, Table 2.6) (Fig. 2.6). In LM-T2, significantly lower values of AG and BG biomass were observed than in LM-T1 (Table 2.3). Also, there were significantly higher AG/BG ratios in deep populations than in shallow ones, which was particularly pronounced in summer (LM-T1 = 0.48 ± 0.01, LM-T2 = 1.67 ± 0.8) (Table 2.3). Shoot density significantly varies among months, with a slightly higher density determined in July (Fig. 2.5). Similarly, the number of shoots was significantly affected by depth (PERMANOVA, $p < 0.001$, Table 2.6), with *Z. marina* plants at the deepest site (LM-T2) characterized by lower annual average densities of 64.8 ± 22.8 shoots m^{-2} than plants at LM-T1 (206.4 ± 39.8 shoots m^{-2}) (Fig. 2.6 A). Eelgrass percentage cover was influenced by temporal changes (PERMANOVA, $p < 0.05$, Table 2.6), with maximum average (LM-T1, TI and FV) values of 87.8 ± 6.6 % attained in summer, and a minimum of 60.6 ± 4.7 %

observed in winter (Fig. 2.6 C). Also, seagrass cover was significantly affected by depth (PERMANOVA $p < 0.01$, Table 2.6), with LM-T1 showing annual average values of 76.1 ± 6.5 %, compared to 34 ± 4.5 % in LM-T2. Furthermore, the presence of maerl was only observed at greater depth (LM-T2) with coverage of around 67.1 ± 15.3 % of the seafloor, which became more widespread at greater depths (*pers. obs*.) (Fig. 2.6 and Fig. S2.3).

The presence of reproductive shoots in all populations was highly influenced by environmental changes, with larger densities observed in July (14 - 17 °C), followed by April (9 - 11 °C) (PERMANOVA, $p < 0.05$, Table 2.6). Only sporadic reproductive shoots were observed in autumn, and no reproductive plants were found in winter. Also, the density of reproductive shoots varied significantly (PERMANOVA, $p < 0.001$) between locations, with the larger meadows, LM-T1 (5.2 ± 0.8 shoot m^{-2}), TI (5.8 ± 1.7 shoot m^{-2}) producing 4 – 6 times more reproductive shoots than plants inhabiting the small and patchy meadows at FV (0.7 ± 0.3 shoot m^{-2}) (Fig. 2.5). In July, the LM-T2 population had a density of flowering plants of 2.0 ± 1.1 shoot m^{-2}, whereas, this population was characterized by having the largest reproductive effort, with 5.4 % of the collected plants having reproductive structures.

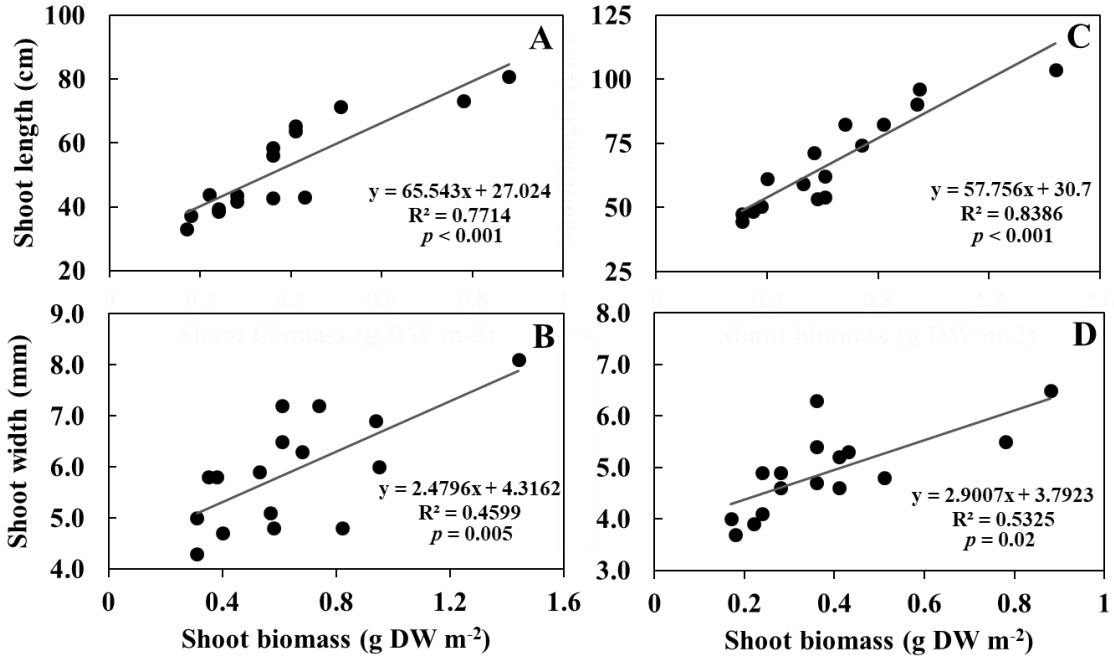

Fig. 2.4 *Zostera marina.* Mean shoot length (Y-exe) versus mean shoot biomass (X-exe) (Panel A, C); mean shoot length (Y-exe) versus mean leaf width (X-exe) (Panel B, D). Left panels (A and B) represent values of all the shoot collected in the 0.5 m x 0.5 m quadrat; Right panels (C and D) represent values of the shoots collected to assess the height canopy (10 largest shoots) in the 0.5 m x 0.5 m quadrat. Black lines represent the linear regression line (n = 24).

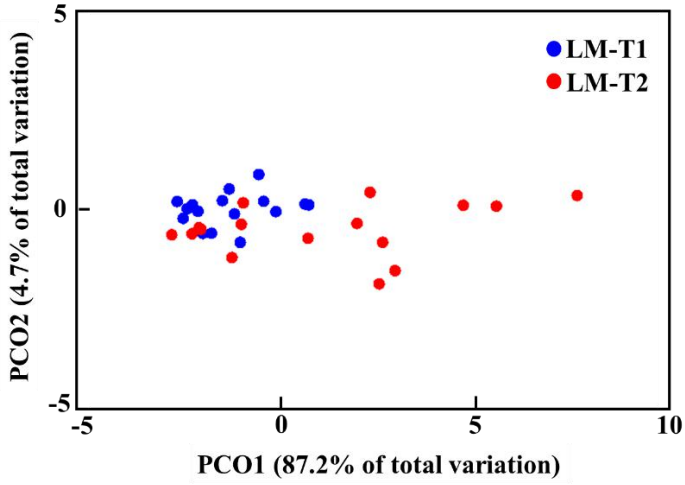

Fig. 2.5 Results of PCO contrasting the dispersion of the morphological characteristics of shoots (from Table 2.2) from LM-T1 and LM-T2.

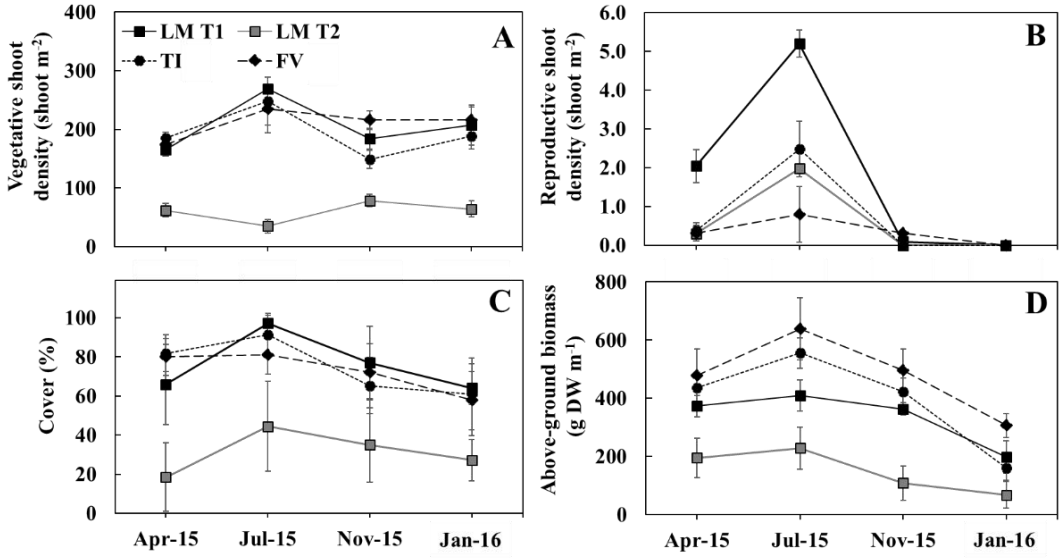

Fig. 2.6 *Zostera marina.* Vegetative (A) and reproductive (B) eelgrass shoots density (m^2) (n= 4) and seagrass (C) and above-ground biomass (n = 4) (D) percentage cover over four seasons assessed by taking random photographs of the 0.5 m x 0.5 m quadrat (n = 10 - 12) at the three locations. Data represent mean ± standard deviation.

Table 2.4 *Zostera marina* production of annual number of rhizome segments per shoot, annual rhizome biomass and annual rhizome elongation at the three locations (n=8-10), and annual (July 2015 and January 2016) average below-ground biomass (n = 6).

Annual productivity	LM-T1	LM-T2	TI-T1	TI-T2	FV-T1	FV-T2
Rhizome internodes (internodes shoot^{-1} yr^{-1})	23.3 ± 1.8	15.1 ± 1.3	20.4 ± 1.5	19.0 ± 0.8	28.9 ± 1.4	29.4 ± 1.8
Rhizome biomass (g DW shoot^{-1} yr^{-1})	0.97 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	1.9 ± 0.4	1.5 ± 0.9
Rhizome length (cm shoot^{-1} yr^{-1})	15.6 ± 1.3	13.2 ± 1.4	17.1 ± 4,4	16.5 ± 1.1	15.9 ± 3.4	15.4 ± 3.5
Below-ground biomass (g DW m^{-1})	796 ± 83	186 ± 73	634 ± 103.2	863 ± 221	1253 ± 209	1127 ± 147

2.3.4. Annual rhizome internodes, rhizome elongation and biomass, and annual leaf formation

Marked differences (t-test, $p < 0.05$) among sites were observed, with higher number of segments produced in FV populations (29.2 ± 1.5), followed by LM-T1 (23.3 ± 1.8) and TI (20.0 ± 1.5) and lowest values observed in the deepest adapted populations **(**LM-T2) with 15.1±1.3. Highest annual rhizome biomass productivity was observed in FV (1.7 ± 0.45) (t-test, $p < 0.05$), followed by LM-T1 (0.97 ± 2.5)

and lowest values observed in plants from the deepest area (LM-T2, 0.60 ± 0.13) (Table 2.4). Longest annual rhizomes were developed in TI (16.79 ± 4.46 cm) and shortest annual rhizomes were measured in plants from LM-T2, with annual values between 13.19 ±1.44 cm (t-test, $p < 0.05$) (Table 2.4).

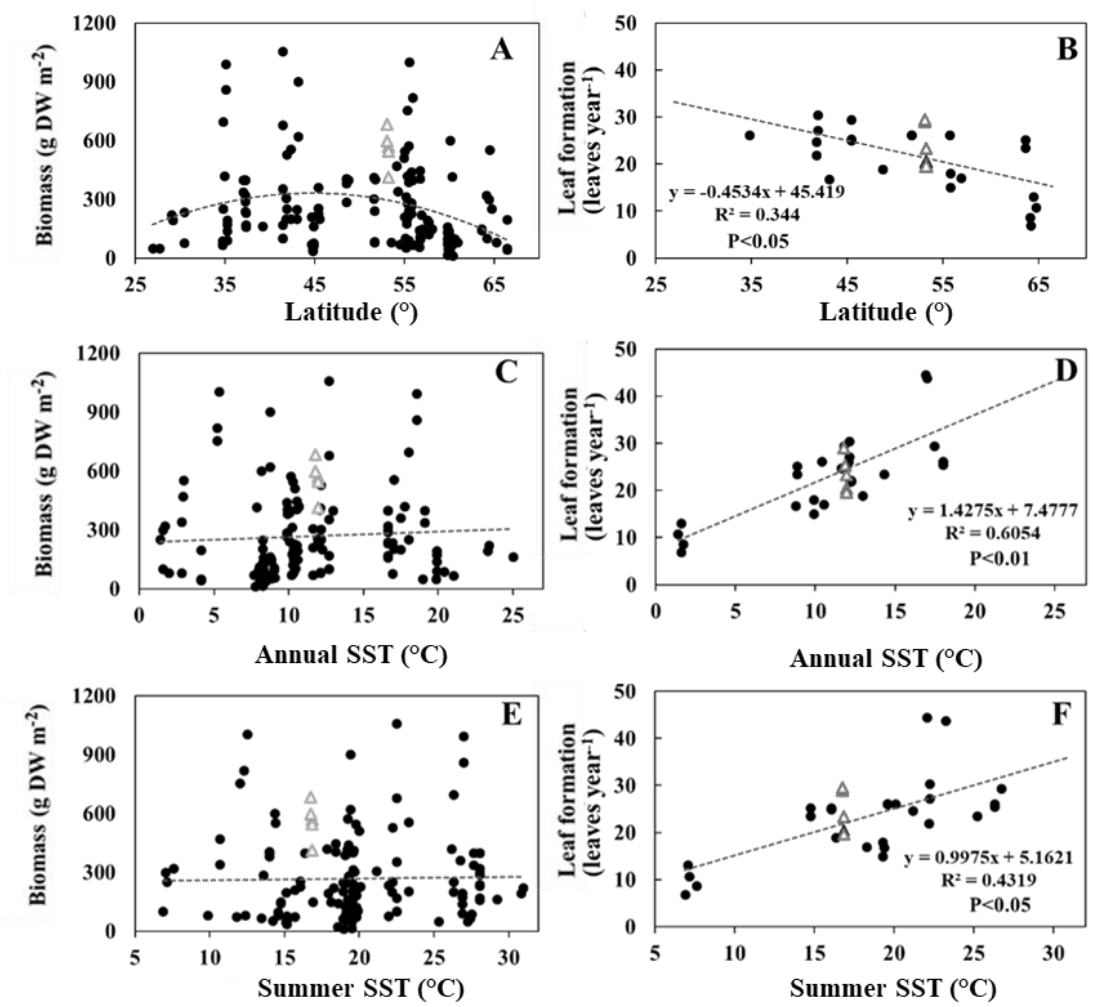

Fig. 2.7 *Zostera marina.* Above-ground biomass and annual leaf formation rate, and annual (Panel C and D) and summer (Panel E and F) sea surface water temperature and corresponding geographical latitudinal distribution range from the literature (Table 2.5 and Table S2.4) (circle symbols), and with results from the present study (triangle symbols). Black lines represent the linear regression line.

Table 2.5 Results of the PERMANOVA analysis of morphological descriptors at shoot level in response to seasonal, populations and depths variabilities**.** Pseudo F-values are shown along with significance levels (**p*<0.05; ***p*<0.01, ****p*<0.001), and degree of freedom (df).

		Mean shoot						***Canopy height shoot***					
Shoot morphometrics			**Shoot biomass** (g DW shoot^{-1})		**Shoot length** (cm shoot^{-1})		**Leaf width** (mm shoot^{-1})		**Shoot biomass** (g DW shoot^{-1})		**Shoot length** (cm shoot^{-1})		**Leaf width** (mm shoot^{-1})
Treatment	***df***	***MS***	***Pseudo-F***	***MS***	***Pseudo-F***	***MS***	***Pseudo-F***	***MS***	***Pseudo-F***	***MS***	***Pseudo-F***	***MS***	***Pseudo-F***
Month (M)	3	0.21	**29.2*****	14.4	**43.5*****	0.04	**50.9*****	0.31	**59.1*****	15.89	**71.8*****	8.78	**175.5*****
Location (L)	2	0.02	**3.2***	3.9	**11.7****	0.03	**35.2*****	0.08	**14.8*****	6.45	**29.1*****	0.55	**10.9****
MxL	6	0.01	**1.9***	0.9	**2.6***	0.00	**3.1***	0.02	**3.8***	1.50	**6.7****	0.81	**16.1****
Residual	68	0.01		0.3		0.00		0.01		0.22		0.05	
Total	79												
Month (M)	3	1.21	**35.4****	0.63	**38.0***	0.10	**22.2****	1.32	**21.9****	0.47	**30.4****	0.53	**77.5*****
Depth (D)	1	2.31	**67.6****	0.10	**6.1***	0.32	**69.8*****	0.81	**13.5****	0.03	2.0	0.13	**19.4****
MxD	3	0.11	3.3*	0.04	2.1	0.002	0.4	0.34	**5.6***	0.11	**7.2***	0.002	0.29
Residual	24	0.03		0.02		0.005		0.06		0.02		0.01	
Total	31												

Table 2.6 Results of the PERMANOVA analysis between descriptors at population level in response to seasonal and depths variabilities**.** Pseudo F-values are shown along with significance levels (**p*<0.05; ***p*<0.01, ****p*<0.001), and degree of freedom (df).

Population descriptors	**Density**						**Cover**			**Biomass**								
			Vegetative S. (no. m^{-2})			**Reproductive S.** (no. m^{-2})			(%)			**AG** (g DW m^{-2})			**BG** (g DW m^{-2})			**AG:BG**
Treatment	***df***	***MS***	***Pseudo-F***	***df***	***MS***	***Pseudo-F***	***df***	***MS***	***Pseudo-F***	***df***	***MS***	***Pseudo-F***	***df***	***MS***	***Pseudo-F***	***df***	***MS***	***Pseudo-F***
Month (M)	3	0.4	**7.3***	3	59.8	**4.3***	3	1.7	**9.9****	3	3.2	**28.9*****	1	0.2	14.8	1	0.5	4.7
Location (L)	2	0.1	1.5	2	20.4	**74.4*****	4	0.2	1.0	2	0.1	2.8	2	0.7	**11.6****	2	0.0	1.3
MxL	6	0.1	1.4	6	14.6	**53.1*****	11	0.2	1.2	6	0.1	**3.6***	2	0.0	0.2	2	0.1	3.1
Residual	68	0.04		68	0.3		201	0.3		68	0.03		24	0.1		24	0.0	
Total	79			79			219			79			29			29		
Month (M)	3	0.2	**3.9***	3	22.6	**312.3*****	3	1.2	**9.7*****	3	1.5	**46.8*****	1	0.0	0.1	1	1.4	**5.2***
Depth (D)	1	11.1	**270.9*****	1	11.9	**164.9*****	1	2.3	**79.9*****	1	1.9	**61.5*****	1	6.7	**55.1*****	1	1.6	**5.8***
MxD	3	0.7	**17.5****	3	4.4	**60.8*****	3	0.9	**4.0*****	3	0.3	**8.1****	1	0.2	1.3	1	0.7	2.5
Residual	24	0.0		24	0.1		90	0.3		24	0.03		8	0.1		8	0.3	
Total	31			31			97			31			12			12		

Table 2.7 Result of PERMADISP analysis comparing morphological descriptors at shoot level of shallow (LM-T1) and deep adapted (LM-T2) *Zostera marina* populations. F-values are shown along with significance levels (**p*<0.05) and marginal significant level (°*p*< 0.1 >0.05).

	Mean shoot			***Canopy height shoot***		
Shoot morphometrics	**Shoot biomass** (g DW shoot^{-1})	**Shoot length** (cm shoot^{-1})	**Leaf width** (mm shoot^{-1})	**Shoot biomass** (g DW shoot^{-1})	**Shoot length** (cm shoot^{-1})	**Leaf width** (mm shoot^{-1})
F	9.804	9.254	0.152	5.019	6.782	0.00103
P (perm)	**0.025***	**0.049***	0.808	0.057°	**0.034***	0.97

2.4. Discussion

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2.4.1. Temporal changes of Irish eelgrass meadows

Results confirm that Irish *Z. marina* populations form monospecific stands and disclose comparable temporal patterns in shoot growth than other perennial populations in temperate regions, which is considered an eelgrass growth strategy in stable environments (i.e. Olesen and Sand-Jensen, 1993, 1994; Cabello-Pasini et al., 2003; Clausen et al., 2014). Our first hypothesis was confirmed since most of the studied descriptors for western Irish seagrass populations described significant variations in their temporal responses which were related to specific environmental patterns such as temperature and irradiance. At both shoot and population level, Irish meadows revealed unimodal patterns with largest values described during warmer month with higher daily light periods, followed by November and April, and lowest in January, whit winter months characterized by colder periods with lower daylight hours (Fig. 2.2, Table 2.1). For instance, shoot biomass and length were generally 2- to 3-fold greater in July than in January. Temporal changes in shoot biomass were mainly related to the temperate eelgrass's capacity to adjust leaf structures by developing larger and wider leaves in favourable periods as these conditions are more suitable for photosynthetic activity. Shorter and thinner leaves were produced in less favourable climatic conditions for photosynthetic activity, thus by reducing

biomass structures, seagrasses reduce metabolic cost and respiration rates. In April, common to the three Irish populations, shorter average shoot lengths were observed than in November, while shoot density remained similar in both months. This can be explained by the seeding and growth of small new shoots in spring as was previously reported in other perennial eelgrass systems (Olesen & Sand-Jensen 1994). In spite of the relatively small spatial scale of this study (< 100 km), the three populations displayed marked morphological differences in shoot characteristics. These differences may be related to the phenotypic plasticity of *Z. marina* and by the specific local environmental conditions such as hydrodynamic conditions or nutrient concentration in the water column which are not tested in this study. This adaptive mechanism allows this species to cope with a wide range of environmental gradients and spatial distribution and is maybe related to its resilient character (Kim et al., 2014; Maxwell et al., 2014).

Comparable averages of vegetative shoot densities (50 to 800 shoots per m^2) were previously observed for some similar cold-temperate populations which displayed larger values in warmer periods (e.g. Aioi et al., 1980; Olesen & Sand-Jensen 1994*b*; Bostrom et al., 2006; Watanabe et al., 2005). By contrast, in other cold temperature eelgrass meadows shoot densities were reported (> 3000 shoots per m^2) ten times higher than in the Irish studied populations (e.g. Wium-Andersen & Borum 1984; Krause-Jensen et al., 2000). Such low shoot densities observed in Irish subtidal-shallow populations may be explained by the development of long shoots which may favour self-shading conditions, thus limiting horizontal propagation. Personal observations in intertidal Irish meadows in Kilkieran Bay reported densities of *Z. marina* ranging from 800 to 1300 shoots per m^2, suggesting that shoot density greatly varies depending on specific *in-situ* environmental conditions.

Temporal changes in percentage cover and AG biomass were positively correlated with the morphological and structural shoot plasticity, as was previously reported by relevant studies (Olesen and Sand-Jensen, 1994). For instance, increases in AG biomass in summer were more attributed with increases in shoot biomass rather than changes in density, which only increased slightly, as was reported in *Z. marina* populations from stable environments (Olesen and Sand-Jensen, 1994; Boström et al.,

2014). This points towards a high renewal of leaf structures with high turnover rates indicating a relevant exportation of organic matter to other systems (Mateo et al., 2006; Boudouresque et al., 2015).

The influence of local environmental fluctuations on the reproductive effort and flowering of *Z. marina* has been extensively documented, where highly variable responses have been observed along its latitudinal distribution range (e.g. Meling-Lopez et al., 2014; Harwell et al., 2002; Lee et al., 2006). In our meadows studied, the presence of early-stage reproductive shoots was observed in March-April (SST of ~ 10 °C) with the development of immature male flowers (and occasionally mature male flowers), but there were no records of either female flowers or seeds. In July, at highest water temperatures (SST of ~ 16 °C) a greater density of reproductive shoots was documented, with plants developing mature male and female flowers and seeds. The presence of flowering plants was observed until the end of November (SST of ~ 10 °C) (*pers*. *obs*.) suggesting that the reproductive period covered 9 months. Prior studies correlated the reproductive effort with light and temperature gradients (Rollon et al., 2003). The lowest density of reproductive shoots was observed at FV, which represented a small and relatively patchy meadow. Such differences in the reproductive effort may be related to both genetic or phenotypic diversity (Alberto et al., 2005; Quin et al., 2014) or to the size of specific meadows, but evidence to support this suggestion was not provided in this study. Prior studies observed lower reproductive efforts in shallower meadows, although we observed the highest values in deep-adapted populations (LM-T2). Sexual recruitment and seed production have previously been related to the capacity of seagrass meadows to maintain a high genetic diversity, enhancing a population's ability to persist during environmental disturbances (Marbà & Walker 1999; Koch et al., 2006; Lee et al., 2007).

2.4.2. Depth-related responses at shoot and population level

Our second assumption stating that populations living at their deepest distribution limit may display a higher temporal morphological plasticity and also may show different morphological and population properties than plants from a shallow site, in

response to different irradiance conditions, was demonstrated since most of the studied descriptors highlighted that deeper plants displayed a larger temporal variation than plants at the shallower sites, and also showed to have different morphological characteristics. Light limitation induced the temporal development of higher shoot size and biomass which seems to be a general adaptive mechanism of seagrasses to increase light absorption efficiency (Enriquez et al., 1993). In July deep-adapted populations (LM-T2) developed shoots with significantly higher weight and size from LM-T1, whereas there were no significant differences in winter. This highlights the plastic capacity of deep *Z. marina* populations to facilitate an adjustment of their AG structures in response to temporal fluctuations in light availability, representing a relevant mechanism for seagrass growth and survival (Touchette and Burkholder, 2000). A reduction in photosynthetic tissues during less favourable periods allow seagrasses to reduce metabolic costs to maintain lower respiration rates (Nielsen et al., 2002). Yet, both shallows and deep plants maintained leaf structures in winter suggesting that they performed some photosynthetic activity (Evans et al., 1986; Olesen & Sand-Jensen 1993). LM-T2 population appeared to be characterized by the presence of larger shoots with higher biomass compare to shallower populations. These morphological characteristics were accompanied by 2-3 fold decreases in AG biomass, eelgrass cover and density with increasing depth; this trend has been widely observed in deep cold-temperate *Z. marina* populations (e.g. Olesen et al., 2002; Nielsen et al., 2002). This indicates that deep-adapted populations favour vertical growth rather that the horizontal growth, which is a well-known response of seagrasses to depletion in irradiance with increases in depth (Ralph et al., 2007). Krause-Jensen (2000) suggested that lower shoot densities at greater depths reduced self-shading and consequently improved the irradiance conditions letting shoots produce larger leaves and biomass, therefore, allowing plants to store greater resources for vertical growth (Watanable et al., 2005). Such results could have been expected since light is considered the controlling factor of eelgrass metabolism, distribution and abundance, especially towards the lower depth limit of a meadow (Duarte 1991; Krause-Jensen et al., 2000; Ralph et al., 2007).

Eelgrass populations inhabiting shallow areas produced significantly higher below-ground biomass and larger numbers of rhizome segments than populations inhabiting

deeper areas. These results concur with previous studies which reported similar patterns in *Posidonia* spp. and subtropical species from eastern Australia, *Zostera mulleri* and *Halophila ovalis* (Serrano et al., 2014; Samper-Villareal et al., 2016), with larger biomass deposits observed in populations inhabiting shallower areas than in deeper plants. Such observations can be explained by low irradiances limiting primary production and carbon balance, thus reducing the capacity to store biomass in the rhizomes as carbon reserves (e.g. Alcoverro et al., 1995; Olesen et al., 2002; Serrano et al., 2015). However, carbon accumulation in the sediment may be attributed to other factors such as rates of decomposition, sedimentation rates or hydrodynamic forces (Fourqurean et al., 2012) which are not tested in this study.

2.4.3. Irish eelgrass leaf productivity in comparison to populations from other latitudes

Eelgrass leaf production is correlated with *in-situ* SST since annual leaf formation rates were found to rise with increases in temperatures (Lee et al., 2007). An increment of 1.0 and 1.4 in annual leaf production is observed for every 1°C rise in annual and summer SST respectively (Fig. 2.7). Similarly, leaf formation rates vary between latitudes where the rate of formation of new leaves increases to 0.56 leaves per degree latitude southward (Fig. 2.7). These observations support the suggestion that leaf production is affected by large-scale factors such as temperature and latitudinal distribution (Olesen et al., 2014). For example, several studies confirm that eelgrass populations adapt their optimal leaf growth rates to local temperature conditions, which can range from 16 to 24 °C (e.g. Sfriso & Ghetti, 1998; Boström et al., 2004; Lee et al., 2007).

Leaf production in shallow eelgrass Irish populations ranged from 29.4 to 19.6 leaves per year, which was similar to values from other cold-adapted seagrasses as well as other populations inhabiting similar latitudinal ranges (i.e. 26.1 leaves shoot^{-1} yr^{-1}, Netherlands, Nienhuis & De Bree, 1980). Higher leaf formation rates were observed at the southern limits of eelgrass distribution in Mexico (22.4 °C) and in Mediterranean regions of Spain (23.2 °C) with 44.5 and 43.8 leaves shoot^{-1} yr^{-1}, respectively (Ibarra-Olbando et al., 1997). Yet, diebacks were observed at these

southern latitudes after periods of anomalously high summer temperatures indicating that their optimal physiological temperature may have been exceeded, compromising their growth and survival (Durako & Moffler 1987; Reusch et al., 2005). On the other hand, the lowest leaf productions were observed in Greenland meadows which inhabit the coldest regions (6.9 – 13.1 leaves shoot^{-1} yr^{-1}, Olesen et al., 2015). Recent investigations suggest that climate change effects already observed in arctic regions, such as rises in SST and reductions in the seasonal sea ice, may improve seagrass environmental conditions, thus favouring their performance and potential dispersion (Krause-Jensen et al., 2015).

Furthermore, above-ground biomass was neither correlated with *in-situ* summer nor with annual seawater surface temperatures (Clausen et al., 2014; Olesen et al., 2015), suggesting that growth patterns of leaf biomass at population level are not controlled by large-scale factors, but local environmental conditions. Greater productivity is observed at intermediate latitudes (404 – 1060 g DW m^{-1}) (e.g. Thorne-Miller et al.,1983; van Lent and Verschuure, 1994; Watanabe et al., 2005). These results are inagreement with the biomass values observed in Ireland, suggesting that Irish populations have similar productivity values to other populations exposed to similar SST.

2.4.4. Implications for management: from local to global scale

The west coast of Ireland, and in particular the bays studied here, represent unpolluted and pristine ecosystems with highly productivity and biodiversity (O´Boyle et al., 2012; SAC, 2013, 2014), rendering the study area to have exceptional ecological and biological value. As it is, to our knowledge, the first ecological assessment of seagrass meadows in western Ireland, this study represents an essential baseline to (i) develop long-term monitoring programs to assess potential ecosystem degradation (ii) to monitor potential effects of heat waves in the west coast of Ireland, and finally, (iii) to provide missing biological data for of global importance of understudied populations. Deep populations of *Zostera* habitats were reported to be key environmental indicators to assess the ecological status of

transitional marine habitats (Krause-Jensen et al., 2007). In particular, in Kilkieran Bay, vast extensions of co-occurrences of seagrass and maerl are commonly observed at the deepest edge of the eelgrass meadows, whilst the bank of maerl became more abundant with depth (SAC, 2013, 2014; Beca-Carretero et al., *under review* (Chapter 4)) (Fig. S2.3). This co-occurrence has so far only been documented from western Ireland (De Grave, 1999; NPWS, 2014) and France (Martin et al., 2005), and is still largely unstudied. Maerl beds form a three-dimensional structure of nodular coralline algae which support a high biodiversity and productivity (Foster, 2001; Wilson et al., 2004). Also, both species are part of the five Annex I habitats in the EU Habitats Directive (92/43/EEC) and both belong to the habitats on the Initial List of OSPAR Threatened and/or Declining Species and Habitats considered to be causes for concern (OSPAR Commission 2005). Further investigations are necessary to assess their interaction.

Furthermore, in the context of climate change with southern populations experiencing die-backs due to anomalously high temperatures, and Arctic distributed populations experiencing more favourable environmental conditions (Krause -Jensen et al., 2015), the response of centrally distributed populations remains unclear (Beca-Carretero et al., 2018*b*). With Irish seagrass populations living at maximum summer sea water temperatures ranging from 15 to 17 ºC, projected increases of 2-3 ºC by the end of this century (IPCC, 2014) may favour seagrass growth and productivity.

Chapter 3

Effects of temporal variations, depth and latitudinal gradients in the synthesis of key biochemical descriptors of *Zostera marina* populations

Beca-Carretero P, Guihéneuf F, Krause-Jensen D, Stengel DB. Effects of temporal variations, depth and latitudinal gradients in the synthesis of key biochemical descriptors of *Zostera marina* populations. Marine Environmental Research.

Author contribution

Pedro Beca-Carretero developed the experimental design and the statistical analysis; Pedro Beca-Carretero and Freddy Guihéneuf performed the biochemical analysis; Dagmar B. Stengel resources and funding for the project; all the authors contributed to the interpretation of the data and the writing of the manuscript

Abstract from original article

Zostera marina is a dominant meadow-forming seagrass in temperate regions in the northern hemisphere, including Irish coasts. Here, the fatty acid content and composition of seagrasses were studied to evaluate their eco-physiological state. For the first time, this investigation assessed the biochemical responses of Irish *Z. marina* populations to temporal changes in temperature and irradiance. Biochemical descriptors were measured in healthy green leaves of seagrass shoots collected in April, July, November 2015 and January 2016. *Z. marina* leaves collected in winter accumulated 2-3 times more TFA and photosynthetic pigments than in summer. Increases in TFA were mainly linked to the larger synthesis of polyunsaturated fatty acids (PUFA) and the lower production of saturated fatty acids (SFA) in colder periods, and in deep-adapted populations during warmer periods than plants living in shallow areas. These results therefore highlight the capacity of *Z. marina* to adjust their lipid composition to achieve optimal membrane fluidity under less favourable environmental conditions. Additionally, a comparison of FA composition of *Z. marina* across its latitudinal distribution range (southern Spain to Greenland) was undertaken. Results suggest that populations adapted to warmer *in situ* seawater temperatures had significantly lower PUFA/SFA ratios, indicating that future warming may negatively affect the nutritional value of seagrass with implications for higher trophic levels.

Key words: Biochemical plasticity; Depth acclimatization; Latitudinal responses; Essential fatty acids (EFA); Polyunsaturated fatty acids (PUFA); Total fatty acids (TFA); Photosynthetic pigments; *Zostera marina*; Nutritional value; Ireland

3.1. Introduction

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Global change is driving effects at different biological levels, from cellular organization and metabolism to community structures, inducing species migration and losses in biodiversity and associated ecological services (e.g. Orr et al., 2005; Doney et al., 2011). Thus, it is crucial for the research community to identify key responsive biological parameters which may help to recognize early signs of stress or vulnerability in indicator species (Walther et al., 2002). Over the last decades, different approaches have been applied to marine plants to assess their ecological status, such as physiological, morphological or structural responses, as well as biochemical descriptors, including, isotope, nutrient or phenol content (e.g. Rottini et al., 2017).

Eelgrass, *Zostera marina* L., is the most widespread marine angiosperm in the northern hemisphere (den Hartog 1979) and is considered an indicator species due its rapid responsivity to climatic fluctuations or to depletions in ambient regimens (Krause-Jensen et al., 2005). Temperature and irradiance are the most important factors driving the eco-physiological responses of *Z. marina* populations which largely explain its temporal and spatial production (Lee et al., 2007). *Z. marina* is adapted to a wide range of environmental conditions from warm and oligotrophic waters in subtropical regions of southern Mexico and Mediterranean coasts (i.e. Den Hartog 1979; Diekmann & Serrao 2012) to temperate climate at its central distribution range (Short et al. 2007), and to cold, dark but nutrient-rich waters in subarctic and Arctic regions (Olesen et al., 2015). Consequently, different adaptive mechanisms, including biometric variation, genetic diversity, biochemical plasticity or metabolic adjustments are required to survive in less favourable environments (e.g. Ehlers et al., 2008). In temperate regions, *Z. marina* populations generally display distinct morphological and biochemical seasonal trends with optimal growth conditions occurring during warmer months and reduced growth during colder and darker periods (e.g. Orth et al. 1986). Light energy controls the vertical distribution of seagrasses, thus affecting shoot characteristics and population dynamics (Dennison 1987; Ralph et al., 2007). An increase in pigment concentrations is a common growing at greater depths to optimize the use of light energy (Cummings et al., 2003; Zimmerman et al., 2007).

Species-specific thermal physiological limits were partially correlated to the membrane composition, particularly to their lipids and fatty acid synthesis (e.g. Murakami et al., 2000). Fatty acids (FAs) are vital structural compounds constituting the membrane lipids of photosynthetic organisms which provide resistance and barriers to environmental surroundings (e.g. Beisson et al., 2007). In marine and terrestrial ecosystems, primary producers biosynthesize the main essential fatty acids (EFAs) which are subsequently transferred to higher trophic levels (Behrens et al., 1996; Dalsgaard et al., 2003). Overall, polyunsaturated fatty acids (PUFAs) are the most abundant FAs constituting the membrane lipids (glycolipids and phospholipids). Their synthesis plays a key role regulating the photosynthetic activity by promoting the fluidity of the thylakoid membranes within the chloroplasts and enhancing the electron transport in the photosystems (Gombos et al., 1994; Sanina et al., 2004, 2008). PUFAs characterized by higher unsaturation levels promote more fluidity of the thylakoid membrane in photosynthetic organisms (Upchurch et al., 2008). On the other hand, saturated fatty acids (SFA) are reserve energy compounds, mainly partitioned into triacylglycerols (TAG); their production highly varies among species and depend on climate drivers as they are generally accumulated under conditions un-favourable for growth (Pal et al., 2011).

The study of fatty acid production in marine primary producers, such as microalgae and seaweeds, has allowed the assessment of their lipid nutritional value (e.g. Tonon et al., 2002), while other recent studies used fatty acids as biomarkers of thermal stress (Feijao et al., 2017). The use of FA synthesis as a tool to assess physiological status has been applied to terrestrial plants, particularly to agricultural species with commercial interest or transgenic plants (e.g. Murakami et al., 2000; Vigh et al., 1998). By contrast, the study of FAs in seagrasses has been less extensive, and only a small number of studies investigated the responses of lipid metabolism and FA synthesis to environmental variations, such as, temperature or irradiance, and also to salinity (e.g. Beca-Carretero et al., 2018*a* (Chapter 7); Viso et al., 1993; Sanina et al., 2004; Sousa et al., 2016). Seagrasses represent an important food source for marine animals (Thayer et al., 1975) so that changes in their nutritional value due to climate changes are a major concern.

The wide latitudinal distribution of *Z. marina* makes this species particularly interesting to assess the biochemical and physiological mechanisms that allow this plant to adjust

to extensive environmental gradients. Here, we hypothesize that biochemical adjustments, in particular the synthesis of FA occur in response to varying climate parameters, such as temperature and irradiance. Specifically we studied (i) potential spatial and temporal changes in total FA content and composition and photosynthetic pigments of shallow populations, (ii) the temporal adaptive biochemical capacities of different eelgrass populations to acclimate to different light regimes (i.e. depth profiles), (iii) the potential correlation between FA and photosynthetic pigment content, and finally, (iv) the variations in TFA synthesis and proportion of PUFA and PUFA/SFA ratios in *Z. marina* across its latitudinal geographical range.

3.2. Materials and methods

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3.2.1. Study site

The study sites were located in Galway Bay, in western Ireland (Fig. 3.1) in two locations recognized as Special Areas of Conservation (SAC) (NPWS, 2014). Lettermore (LM) (53°17'58" N, 9°42'43" W) and Tír an Fhia (TI) (53°15'37.5" N, 9°38'28.8" W) are semi-exposed areas and were situated in Kilkieran Bay in northern Galway; the third location, Finavarra (FV) (53°8'50" N, 9°07'43" W) was a sheltered bay and located in southern Galway Bay. At each location, we placed two 50 m-long permanent transects (T1 and T2) parallel to the shoreline. At TI and FV, both T1 and T2 were fixed at 2-2.5 m (shallow), while at LM, T1 was situated at 2-2.5 m and T2 at 4.5-5 m (shallow and deep, respectively).

3.2.2. Environmental descriptors

We obtained the temperature (˚C) data for the two locations of Kilkieran Bay (TI and LM) from the Marine Institute (http://www.marine.ie/Home/). At FV a submersible HOBO temperature logger (U22-001, Onset) provided seawater temperatures at ca. 2 m depth close to the meadow over two seasons (spring and summer 2015). Daily

irradiance was obtained using a light sensor (Li-1400, Li-Cor, USA) placed on the roof of the Martin Ryan Building, NUI Galway campus (53°16'43" N, 9°03'40" W, Galway City, about 25 km from FV and 40 km from LM and TI). Total daily irradiance was expressed as mol photons m^{-2} d^{-1}. Moreover, at each location during sampling time, one Odyssey integrating PAR sensor (Dataflow Systems PTY Limited) was placed in the sediment at a 90° angle. Loggers recorded underwater *in-situ* instant irradiance (µmol photon m^{-2} s^{-1}) every 15 min for 1-2 h.

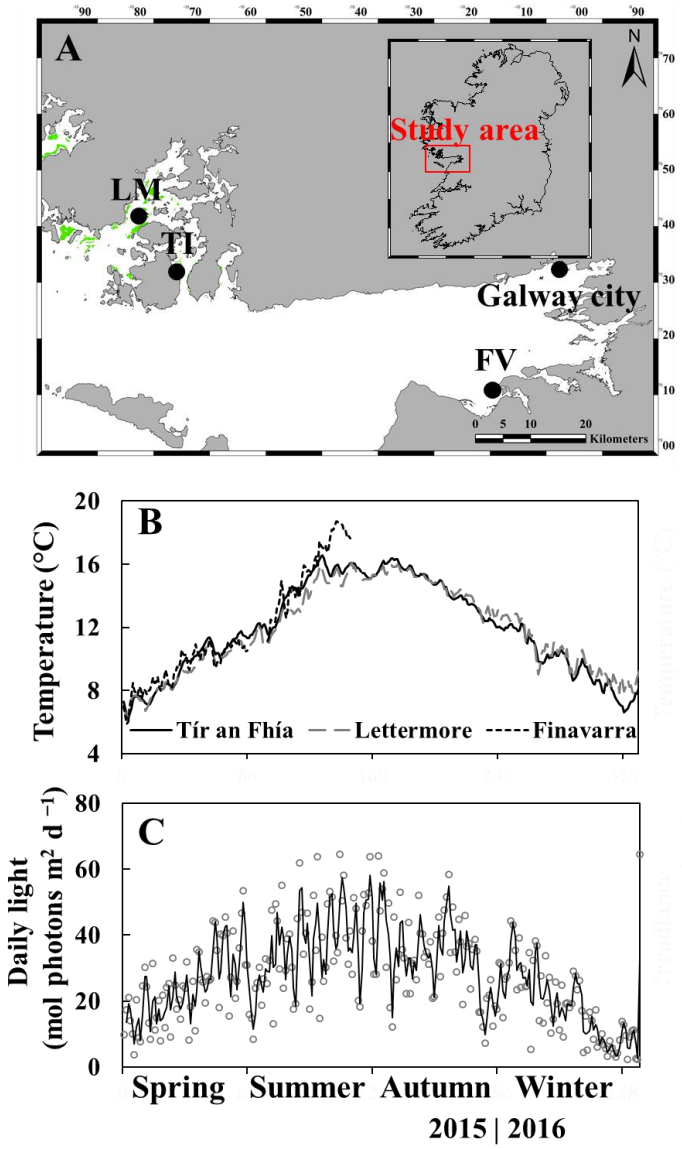

Figure 3.1 Map of study area with the three *Zostera marina* meadows (green colour) at Lettermore (LM), Tír an Fhia (TI) and Finavarra (FV) in western Ireland (Panel A). Data of daily sea surface temperature (SST) (Panel B) and daily measurements of daily irradiance (mol

photons m^{-2} d^{-1}) measured on the roof of the Martin Ryan Building, NUI Galway, Galway City (Panel C). Blue arrows represent the period time of sample collection over the seasons.

3.2.3. Sample collection

We conducted the sample collection over four times, April, July and November 2015, and January 2016, by SCUBA diving or by snorkelling. We collected healthy apical shoots of *Z. marina* from each transect at intervals of 4-6 meters to potentially prevent resampling of the same genotype (n = 10). We transported the biomass in seawater in cooler boxes, to achieve similar temperatures to *in-situ* field conditions, in darkness to the laboratory within 2 h. Subsequently, the first and second youngest leaves per shoot were selected for biochemical analysis. Intermediate parts of the leaves, (without epiphytes) were selected (~ 1.5-2.5 g FW (fresh biomass) per sample), cleaned in distilled water, kept in small plastic bags and frozen at -20 °C. After 48 h, frozen samples were freeze-dried (Labconco Freezone, Kansas City, MO, USA, Freeze-dryer System) for 24-48 h. Freeze-dried Samples were stored for no longer than 1-2 months at -40 °C. Prior to biochemical analyses, we processed the selected biomass into a fine powder using a bead mill homogenizer Beadmill 4 (Fisher Scientific, USA) machine at 5 m s^{-1} for 3 min.

Leaf samples collected from different latitudinal regions, where was no freeze-drying facilities were available, were dried (1.5-2 g of FW) in 15-20 g of silica gel in small plastic bags for 24-48 h in darkness until the biomass was completely dried. Samples were then kept in the same conditions as the (Irish) freeze-dried samples prior to biochemical analysis.

Table 3.1 Geographic coordinates of the permanent transects 1 (T1) and transects 2 (T2) at each location Lettermore (LM), Tír an Fhia (TI) and Finavarra (FV) (water depth of each transect relative to the mean water level). Current velocity was measured on the seafloor (not determined at FV). Seawater temperature measured at times when *Z. marina* biomass was collected. *In-situ* irradiance (PAR, µmol photon m^{-2} s^{-1}) was measured at each location during sampling.

	LM-T1	LM-T2	TI-T1	TI-T2	FV-T1	FV-T2
Latitude	53.293	53.293	53.260	53.260	53.147	53.147
Longitude	-9.712	-9.713	-9.641	-9.641	-9.128	-9.128
Depth (m)	[2-2.5]	[4.5-5]	[2-2.5]	[2-2.5]	[2-2.5]	[2-2.5]
Temperature (°C)						
April	11	11	10	10	11	11
July	16	15	15	16	17	17
November	12	11	11	11	11	11
January	7	7	7	7	6	6
Irradiance (µmol photons m^{-2} s^{-1})						
April	66.8 ± 19	15 ± 0.6	93.7 ± 48.0	93.7 ± 48.0	71.7 ± 6.8	71.7 ± 6.8
July	43.5 ± 15.10	14.8 ± 4.2	60.0 ± 11.3	60.0 ± 11.3	63.0 ± 15.7	63.0 ± 15.7
November	38.8 ± 20.8	7.1 ± 2.7	35.0 ± 5.8	35.0 ± 5.8	18.4 ± 5.5	18.4 ± 5.5
January	19.3 ± 8.1	4.3 ± 0.6	37.5.0 ± 16.0	37.5.0 ± 16.0	45.3 ± 9.9	45.3 ± 9.9

3.2.4. Fatty acid analysis

We analysed the fatty acids from the selected leaf biomass of *Z. marina* applying the protocol previously used with microalgae, algae and seagrasses (Schmid et al., 2014; Beca-Carretero et al., 2018*a*). Fatty acid methyl esters (FAME) were obtained by direct transmethylation of ~20-30 mg of powdered leaf biomass with dry methanol containing 2 % (v/v) H_2SO_4. To prevent oxidation, vials were closed with nitrogen gas and heated at 80 °C for 2 h under continuous stirring conditions. After transmethylation, we added 1 mL of milli-Q water and later extracted the FAME using 0.5 mL of n-hexane. Analysis of FAME followed the protocol described by Beca-Carretero et al. (2018*a*) using an Agilent 7890A /5975C Gas Chromatograph/mass selective detector (GC/MSD) Series (Agilent Technologies, USA) equipped with a flame ionization detector and a fused silica capillary column (DB-WAXETR, 0.25 mm × 30 m × 0.25 µm, AgilentTechnologies, Catalog No.: 122-7332). Identification of FAME was achieved by co-chromatography with authentic commercially available FAME standard of fish oil (Menhaden Oil, catalogue no. 47116, Supelco). Total and individual fatty acid contentswere quantified by comparison with a known quantity of added pentadecanoic acid 15:0

(99 %, catalogue no. A14664-09, Alfa Aesar, UK) as internal standard. We added the internal standard (10 µl, 5 mg mL^{-1}) prior to start the direct transmethylation and expressed the results as the mean values of 5 replicates (n = 5) for each treatment.

3.2.5. Photosynthetic pigment extraction

We carried out two consecutive pigment extractions, using 5 mL of 80 % (v/v) acetone each time, from ~20-30 mg of powdered leaf biomass. The first extraction was conducted over 20 h, and the second over 4 h. To ensure optimal extraction and to avoid pigment oxidation, the two extractions were performed in darkness at 4 °C and with continuous stirring, and the vials were closed under nitrogen gas. After the first extraction, we centrifuged the samples and the supernatant (5 mL) was kept in darkness at 4 °C. The remaining biomass was used for the second extraction. The supernatants of both extractions were combined to a final volume of 10 mL which was then used for pigment analysis. Where necessary, 80 % acetone was added to ensure the target final volume (10 mL). We quantified chlorophyll *a*, chlorophyll *b* and carotenoids with spectrophotometric absorbance reading (CARY 50 Scan UV-Visible Spectrophotometer), following the equations of Lichtenthaler & Buschmann (2001).

Chl *a* (µg mL^{-1}) = $12.21E_{663} - 2.81E_{646}$

Chl *b* (µg mL^{-1}) = $20.13E_{646} - 5.03E_{663}$

Carotenoids (µg mL^{-1}) = $(1000\ A_{470} - (3.27\text{Chl } a) - (104\text{Chl } b))/227$

Where: E_{xxx} = Absorbance at xxx nm – Absorbance at 725 nm

3.2.6. Latitudinal comparison

Leaf biomass from 7 different *Z. marina* populations from 4 different latitudinal regions were collected between 28th August and 9th September 2016 (see details in S3.1); southern Spain, Cadiz, (Ruiz et al., 2015; Brun et al., 2015), northern Spain, Bouzas (Vigo) and Sada (A Coruña) (Ruiz et al., 2015), western Ireland (Finavarra and

Lettermore) (Beca-Carretero et al., *under review* (Chapter 2)) and western Greenland (Kappissillit and KobbeFjord) (Olesen et al., 2016; Beca-Carretero et al., 2018). Sample collection was conducted following the same procedure as for samples from Irish transects.

At Cadiz, the *Z. marina* population was represented by a few small patchy meadows which is the only record of this species in the south of Spain. From this population, we randomly collected samples from two separate patchy meadows (M1 and M2). All samples derived from the different latitudinal regions were obtained from subtidal populations which occurred at depths ranging from 2-3.8 m. Sea surface temperatures (SST) (°C) at the collection time were obtained using different *in-situ* temperature meters, such as, HOBO loggers or dive computers. Moreover, annual SST data was derived from the Bio-ORACLE database (http://http://www.bio-oracle.org) (Tyberghein et al., 2012) with a resolution of 0.5 km^2.

3.2.7. Statistical analyses

Prior to performing statistical analyses, we tested homogeneity (Levene's test) and normality (Kolmogorov–Smirnov test); non-conforming data were ln transformed to meet the criteria of ANOVA. Seasonal data of pigments and fatty acids from different transects, T-1 and T-2, across the study sites on the Irish west coast (TI, LM and FV) were compared by applying *t*-tests ($p < 0.05$). Subsequently, we conducted two two-way ANOVAs and *post hoc* Tukey's pairwise test to (i) evaluate temporal differences in the biochemical composition between months (April, July, November and January) and shallow locations (2-2.5 m; TI, FV and LM-T1), and (ii) to assess different biochemical responses between months and depths (LM-T1 (2-2.5 m) and LM-T2 (4.5-5 m)).

For the latitudinal study, we conducted a one-way ANOVA and *post hoc* Tukey's pairwise test to assess differences between the seagrass populations (southern Spain, northern Spain, western Ireland and western Greenland). We carried out all data treatments and statistical analyses using IBM SPSS Statistics V13.0 (IBM Corporation, USA). All values are reported as means and standard deviation (SD).

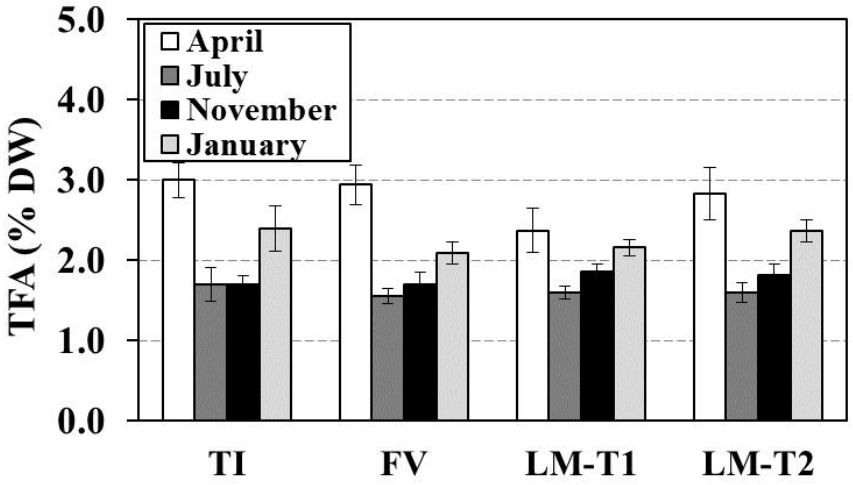

Figure 3.2 Total fatty acid contents (% DW) of leaves of Irish populations of *Zostera marina* collected in Spring, Summer, Autumn and Winter. Transect (T), Lettermore (LM) (Transect 1 (LM-T1-M1) and Transect 2 (LM-T2)), Tír an Fhia (TI), Finavarra (FV).

Table 3.2 Total fatty acid content (% DW) and composition (% of TFA) of leaves of Irish *Zostera marina* populations, Lettermore (LM), Tír an Fhia (TI) and Finavarra (FV). Results are expressed as mean ± SD (n = 5).

Location		**TI**	**TI**	**TI**	**TI**		**FV**	**FV**	**FV**	**FV**		**LM-T1**	**LM-T1**	**LM-T1**	**LM-T1**		**LM-T2**	**LM-T2**	**LM-T2**	**LM-T2**
Season	**CV (%)**	**Spring**	**Summer**	**Autumn**	**Winter**	**CV (%)**	**Spring**	**Summer**	**Autumn**	**Winter**	**CV (%)**	**Spring**	**Summer**	**Autumn**	**Winter**	**CV (%)**	**Spring**	**Summer**	**Autumn**	**Winter**
Total fatty acids (% DW)	**24.7**	**3.0**	**1.7**	**1.7**	**2.4**	**24.7**	**2.9**	**1.6**	**1.7**	**2.1**	**15.0**	**2.2**	**1.6**	**1.9**	**2.4**	**22.2**	**2.8**	**1.6**	**1.8**	**2.4**
Saturated FA (% of TFA)																				
14:0		0.3	0.4	0.4	0.3		0.4	0.7	1	1.1		0.2	0.2	0.4	0.2		0.2	0.2	0.4	0.2
16:0		18.1	24.9	26.4	23.0		16.9	19.1	26.7	21.3		19.9	21	27.7	22.7		18.4	21.3	29	24.2
18:0		0.9	1.6	1.7	0.8		1.2	1.1	1.3	1		1.1	2.4	1.2	0.9		1.6	1	1.7	0.8
23:0		1.9	0.3	0.4	0.4		1.8	1	1.1	0.9		0.9	0.1	0.4	0.3		0.1	0.5	0.4	0.3
Sum of SFA	**11.4**	**21.2**	**27.2**	**28.9**	**24.5**	**15.4**	**20.3**	**21.9**	**30.1**	**24.3**	**11.5**	**22.1**	**23.7**	**29.7**	**24.1**	**16.5**	**20.3**	**23.0**	**31.5**	**25.5**
Monounsaturated FA (% of TFA)																				
14:1		0.7	0.9	1.0	1.0		0.8	0.5	0.5	1.1		0.5	0.8	0.7	0.6		0.8	0.5	0.9	0.7
16:1 n-7		0.3	0.4	0.3	0.3		0.2	0.4	0.4	0.5		0.6	0.5	0.4	0.3		0.3	0.4	0.3	0.3
18:1 n-7		1.1	0.5	0.4	0.4		0.4	0.5	0.5	0.3		0.6	0.5	0.6	0.3		0.4	0.4	0.6	0.3
18:1 n-9		0.4	0.9	0.9	1.0		1.4	1.9	1.4	1		1.2	1	1.5	1.2		0.7	1.1	1.3	0.9
Sum of MUFA	**3.2**	**2.5**	**2.7**	**2.6**	**2.7**	**7.0**	**2.8**	**3.3**	**2.8**	**2.9**	**10.1**	**2.9**	**2.8**	**3.2**	**2.4**	**14.9**	**2.2**	**2.4**	**3.1**	**2.2**
Polyunsaturated FA (% of TFA)																				
16:2 n-3		0.4	0.2	0.2	0.2		0.3	0.4	0.3	0.3		0.3	0.3	0.2	0.2		0.5	0.1	0.2	0.2
16:3 n-3		8.6	4.7	4.7	6.8		8.5	7.5	4.7	6.8		8.5	7.5	4.9	7		9.4	7.7	4.5	7.3
16:3 n-4		1.0	1.8	1.5	1.1		1	1.1	1.8	1.8		1.2	0.7	0.8	0.9		0.8	0.7	0.7	0.9
18:2 n-6		10.6	13.6	13.6	10.7		10.7	12.5	14.4	11.8		11.3	15	14.7	13.1		10.6	14.9	13.5	11.9
18:3 n-3		49.6	41.8	40.5	45.9		49.5	47.9	40.3	46.9		45.4	46	37.6	45		49	46.9	37	44.9
20:5 n-3		0.7	1.2	1.2	1.4		0.8	0.2	0.4	0.4		2.3	0.2	2.6	2.3		1.8	1.2	2.6	2
22:5 n-3		0.8	1.0	1.1	0.8		0.7	1	0.7	1		1	0.1	1.4	0.8		0.6	0.1	1	0.8
Sum of PUFA	**5.1**	**71.7**	**64.3**	**62.8**	**66.9**	**5.1**	**71.5**	**70.6**	**62.6**	**69.0**	**4.8**	**70.0**	**69.8**	**62.2**	**69.3**	**7.6**	**72.7**	**71.6**	**59.5**	**68.0**
Others		4.6	5.8	5.7	5.9		5.4	4.2	4.5	3.8		5	3.7	4.9	4.2		4.8	3	5.9	4.3
PUFA/SFA	**17.0**	**3.4**	**2.4**	**2.2**	**2.7**	**18.1**	**3.5**	**3.2**	**2.1**	**2.8**	**14.7**	**3.2**	**2.9**	**2.1**	**2.9**	**22.0**	**3.6**	**3.1**	**1.9**	**2.7**
n3/n6	**21.2**	**5.7**	**3.6**	**3.5**	**5.1**	**19.0**	**5.6**	**4.6**	**3.2**	**4.7**	**17.8**	**5.1**	**3.6**	**3.2**	**4.2**	**20.8**	**5.8**	**3.8**	**3.4**	**4.6**
% n3 (TFA)	**9.4**	**60.1**	**48.9**	**47.7**	**55.1**	**9.2**	**59.8**	**57.0**	**46.4**	**55.4**	**7.6**	**57.5**	**54.1**	**46.7**	**55.3**	**10.6**	**61.3**	**56.0**	**45.3**	**55.2**

3.3. Results and discussion

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This study is, to our knowledge, the most detailed analysis of fatty acid (FA) changes occurring in response to temporal variations and depth adaptations in marine plants; it includes an extensive assessment of samples of a single seagrass species across a large latitudinal range. Temporal variations in temperature and irradiance induced significant changes in FA and photosynthetic pigment profiles in *Z. marina* leaves. The production of TFAs, particularly changes in n-3/n-6 (PUFA) and SFA (16:0), displayed clear responses to environmental gradients. Reductions in temperature and irradiance appeared to induce an increase in the levels of FA unsaturation of *Z. marina* leaves, while a marked increase in SFA was linked to increases in temperature, accentuated in southern eelgrass populations. Finally, the temporal accumulation of FAs, especially PUFAs, was positively correlated with the production of photosynthetic pigments in eelgrass leaves.

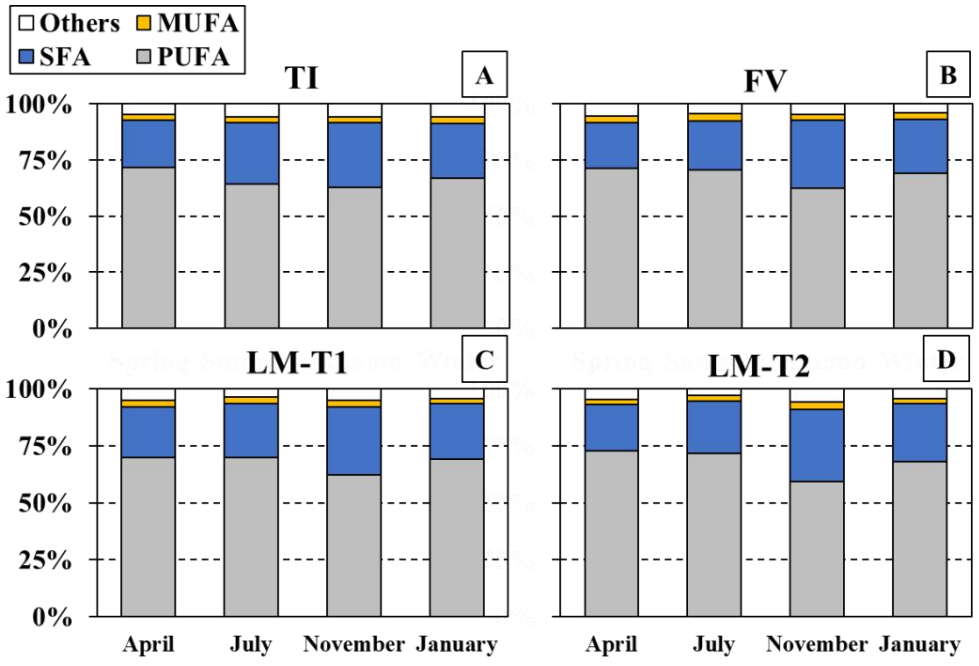


Figure 3.3 Proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and non-identified (Others) fatty acids (% of TFA) of leaves of Irish *Zostera marina* populations of collected in Spring, Summer, Autumn and Winter. Transect (T), Lettermore (LM) (Transect 1 (LM-T1-M1) and Transect 2 (LM-T2)), Tír an Fhia (TI), Finavarra (FV).

3.3.1. Environmental descriptors

Maximum sea surface temperatures (SST) were observed in July (16.6 ± 0.7 °C) and lowest in January (4 - 5 °C). Intermediate temperatures of 10 - 12 °C were attained in November and April (Table 3.1, Fig. 3.1). Maximum (surface) irradiance (40.9 ± 14.2 mol photons m^{-2} d^{-1}) coincided with the warmest months, and minimum values of 11.1 ± 7.4 mol photons m^{-2} d^{-1} were observed in winter. Furthermore, annual underwater *in-situ* instant irradiances measured *in-situ* during sampling in the deepest transect (LM-T2 (4.5-5 m: 10.3 µmol photons m^{-2} s^{-1}) were 75.5% lower (*t*-test, $p < 0.01$) than in the shallowest area (LM-T1; 2-2.5 m: 42.1 µmol photons m^{-2} s^{-1}) (Table 3.1). In the study areas salinity was reported to remained constant throughout the seasons (range of 32 and 34 PSU), with no differences between sampling locations (O'Connor et al., 1990).

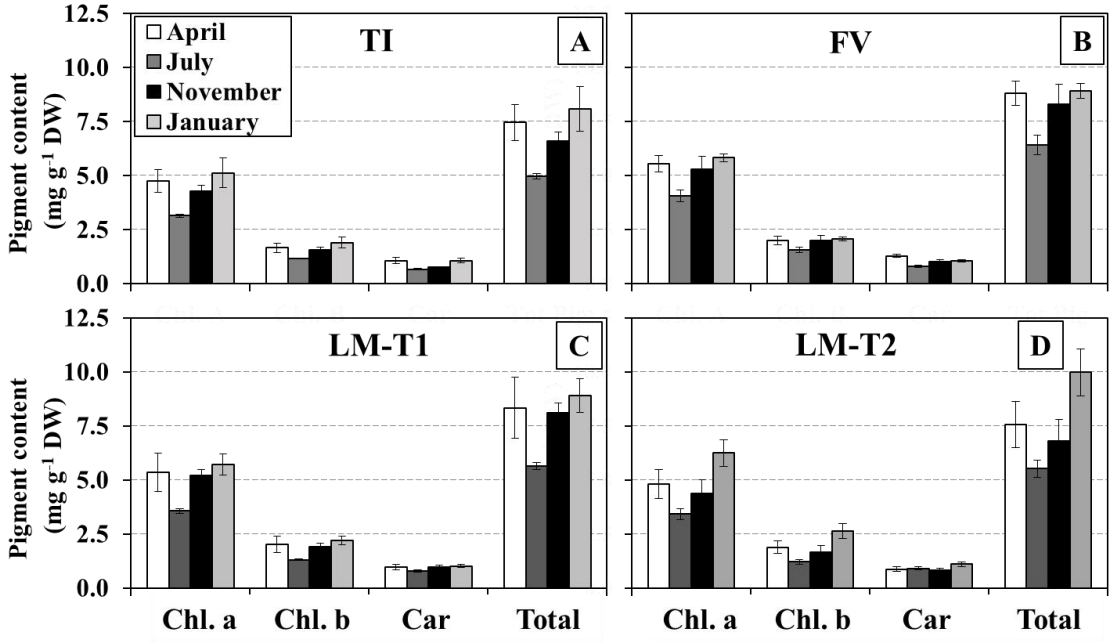


Figure 3.4 Total pigment contents, chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*) and carotenoids (Car) (mg g^{-1} DW) of leaves of Irish populations of *Zostera marina* collected in Spring, Summer, Autumn and Winter. Lettermore (LM) (Transect 1 (LM-T1-M1) and Transect 2 (LM-T2)), Tír an Fhia (TI), Finavarra (FV).

3.3.2. FA content and composition of leaves of Irish *Z. marina* populations

Previously, variations in levels of TFA in primary producers, including seagrass species were associated with species-specific characteristics such as variations in photosynthetic structures and morphology (Iba et al., 2002; Upchurchet al., 2008; Beca-Carretero et al., 2018*a* (Chapter 7)). Annual average content of TFA presented in the leaves of the shallow seagrass populations studied, TI (2.2 ± 0.5 mgg^{-1} DW), FV (2.1 ± 0.5 mg g^{-1} DW), LM-T1 (2.0 ± 0.3 mg g^{-1} DW) did not display significant differences (Table 3.2, Fig. 3.2).

Detailed FA composition of annual average values of the studied Irish eelgrass populations indicated that PUFA (67.6 ± 3.5 % of TFA) was the most dominant group of FAs, followed by SFA (24.8 ± 3.2 % of TFA) and finally MUFA (2.8 ± 0.2 % of TFA) (Table 3.2). These results are similar to previous data on FA composition of seagrass leaves (e.g. Sanina et al., 2004). In this study, the most abundant PUFAs were alpha-linolenic acid (ALA, 18:3 n-3) representing 44.7 ± 3.7 % of TFA, hexadecatrienoic acid (HTA, 16:3 n-3) accounting for 6.7 ± 1.5 % of TFA, and linoleic acid (LA, 18:2 n-6) representing 12.7 ± 1.6 % of TFA (Table 3.2). Generally, trienoic fatty acids are the major FAs detected in plant membrane lipids, and their levels vary according to environmental fluctuations and to species (Upchurch et al., 2008). Here, the second most dominant group of FAs was SFA, where the major FAs were palmitic acid (16:0) and stearic acid (18:0) with 22.3 ± 3.4 and 1.3 ± 0.4 % of TFA respectively (Table 3.2). Finally, the most abundant MUFA was 18:1 n-9 (1.2 ± 0.4 % of TFA), followed in lower presence (< 1.0 % of TFA) by 18:1 n-7, 16:1 n-7 and 14:1 (Table 3.2). In general, both seagrasses and terrestrial plants, are characterized by the synthesis of short chain FAs (< 18 carbons), while microalgae and macroalgae are characterized by the production of long-chain FAs (> 20 carbons), such as LC-PUFAs (long-chain polyunsaturated fatty acids) (e.g. Schmid et al., 2014, 2018).

Table 3.3 Effect of season (spring, summer, autumn and winter) (S) and location (TI, FV and LM-T1) (L); effect of seasons (S) and depth (2-2.5 and 4.5-5 m); and effect of geographical distance in the synthesis of TFA and composition on leaves of *Zostera marina*. F-values of two-way and one-way ANOVA are shown along with significance levels (*p < 0.05; **p < 0.01; ***p < 0.001).

Parameter		TFA		PUFA		SFA		MUFA	
Treatment	**df**	**MS**	***F***	**MS**	***F***	**MS**	***F***	**MS**	***F***
Month (M)	3	1.3	**90.5*****	222.9	**47.8*****	184.1	**63.5*****	1.5	**6.6****
Location (L)	2	0.3	**2.7°**	97.4	**20.4*****	27.1	**9.3****	2.4	**10.3*****
MxL	6	0.7	**5.6****	87.5	**18.4*****	54.2	**18.7*****	0.9	**3.8***
Month (M)	3	1.7	**14.7****	365.4	**66.3*****	231.5	**67.4*****	1.7	**14.7****
Depth (D)	1	1.1	**9.5***	0.2	0.0	0.2	0.1	1.1	**9.5***
MxD	3	0.2	**1.6***	17.4	**3.1***	9.4	**2.7***	0.2	1.6
Latitudinal comparison									
Location (L)	7	0.26	**3.7***	12.5	**19.8****	18.7	**20.7*****	0.18	**5.6***

3.3.3. Temporal responses of biochemical descriptors in *Z. marina* leaves

The synthesis of FAs and photosynthetic pigments in the leaves from shallow *Z. marina* populations (TI, FV and LM-T1) displayed similar clear temporal patterns in response to climate ranges (Fig. 3.2 and 3.4). Particularly, average (TI, FV and LM-T1) content of TFA in April was 65.3 % higher than in January, 52.8 % higher than in November and finally 17.4 % higher than in July (Table 3.2) (Tukey's, p < 0.001). Variations in TFA levels were mainly related to the synthesis of PUFAs, specifically16:3 n-3, 18:2 n-6 and 18:3 n-3, and SFA, mainly 16:0 (Table 3.2). Average (TI, FVand LM-T1) content of pigments in winter (8.8 ± 1.0 mg g^{-1} DW) was 9.4 % larger than in April, 18.5 % larger than in November and finally 38.9 % larger than in July (Tukey's, p < 0.001) (Table 3.6, Fig. 3.4). Comparable seasonal variations inpigments were previously observed in other seagrass species at similar latitudes (e.g.Bargain et al., 2013).

In April, in the three shallow populations (TI, FV and LM-T1) we observed the highest average level of PUFAs (71.1 ± 0.8 % of TFA), specifically, the largest ratio of n-3/n-6 (3.4 ± 0.1), while the lowest proportion of SFA (21.2 ± 0.7 % of TFA) were recorded (Tukey's, p < 0.001) (Table 3.2, Fig. 3.3). Interestingly, coastal water conditions in Spring in Ireland are characterized by (i) higher concentrations of nutrients after the winter mixing, (ii) increases in irradiance and temperature and (iii)

the formation of a thermocline on the sea surface (Richardson et al., 1985; Raine et al., 1998). Therefore, the reported biochemical adjustments may be an acclimatization mechanism of *Z. marina* to optimize the use of environmental resources, thus favouring eelgrass growth and productivity after low winter temperatures.

Table 3.4 Geographic coordinates of the sampling sites of the *Zostera marina* populations across the latitudinal range; water depth of each transect relative to the mean water level. Collection date; Seawater temperature measured at times when *Z. marina* biomass was collected, and Annual average of sea surface temperature (SST). Southern (Meadow 1 (M1) and Meadow 2 (M2)) and northern Spain (Vouzas (VZ) and (Sada) (SA)), Ireland (Tír an Fhia (TI) and Finavarra (FV)) and Greenland (Kapossillit (KA) and Kobbejord (KB)).

Region	**Southern Spain**		**Northern Spain**		**Ireland**		**Greenland**	
Location	**M1**	**M2**	**VZ**	**SA**	**FV**	**LM**	**KA**	**KB**
Latitude	36.52	36.52	42.23	43.35	53.15	53.30	64.40	64.20
Longitude	-6.19	-6.19	-8.76	-8.25	-9.13	-9.71	-50.30	-51.50
Depth (m)	2	2	2	2	2-2.5	2-2.5	3.8	2.5
Date (2016)	26/08	26/08	29/08	30/08	01/09	31/08	01/09	02/09
Temperature (°C)								
Collection time	20.0	20.0	18.0	16.0	15.0	15.0	11.0	8.0
Annual average	16.8	16.8	14.1	13.8	11.8	12.0	7.04	6.87

From April to July, we observed a decrease in TFA levels by 56.7 ± 0.8 % in FV, 55.2 ± 1.3 % in TI, and 72.7 ± 1.8 in LM-T1; and in photosynthetic pigments, by 63.4 ± 1.1 in TI, 66.5 ± 0.6 % in FV, and 73.0 ± 0.9 % in LM-T1 (Table 3.2, Fig.3. 4). The reductions in TFA levels were related to (i) the lower production of PUFAs, mainly associated with a decrease in the n-3/n-6 ratio, and (ii) the higher accumulation of SFAs. Increases in water temperature (average increase of ~7 °C) may explain the reduced requirements for PUFAs and lower levels of FA unsaturation of chloroplast thylakoids to promote optimal membrane fluidity. In July, we also observed the lowest content of photosynthetic pigments over the study period (TI = 5.6 ± 0.2 mg g^{-1} DW; FV = 5.5 ± 0.4 mg g^{-1} DW; LM-T1 = 5.0 ± 0.8 mg g^{-1} DW) (Tukey's, $p < 0.01$). Such results were previously documented for other seagrass systems as higher irradiances and longer day length are thought to reduce

the requirements for pigment production to ensure optimal light absorption (e.g. Cummings et al., 2003; Olive et al., 2013).

From July to November we observed a slight increase in TFA production of 3.1 ± 0.1 %; 6.0 ± 0.1 %; 18.8 ± 0.7 % in TI, FV and LM-T1, respectively (Fig. 3.2). However, this was accompanied by a marked change in the proportion of the FA composition in the eelgrass leaves (Table 3.2, Fig. 3.3). The average (TI, FV and LM-T1) proportion of SFAs significantly increased by 21.2 ± 2.3 % from July to Autumn, being in November the highest SFA levels observed in the temporal study (Table 3.2, Fig. 3.3) (ANOVA, $p < 0.01$). For instance, in LM-T1, TFA increased from 23.7 ± 1.1 % in July to 29.7 ± 1.4 of % in November. In autumn, average values among the three populations of PUFA (62.5 ± 0.2 % of TFA), especially n-3 PUFA (46.9 ± 0.6 % of TFA), and n-3/n-6 PUFA ratios (3.3 ± 0.1) reached their lowest annual values over the study period (Table 2) (Tukey's, $p < 0.05$). The accumulation of storage lipids, such as TAG containing mostly SFAs, was widely described as a physiological mechanism to transform and store the excess energy generated by photosynthesis (Goncharova et al., 2004; Pal et al., 2011; Solovchenko 2012). Thus, the production of these compounds may be an adaptive strategy of eelgrasses to survive coming colder and darker months in winter, where the photosynthetic activity is expected to be limited (Alcoverro et al., 2011). Similarly, in this sense, prior studies reported that some Irish *Z. marina* populations accumulated highest annual levels of energetic compounds, such as soluble carbohydrates in leaves and rhizomes in November (Dawes et al., 1992), which are critical reserves to maintain optimal carbon balance less favourable conditions (Burk et al., 1996). It is noteworthy that morphological and biochemical responses of some seagrass species are controlled by an internal biological clock allowing the pre-adapt to seasonal environmental cycles (Buia et al., 1992; Marbà et al., 1996; Beca-Carretero et al., 2018*a* (Chapter 7)). Previous studies observed similar trends in Antarctic terrestrial plants, which also store higher levels of SFAs before winter as accessible energy sources to survive non-favourable periods with negative carbon balance (Alberti et al., 1991). Similarly, some kelp species store higher content of carbohydrates during summer to cope with less favourable conditions in winter (e.g. Schaffelke & Lüning 1994).

Finally, from November to January, TFA contents increased markedly by 41.2 ± 0.7 % in TI, 23.5 ± 1.0 % in FV and 26.3 ± 0.8 % in LM-T1 (Table 3.2, Fig. 3.2)

(Tukey's, $p < 0.05$). These variations were associated with higher average (TI, LM and LM-T1) levels of PUFA (68.4 ± 0.2 % of TFA) and significant increases in n-3/n-6 ratios. Pigment levels reached a peak in January with average values of 8.8 ± 1.0 mg g^{-1} DW (Fig. 4). These results indicate that combined effects of annual minimum temperatures (5-7 °C) and reductions in irradiance (and daylength) (Table 3.1, Fig. 3.1B – C) stimulate the synthesis of TFAs and the degree of unsaturation (n-3 PUFA), as well as the production of photosynthetic pigments. Low temperatures on the FA metabolism were also shown to increase the degree of unsaturation of thylakoid membrane lipids in leaves of terrestrial plants, as a physiological mechanism to maintain optimal fluidity conditions in cold seasons (Murata et al., 1982).

Table 3.5 Total fatty acid content (% DW) and composition (% of TFA) of leaves of *Zostera marina* populations across the latitudinal composition. Results are expressed as mean ± SD (n = 5).

Region	**South Spain**		**North Spain**		**Ireland**		**Greenland**	
Location	**M1**	**M2**	**VZ**	**SA**	**FV**	**LM**	**KA**	**KB**
Total fatty acids (% DW)	**1.9**	**1.8**	**2.2**	**2.0**	**2.1**	**2.2**	**1.3**	**1.2**
Saturated fatty acids (% of TFA)								
14:0	0.8	0.7	0.3	0.4	0.6	0.7	0.3	0.3
16:0	22.3	22.7	19.1	20.0	21.1	19.8	18.5	16.8
18:0	1.5	1.5	1.2	1.3	1.4	1.4	1.2	1.0
23:0	0.9	0.7	0.4	0.3	0.1	0.3	0.4	0.1
Sum of SFA	**25.5**	**25.6**	**21.0**	**22.0**	**23.2**	**22.2**	**20.4**	**18.2**
Monounsaturated fatty acids (% of TFA)								
14:1	0.6	0.6	0.6	0.5	0.7	0.7	0.7	0.8
16:1 n-7	0.6	0.7	0.4	0.4	0.4	0.4	0.2	0.3
18:1 n-7	0.5	0.5	0.4	0.4	0.4	0.5	0.2	0.3
18:1 n-9	1.0	1.0	1.5	1.1	1.3	1.2	1.4	1.3
Sum of MUFA	**2.7**	**2.8**	**2.9**	**2.4**	**2.8**	**2.8**	**2.5**	**2.7**
Polyunsaturated fatty acids (% of TFA)								
16:2 n-3	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0
16:3 n-3	4.4	4.4	7.2	7.2	7.3	7.8	8.6	9.7
16:3 n-4	0.9	0.8	0.8	0.7	0.9	1.0	1.0	1.0
18:2 n-6	23.2	22.8	13.2	13.6	12.3	10.5	11.0	9.3
18:3 n-3	39.3	39.4	47.4	46.1	47.4	49.1	51.2	53.9
20:5 n-3	0.8	0.6	1.6	1.5	0.7	0.1	0.7	0.4
22:5 n-3	0.1	0.0	0.4	1.1	0.7	0.9	0.2	0.3
Sum of PUFA	**68.8**	**68.0**	**70.7**	**70.2**	**69.4**	**69.5**	**72.7**	**74.6**
Others	3.0	3.6	5.4	5.4	4.6	5.5	4.4	4.5
PUFA/SFA	**2.7**	**2.7**	**3.4**	**3.2**	**3.0**	**3.1**	**3.6**	**4.1**
n3/n6	**1.9**	**1.9**	**4.3**	**4.1**	**4.6**	**5.5**	**5.5**	**6.9**
% n3 (TFA)	**44.7**	**44.4**	**56.7**	**55.9**	**56.2**	**58.0**	**60.7**	**64.3**

3.3.4. Depth-induced responses of biochemical descriptors

Average TFA levels in leaves of plants adapted to different irradiance levels displayed only slight, but significant, annual differences (LM-T1, 2.2 ± 0.5 mg g^{-1} DW; LM-T2, 2.0 ± 0.3 mg g^{-1} DW) (Table 3.2, Fig. 3.2). Deeper plants appeared to have a greater biochemical plasticity as they had a higher coefficient of variation (CV) than shallow plants (Table 3.2). TFA content and composition from plants living at different depths revealed markedly different temporal patterns (Table 3.2 and 3.5) (ANOVA, $p < 0.05$). Particularly, in April, plants from LM-T2 accumulated 27.3 % more TFA (2.8 ± 0.4 mg g^{-1} DW) than LM-T1 (2.2 ± 0.4 mg g^{-1} DW) (Table 3.2) (Tukey´s, $p < 0.01$). These dissimilarities were mainly associated with the greater content of n-3 PUFAs (16:3 n-3 and 18:3 n-3) (LM-T1 = 57.5 ± 0.5 %; LM-T2 = 61.3 ± 1.2 %) and lower content of SFA (LM-T1 = 22.1 ± 0.5 %; LM-T2 = 20.3 ± 0.9 %) (Table 3.2) (ANOVA, $p < 0.05$). Similarly, in July, plants from LM-T1 and LM-T2 contained similar proportions of PUFAs and SFAs as in April, however, there were no marked differences in their TFA contents (LM-T1, 1.6 ± 0.9 mg g^{-1} DW; LM-T2 1.6 ± 0.2 mg g^{-1} DW) (Fig. 3.2). Regarding photosynthetic pigments, maximum annual differences were observed in July, the pigment production being 29.4 % higher in plants adapted to darker regimes (Table 3.6, Fig. 3.4) (Tukey´s, $p < 0.01$).

Overall, these results suggest that *Z. marina* plants adapted to darker environments (LM-T2) adjust their thylakoid membranes by increasing the degree of FA desaturation and their photosynthetic pigments in a manner that is different to shallower populations in warmer periods. Biochemical adjustments in seagrasses inhabiting light-limited habitats appear to be a common mechanism to optimise the light absorption and thus favour optimal photosynthetic responses (Olesen et al., 1993; Cummings & Zimmerman 2003).

Both total annual contents of total pigment (mg g^{-1} DW) and Chl. *a* (mg g^{-1} DW) were significantly correlated ($R > 0.6$, $p < 0.05$) with TFA (mg g^{-1} DW) and PUFA (% of TFA), respectively. These results suggest that *Z. marina* populations adjust the synthesis of both key biochemical components in response to temporal environmental changes and irradiance gradients as a photo-acclimatory strategy.

Previous studies conducted in the Gulf of Aqaba (GoA; northern Red Sea) with the tropical *Halophila stipulacea* revealed a correlated increase of 25.1 % in TFA and 22 % in photosynthetic pigments as a result of reductions in PAR from shallow (6/9 m) to deep areas (21 m) (Beca-Carretero *under review* (Chapter 8)). Also, experimental studies of microalgae reported that under low light conditions, PUFA accumulation was induced, alongside the development of larger thylakoid membranes in the chloroplasts (e.g. Goss & Wilhelm 2010).

Surprisingly, in autumn and winter, slightly, but not significantly, higher levels of PUFAs and lower levels of SFAs in LM-T1 (2-2.5 m) than in LM-T2 (4-4.5 m) were observed (Table 3.2, Fig. 3.3). For instance, in November and January, PUFA level in LMT1 (65.8 ± 3.5 % of TFA) was 3.1 ± 0.1 % higher than in LM-T2 (63.7 ± 4.2 % of TFA) while there were no differences in TFA content among populations (LM-T1= 2.4 ± 0.4 mg g^{-1} DW, LM-T2 = 2.4 ± 0.3 mg g^{-1} DW) (Table 3.2, Fig. 3.2). Such biochemical responses coincided with the smallest (and non-significant) temporal changes in pigment contents observed in January between LM-T1 (8.1 ±1.1 mg g^{-1} DW) and LM-T2 (8.9 ± 0.3 mg g^{-1} DW) (Table S3.1, Fig. 3.4). Thus, under less favourable periods for growth (Beca-Carretero *under review a*), plants exposed to different light intensities (Table 3.1) displayed a similar biochemical composition; this may imply that during colder periods, deeper plants substantially reduced their levels of n-3 PUFAs, thus potentially adjusting photosynthetic activity to lessen their metabolic costs.

Table 3.6 Effect of month (spring, summer, autumn and winter) (S) and location (TI, FV and LM-T1) (L); effect of seasons (S) and depth (2-2.5 and 4.5-5 m) in the synthesis of total pigment contents, chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*) and carotenoids (Car) (mg g^{-1} DW) of leaves of *Zostera marina*. F-values of two-way ANOVA are shown along with significance levels (*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$).

Parameter		**Chl. *a***		**Chl. *b***		**Carotenes**		**Total pigments**	
Treatment	**df**	**MS**	***F***	**MS**	***F***	**MS**	***F***	**MS**	***F***
Month (M)	3	1.7	1.3	0.3	1.0	0.1	**4.1****	4.1	1.3
Location (L)	3	24.3	**18.5*****	4.6	**16.9*****	0.3	**10.5*****	57.6	**17.8*****
MxL	9	-6.6	-5.1	-1.2	-4.6	-0.1	-4.0	-15.8	-4.9
Month (M)	3	8.1	**38.7*****	0.8	**25.3*****	0.5	**64.4*****	18.9	**37.7*****
Depth (D)	1	9.3	**44.6*****	1.3	**39.2*****	0.3	**34.0*****	22.0	**43.9*****
MxD	3	0.1	0.3	0.0	**1.3**	0.1	**6.8****	0.4	0.8

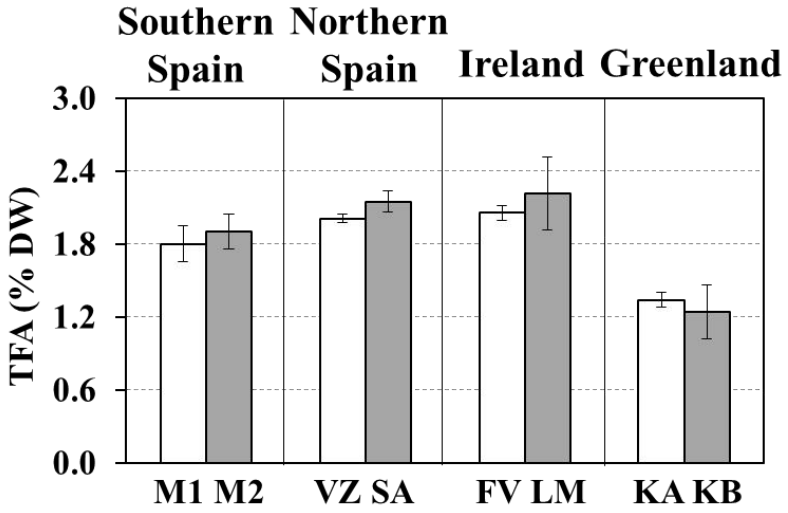


Figure 3.5 Total fatty acids (% of DW) of leaves *Zostera marina* populations across the latitudinal range. Southern (Meadow 1 (M1) and Meadow 2 (M2)) and northern Spain (Vouzas (VZ) and (Sada) (SA)), Ireland (Tír an Fhia (TI) and Finavarra (FV)) and Greenland (Kapossillit (KA) and Kobbejord (KB)).

3.3.5. Latitudinal comparison

The latitudinal comparison revealed a high sensitivity in the production of FAs in *Z. marina* populations adapted to different *in situ* environmental conditions (Table 3.3, Fig. S3.3). TFA varied significantly among populations with higher values observed in Irish populations (FV = 2.1 ± mg g^{-1} DW; TI = 2.21 ± mg g^{-1} DW), followed by northern Spanish (VZ = 2.01 ± 0.03 mg g^{-1} DW; SA = 2.25 ± 0.1 mg g^{-1} DW), southern Spanish (G1 = 1.9 ± 0.1 mg g^{-1} DW; G2 = 1.8 ± 0.2 mg g^{-1} DW) and finally Greenland plants (KA = 1.3 ± 0.1 mg g^{-1} DW; KB = 1.2 ± 0.2 mg g^{-1} DW) (Table 3.5, Fig. 3.5) (ANOVA, $p < 0.05$). Differences in TFA among populations may be related with phenotypical or genetic differences among populations, or due a biomass dilution effect regarding different growth rates (Hernandez et al., 2006; Bermejo et al., 2008), although, further studies are needed to confirm these speculations. Among the latitudinal populations studied, substantially larger (Tukey's, $p < 0.01$) PUFA/SFA ratios were found in both Greenland populations, in KobbeFjord (4.1 ± 0.2) which is the *Z. marina* population living under *in-situ* coldest temperatures (~ 7-8 °C in summer), followed by plants from Kappissillit (3.6 ± 0.1) (Fig. 3.6). On the

contrary, lowest values were observed in samples from Cadiz (24 °C), with an average PUFA/SFA ratio of 2.2 ± 0.4 (Table 3.4). Intermediate values were reached in populations from north western Spain and western Ireland which are adapted to intermediate temperatures (15-18 °C). More pronounced were the significant differences (ANOVA, $p < 0.05$) in the n-3/n-6 PUFA ratios in the populations living at different latitudes (Table 3.4 and 3.5). Higher n-3/n-6 PUFA ratios were observed at KobbeFjord (6.9 ± 0.2) and Kappissillit (6.9 ± 0.2), which were 222.7 ± 36.2 % significantly higher than the n3/n-6 PUFA ratios reported for plants from southern Spain (2.7 ± 0.1) (Fig 3.6, Table 3.5) (ANOVA, $p < 0.01$). Noticeably, the levels of PUFA (75 % of TFA) in KobbeFjord represent the highest ever recorded in a seagrass species worldwide. Finally, larger SFA contents were observed in plants from the southern Spanish population (25.6 ± 0.1 of TFA), which were significantly (18.8 %) higher than in plants from northern Spain, 11.2 % higher than in Irish plants, and 32.4 % higher than in Greenland populations (Table 3.4, Fig. 3.6) (ANOVA, $p < 0.05$).

Overall, these findings highlight that Irish *Z. marina* possesses the capacity to rearrange the physiochemical consistency of their leaves through the synthesis of key fatty acids in response to *in-situ* environmental conditions across its latitudinal distribution range. Previous studies observed a thermal and latitudinal correlation among the content of TFA (primarily in PUFA and SFA) in leaves across different species, with species inhabiting colder regions and higher latitudes synthetizing larger levels of n-3 PUFA (Beca-Carretero et al., 2018*a* (Chapter 7)), thus supporting the differences observed in the present latitudinal study. Similar observations have been reported for seaweed species along a thermal gradient, with larger PUFA content in populations living in colder regions (Colombo et al., 2016). High levels of PUFA, and specifically n-3 PUFA, were also reported previously in leaves of Antarctic plants which allowed optimal metabolic reactions and membrane fluidity and cryoprotection of photosynthetic tissues (Alberdi & Corcuera 1991). These outcomes may explain the exceptional high contents of PUFA and n-3/n-6 PUFA ratios observed here in Greenland population to survive in extreme cold waters, and also the low seagrass diversity in this region.

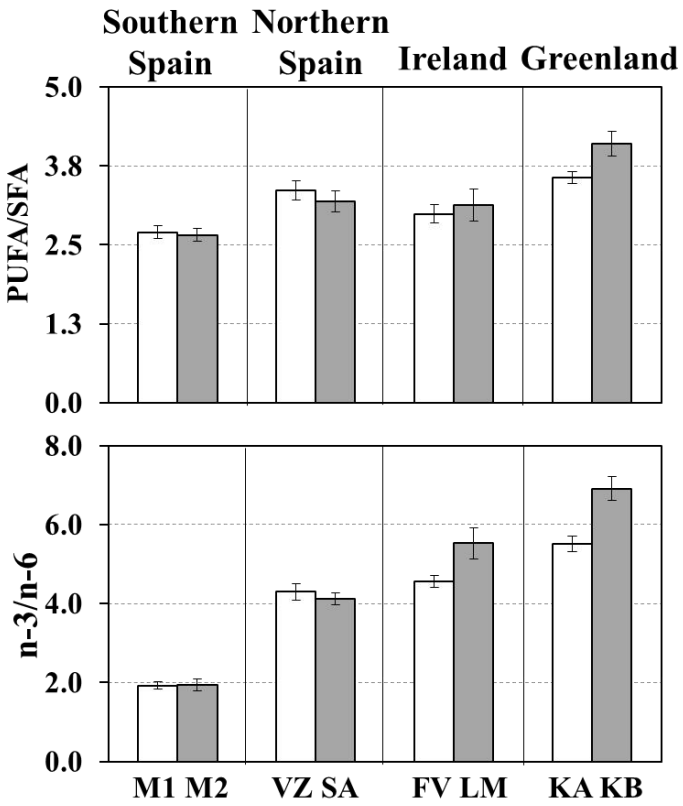


Figure 3.6 PUFA/SFA ratios and n-3/n-6 PUFA ratios of leaves *Zostera marina* populations across the latitudinal range. Southern (Meadow 1 (M1) and Meadow 2 (M2)) and northern Spain (Vouzas (VZ) and (Sada) (SA)), Ireland (Tír an Fhia (TI) and Finavarra (FV)) and Greenland (Kapossillit (KA) and Kobbejord (KB)).

Increases in the proportion of SFAs in response to high temperatures have previously been widely observed in primary producers, including seagrasses. For example, effects of an experimental heat wave induced a remodelling of the thylakoid membranes in *P. oceanica* and *C. nodosa* by reducing TFA contents, specifically depletions in PUFA and increases in SFA proportions (Beca-Carretero et al., 2018*a* (Chapter 7)). Supporting these outcomes, a comparison among different seagrass species distributed worldwide, indicates that tropical and subtropical species biosynthesize significant larger amounts of SFA than cold-adapted species, such as *Halophila ovalis* or *Thalassia hemiprichii*, (Nicholst & John 1985). This may be related to (i) the role of SFA in the protection of the photosynthetic machinery at high temperatures (ii) low levels of fatty acid unsaturation to tolerate high thermal conditions. For instance, in tobacco plants, higher levels of SFA in plants acclimated

to high temperatures showed an ability to silence the gene responsible to synthetize n-3 PUFA, rendered the plants more resistance to warming (Murakami et al., 2000). Similarly, previous studies within Arabidopsis and soybean mutants with lower PUFA/SFA ratios positively correlated with a larger tolerance to survive to high temperatures (Hugly et al., 1989; Alfonso et al., 2001). Therefore, the unusual high levels of SFA of Cadiz populations adapted to summer SST of ~25 °C and to air temperature of ~35 °C, may be a mechanism of *Z. marina* to resist high temperatures as was previously observed in terrestrial plants. Interestingly, Cadiz population represent one of the southernmost distributed eelgrass population worldwide; the FA proportions, such as PUFA/SFA or n-3/n-6 PUFA ratios in photosynthetic structures, may this be used as an eco-physiological indicator to assess seagrass healthy state and to detect early signals of thermal stress. As samples for this study were only collected during a single sample event at each site, a more extensive and detailed study would be required to confirm the temporal responses of the latitudinal *Z. marina* populations.

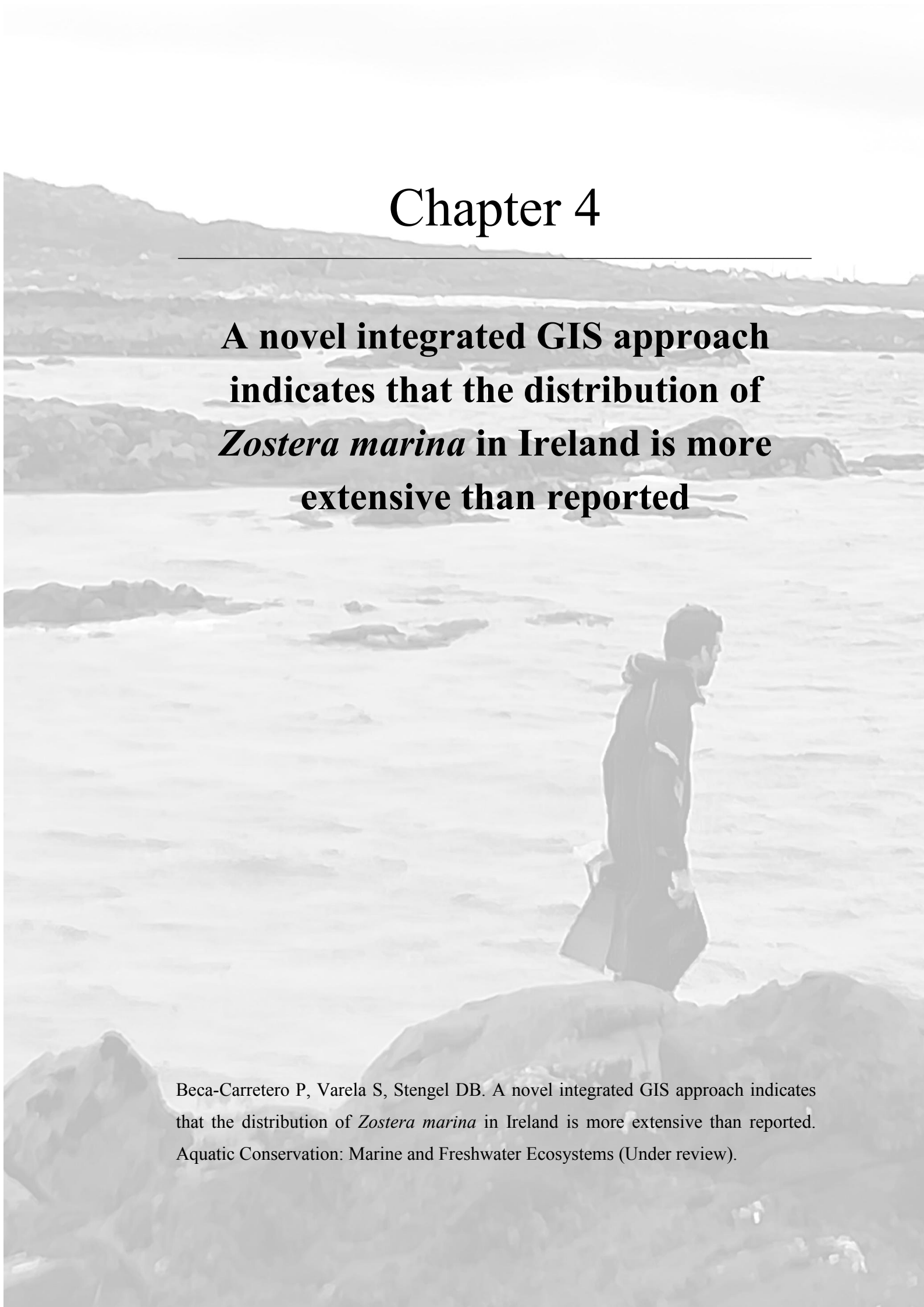
3.3.6. Role of climate regimes in the nutritional value of seagrasses

Primary producers such as seagrasses represent an important food source for aquatic organisms, producing vital compounds which are transferred to next trophic levels, such as vitamins, proteins, carbohydrates or essential fatty acids EFA (i.e. ALA and LA) (Kanan et al., 2013; Nancy et al., 2015). These compounds have a relevant role in critical physiological processes and healthy state of consumers which include survival, growth and metamorphosis to favour optimal neural, hormonal, reproductive or visual functions (Sargent et al., 1999; Tocher 2003). Seagrasses represent about ~12-15 % of the total marine primary production, thus representing some of the main source of nutrition for key stone herbivores worldwide, such as, *Salpa salpa* (Sparidae) and the sea urchin *Paracentrotus lividus* (Echinidae) in Mediterranean Sea, the isopod *Idotea resecata* or gastropod *Smaragdia viridis* in temperate ecosystems, or some charismatics species, such as manatees (*Trichechus manatus*) or dugongs (*Dugong dugon*) in some tropical or subtropical climates, or the green turtle (*Chelonia mydas*) (Christianen et al., 2014, 2018). Our findings suggest a

significant reduction of PUFA/SFA and n-3/n-6 ratios in response to an increment in water temperature across seasons and different latitudes (Table 3.2, Fig. 3.8). A recent experimental study simulating the heat wave effects in *Posidonia oceanica* and *Cymodocea nodosa* reached similar conclusion with proportions of PUFA decreasing, and the percentage of SFA increasing, significantly after heat treatment (Beca-Carretero et al., 2018 (Chapter 7)). Projected climate regimes will induce biochemical acclimatization in marine primary producers which will therefore affect their lipid nutritional status alongside with changes in productivity and population dynamics, and interactions among species (Harley et al., 2012; Wernberg et al., 2010). In Ireland, future warming is expected to rise the annual summer temperature (~16 °C) by ~ 2-3 °C (IPCC, 2014) which may favour seagrass growth and production, however, these structural improvements may be accompanied by a potential reduction in n-3 PUFA levels and increases in SFA. In Arctic regions, biomass productivity of eelgrass populations is expected to increase with projected climate, so that a larger food source and refuge area may be available for consumers (Krause-Jensen & Duarte 2014; Beca-Carretero et al., 2018*b* (Chapter 7)). On the other hand, south-adapted eelgrass communities may experience die-backs and losses in habitat distribution which may, in turn, result in a net loss in fatty acid sources, as was recently reported (Durako & Moffler 1987; Reusch et al., 2005). In conclusion, with reported variations in the metabolic demands of herbivores and in their diet preferences under projected climate scenarios (Hernan et al., 2007, 2018; Deutsch et al., 2015), a cascade effect across higher throphic levels may occur with uncertain consequences for marine ecosystems.

Chapter 4

A novel integrated GIS approach indicates that the distribution of *Zostera marina* in Ireland is more extensive than reported



Beca-Carretero P, Varela S, Stengel DB. A novel integrated GIS approach indicates that the distribution of *Zostera marina* in Ireland is more extensive than reported. Aquatic Conservation: Marine and Freshwater Ecosystems (Under review).

Author contribution

Pedro Beca-Carretero and Sara Varela developed the experimental design and performed the models and the statistical analysis; Dagmar B. Stengel resources and funding for the project; all the authors contributed to the interpretation of the data and the writing of the manuscript.

Abstract from original article

Seagrasses such as *Zostera marina* L. play a key role in coastal ecosystems due to the ecological goods and services they provide, enhancing biodiversity, productivity and carbon sequestration. Despite their ecological relevance, the distribution of seagrass meadows in Ireland is, to date, insufficiently documented and records of their distribution are scarce and mostly outdated. Here, we applied a novel approach based on species distribution models (SDMs), remote sensing and field surveys to record new eelgrass populations. First, SDM allowed the identification of the relative importance of different biotic and abiotic factors determining the presence of the *Z. marina* and to estimate habitat suitability in three bays in western Ireland. We then visually compared the obtained habitat suitability maps with remote sensing images, to appraise the potential presence of the species. Finally, we conducted several field surveys to validate the presence or absence of the seagrass in areas predicted from SDM and satellite images. Using this integrated approach, we mapped and categorized 21 new regions of seagrass habitats which accounted for a total of 2,728,948.3 m^2, which increased the previously documented distribution by 45.6%, within an estimated economical (blue carbon) value of 6,576,765 €. Focusing on three selected areas on the Irish west coast (Kilkieran Bay, Bertraghboy Bay, Chasla Bay) as a case study, results demonstrate that the actual distribution of seagrass along the coast of Ireland, and in particular, on the western coast, is considerably larger than currently understood. As eelgrass populations are under threat globally, the development of new mapping strategies that accurately define their distribution and extent is critical to support adequate future conservation policies. The mapping of previously not recorded submerged meadows in Ireland constitutes an essential contribution to current efforts in seagrass monitoring and management across Europe.

Key words: *Zostera marina;* maerl; geographic information system (GIS); mapping; species distribution model (SDM); field survey; Remote sensing; MAXENT; Ireland.

4.1. Introduction

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Marine angiosperms, commonly known as seagrasses (see Chapter 1) are marine habitat-founding species that can colonize intertidal or subtidal coastal areas, providing important ecological services such as protection of the coastline, carbon sequestration and nutrient retention (Barbier et al., 2011; Hemminga & Duarte, 2000). These functions render seagrass meadows unique, ranking them amongst the most valuable ecosystems, comparable with coral reefs or terrestrial forests (Costanza et al., 2014; Norlund et al., 2017). Their global distribution is estimated to cover 600,000 km^2, within an approximated economic annual value of US$ 3.8 million based on the ecological goods and services they provide (Costanza et al., 1997; Mcleod et al., 2011; Waycott et al., 2009). *Zostera marina* L. is the dominant seagrass in the northern hemisphere, distributed from the Arctic to subtropical regions (Short et al., 2007; Olesen et al., 2015), and is commonly considered an indicator species due to its high sensitivity to shifts in water quality, providing early warnings of the status of the marine environment (Krause-Jensen et al., 2005). *Z. marina* meadows form part of the five Annex I habitats in the EU Habitats Directive (92/43/EEC) and are included in the Initial List of OSPAR Threatened and/or Declining Species and Habitats (OSPAR Commission 2005).

Over the last number of years, novel and powerful approaches such as species distribution models (SDMs) have provided an advanced insight into the biogeography of ecologically important terrestrial and marine species. Such tools facilitate the quantification species-environmental relationships and predict potential species habitat distribution in response to abiotic or biotic factors (Guisan and Zimmermann, 2000). Although, SDMs have been more commonly applied to terrestrial than aquatic systems (Frankiln et al., 2010), their value to marine research has also been demonstrated in recent years (i.e. Wright et al., 1999; Perry et al., 2005). SDMs have been used to identify suitable areas for long-term monitoring programs, the prediction of potential impacts of climate change or the recovery of

seagrass systems (i.e. Heide et al., 2009; Bekkby et al., 2008; Valle et al., 2013 and 2014; Zucchetta et al., 2016).

Remote sensing tools, such as the use of satellite-derived image technology (i.e., Landsat satellite sensors or LIDAR and RADAR products), have assisted with species identification, quantification, and the long-term monitoring to assess changes in spatial distribution in both terrestrial and marine species, including seagrasses (i.e. Dahdouh-Guebas et al., 2002; Lathrop et al., 2006; Gullstrom et al., 2016). Previous studies effectively employed these efficient, reliable and low-cost approaches along with other GIS techniques such as airborne hyperspectral or ecological time-series analysis (i.e. Phinn et al., 2008; Osborne et al., 2001).

Contrary to terrestrial ecosystems where geographic information approaches, such as SDM or remote sensing tools, generate accurate mapping results (i.e. Frankiln et al., 2012; Roy et al., 2014), marine coastal ecosystems are characterized by the presence of the water column and the exposure to natural disturbances. These intrinsic characteristics make it more challenging to develop precise species distribution maps (Roelfsema et al., 2013; Leslie et al., 2007) with the exception of seagrass species/populations inhabiting shallow or clear waters allowing easy access (Phinn et al., 2008; Wang et al., 2007). As a large proportion of seagrass meadows worldwide potentially remain unmapped to date, creating novel mapping strategies may aid the discovery of new seagrass meadows.

Here, we hypothesised that the actual distribution of seagrass along the west coast of Ireland, and in particular Kilkieran Bay, in Bertraghboy Bay and in Chasla Bay, was larger than reported to date. Hence, we aimed to develop an updated more accurate map of eelgrass distribution and estimate the potential economic value of the seagrass meadows in the studied area. To achieve this, we (i) determined the relative importance of different factors outlining the presence of eelgrass populations on a local scale; (ii) predicted the potential habitat suitability of *Z. marina* at a fine-scale integrating both species distribution model (SDM) and remote sensing products focussing on submerged populations; (iii) validated the predictive ability of our method by undertaking field surveys in areas where the data suggested eelgrass presence; and, finally (iv) constructed an updated map of the eelgrass distribution for the region under investigation.

4.2. Methods

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4.2.1. Study sites

The study area was located in western Ireland in the northern Galway Bay (Fig. 4.1), more specifically encompassing Kilkieran Bay, Bertraghboy Bay and Chasla Bay (53,394 N, -9,869 W; 53,222 N, -9,536 W). Kilkieran Bay represented an area with the most widely studied distribution of seagrasses in Ireland (NPWS 2014), including extensive areas of *Z. marina* occurring at depths of 1-10 meters, with a total distribution of 5,988,298 m^2; there were no records of the presence of any seagrass species in Bertraghboy or Chasla Bay (NPWS 2010, 2015).

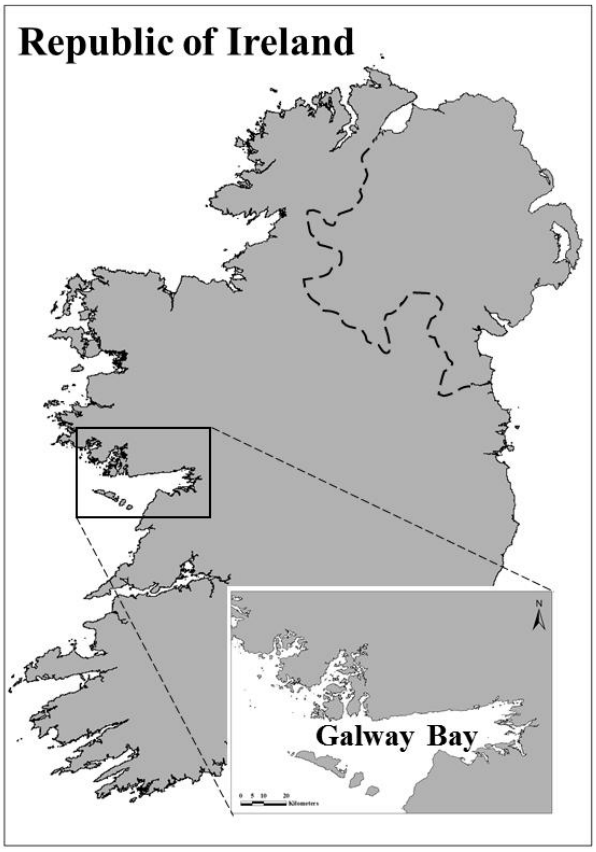

Figure 4.1 Map of the study site showing the *Zostera marina* distribution in Galway Bay along the west coast of Ireland.

4.2.2. Data sources for species distributions

We obtained distribution maps of *Z. marina* from three different sources; (i) the MESH Atlantic project (http://www.emodnet-seabedhabitats.eu/map), (ii) the Site-Specific Conservation Objectives Marine Community Types project (ttps://data.gov.ie), and (iii) the OSPAR Habitats project (http://www.ospar.org/). Data of species distribution in the study site were derived from previous studies conducted over several years: 1997 (as a part of the BioMar program; Picton & Costello, 1997), 2001 and 2002 (SSI, 2003), 2005 (MERC, 2005, 2006), 2010 (APEM, 2011), and finally a Marine Institute survey in 2010-2011 (Tully & Clarke 2012) (RPS, 2012).

4.2.3. Predictor variables and pre-processing the GIS layers

Different environmental variables affect the eelgrass survival and distribution, such as, temperature, irradiance, sediment type and hydrodynamic forces (i.e. Duarte 1989; Borum et al., 2004; Lee et al*.,* 2007). Here, we employed a set of seven types of environmental predictors, six of which were continuous variables (temperature, current velocity, bathymetry, slope, orientation and distance from the coast), and one variable was designated as categorical, sediment type. We derived temperature (°C) and current velocity (m s^{-1}) at the seafloor from the Marine Institute (http://www.marine.ie). The data were generated using ROMS models (Regional Ocean Modelling System) (Jackson *et al*., 2012) which provided annual daily averages, maxima, minima and also the differences between maximum and minimum values of seawater temperature and current velocity from January 2011 to June 2015. The ROMS models developed data with a horizontal resolution of 200 meters (Nolan et al., 2010). Bathymetry data were based on the Irish National Seabed Survey (https://jetstream.gsi.ie). The bathymetry layer is a high-resolution map obtained from the Lidar survey method with a resolution of 0.25 m^2. From this layer, we created two new layers: (i) the slope, expressed in percentage rise, and (ii) the orientation layer, expressed in degrees, utilising the *slope* and *aspect* tools respectively, from the Spatial Analyst extension of ArcGIS 10.2 software (ESRI ®).

Also, we calculated the distance from the shoreline using the *Euclidean distance* tool of the Spatial Analyst extension. Sedimentological data were available from the European Union Nature Information System (https://data.gov.ie) which is categorised following the EUNIS classification system (Galparsoro et al., 2012). From the different sediment types, three sedimentological layers were created as, (i) soft sediment including mud, mud-sand and sand-mud; (ii) mixed sediment comprised of sand, mixed and coarse sediment, and finally, (iii) hard sediment consisting only of rock and reef. Moreover, the presence of maerl community was incorporated as a fourth sedimentological GIS layer. Maerl deposits form extended beds in the study area, and was previously characterized as biogenic substrata (i.e. Kamenos et al., 2004; Jackson et al., 2004). We downloaded the data of maerl distribution from the same sources as data of *Z. marina* and sediment information (See details in Section 4.2.2). All the predictable variables were transformed into raster files ensuring the same resolution extent and projection (WGS_98); we selected the grain based on the raster with the larger pixel value, which was the temperature and velocity data with a dimension of 320 m^2.

4.2.4. SDMs

We applied MAXENT model to determine the main environmental factors controlling the eelgrass presence and to assess the potential habitat distribution of the target species. We run MAXENT model with the default response settings (Table 4.2) (Phillips et al., 2006). For computing the model, we assumed that (i) the seagrass depth distribution must be equal to or below 0 meters, (ii) the distribution of the eelgrass has remained constant since being mapped from 1997 to 2011, (iii) the distribution of the target species is in equilibrium with the current environmental conditions, (iv) and there is no exclusion through competition with other species. MAXENT generated a continuous raster file with a pixel value ranging from 0 to 1, with 1 representing the highest probability for potential habitat, suitability, and 0 the absence of the target species, within a cell size of 320 m^2.

4.2.5. Model evaluation

We evaluated the performance of the model using two different evaluation measurements chosen according to the nature of our data and the kind of SDMs used. The threshold- independent metric AUC - area under the "receiver operating characteristic" (ROC) curve rank from 0.5 to 1, where models with values of 0.5-0.7 are poorly performing, 0.7-0.9 are considered to have a moderate discriminatory ability and models with values higher than 0.9 are considered to perform excellently (Manel et al., 2001). The significance of the AUC was tested using a cross-validation procedure covering 100 interactions. The second evaluation tool was the sensitivity parameter, which is the proportion of the presence of the target species that is precisely predicted by the SDM.

4.2.6. Remote sensing map

Using high resolution satellite-derived images downloaded from the map base of the world imagery from ArcGIS 10.2 software (ESRI ®) in June 2017, we created a remote sensing map with the potential presence of *Z. marina* with a 0.3 m resolution. To define precisely the extent and shape of the meadows, we manually ascertained and illustrated using the ArcGIS 10.2 software (ESRI ®). We then obtained a polygon shapefile with the potential eelgrass distribution in the study area.

To determine locations that may represent seagrass meadows in the study area, firstly, we compared sites where previous records existed (see details in *Data sources for species distributions, Section 4.2.2.*) with aerial satellite images. Thus, we identify the meadow characteristics such as shape and colour, and thus allow us to deduce where the *Z. marina* may or may not exist in unmapped areas. For instance, in several regions, the aerial shape of some seagrass meadows developed striped forms potentially induced by the high current velocities or by the wave action (Fonseca et al., 1998), which led to an easy satellite identification (Fig. 4.4, panel A). In some cases, aerial views of maerl systems may cause confusion as they also develop similar forms. However, in most cases, the shallow (2-10 m) marine coralline deposits appear darker in colour (Fig. 4.4, panel B) than seagrass meadows which are

characterized by a lighter shade of green (Chauvaud et al., 1998). Also, it was possible to distinguish the seagrass meadows from seaweeds located in sheltered or non-exposed areas. Here, seagrasses are characterized by a light green colour, while seaweeds (such as subtidal kelp) are easily distinguishable by their darker pigmentation and attachment to hard substrates (Fig. 4.4, panel C). Hence, applying these background checks it was possible to forecast the presence of submerged seagrass meadows in the study area with a high degree of confidence.

4.2.7. Integrated map generation

The next step of the integrated method consisted of contrasting the results of (i) the developed SDM map with (ii) the predicted map produced with the aid of satellite-derived images. Subsequently, we created a final map of the distribution of the eelgrass by overlaying both predicted maps and extracting the matched spatial information using the *clip* tool from ArcGIS 10.2 software (ESRI ®).

4.2.8. Integrated map validation

The accuracy of the final predictive map of seagrass distribution was evaluated by carrying out field surveys. In 64 site visits conducted over 3 years, 20 potential seagrass areas were assessed; these were selected based on data visualised on the integrated map, and accessibility. The accuracy of the created map was quantified by dividing the presence of the target species between the total evaluated areas, and subsequently expressed % correctness.

As the distribution of eelgrass meadows was largely at shallow to intermediate depths (1-10 m), we conducted mostly of the field surveys by snorkelling which allowed good manoeuvrability with light equipment. To determine the presence of *Z. marina,* we GPS-traced georeferenced zig-zag transects (Garmin Montana 600) (4 m resolution), parallel to the shoreline. We took several GPS measurements (GCS_WGS_84) every 10 to 20 meters, recording the presence or absence of the eelgrass, as well as meadow borders. In parallel, at the designated points we took

underwater photographs (water-proof HD action-camera SJCAM sj5000). This method was a comparatively fast technique (~1 km^2 h^{-1}) which allowed large seagrass areas to be covered with a relatively high resolution (1-5 m). In Bertraghboy Bay, we conducted a survey using a boat, following a similar methodology. When the tides were particularly low, we performed field work on foot. In some cases, it was not possible map the entire meadow or to reach their edges due to logistical constraints, or because of the inaccessibility of some regions in the area. To allow for the large extension of some eelgrass meadows, multiple trips were required for the evaluation of one specific location.

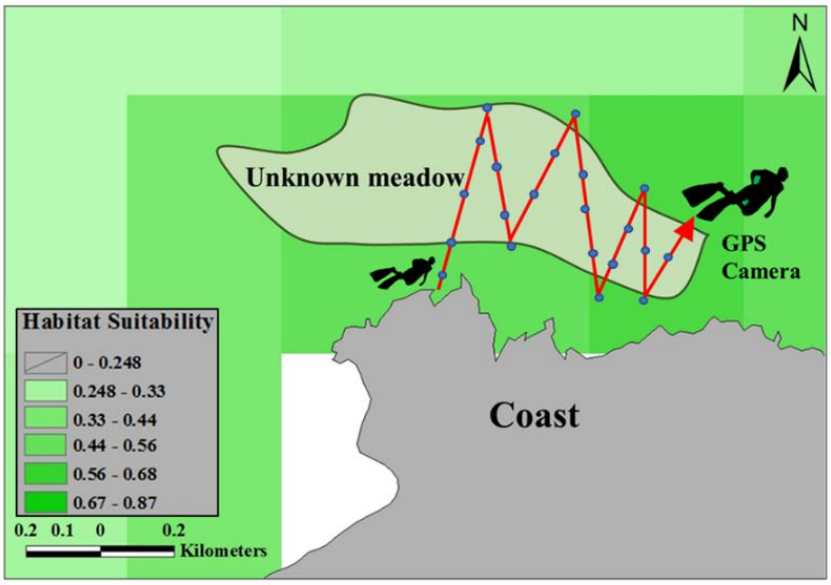

Figure 4.2 Example of the snorkelling field work performed to determine the seagrass presence and map distribution by tracing zigzag temporal and GPS-georeferenced route transects. In parallel, at the designated points, underwater photographs were taken every 10 to 20 meters indicating the presence or absence of the eelgrass and the limits of the meadows (Blue points).

4.2.9. Final distribution map

We used the spatial information generated from the acquired transects to create a final polygon shapefile with the precise distribution of the *Z. marina* in our study area. Firstly, we transformed the obtained poly-point with the presence or absence of

the seagrass into a polyline in ArcGIS 10.2 software (ESRI ®). Subsequently, we overlaid this spatial information onto satellite images and manually defined the exact distribution and extent of the meadows in the study area.

In addition, we visually estimated eelgrass cover by using the underwater photographs obtained at each location. Three categories of cover based on the seagrass coverage were defined: sparse (10–40%), moderate (40–80%) and dense (80–100%) (Lathrop et al., 2006).

4.2.10. Seagrass meadow economic value

Using the formula proposed by Costanza (2014) which estimates seagrass meadow value in relation to the ecological services they provide and the area they cover, the annual economic value of the new *Z. marina* meadows discovered in the study, was estimated, using the figure of € 2.41 m^2 yr^{-1} (Costanza et al., 2014). We converted the value of US $ to EU € (based on $ 1.20 $ = € 1.00).

Seagrass meadow value = Cover area (m^2) × Seagrass value (€ 2.41 m^{-2} yr^{-1}) (Eq. 4.1)

4.3. Results

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4.3.1. SDMs

The MAXENT model predicted a large distribution area for the target species, larger than based on previously published information (5,988,294 m^2), which represents 3.6 % of the total coastal area (Table 4.4) (Fig. 4.3). MAXENT predicted a total suitable area of 31,667,855 m^2, representing 19.3 % of the investigated coastal area (Table 4.4). *Z. marina* was predicted for areas where no previous records existed (Bertraghboy and Chasla Bay; Fig. 4.3 and 4.8), as well as previously unmapped areas of Kilkieran Bay. MAXENT demonstrated a high discriminatory ability within

an AUC value of 0.938 (Table 4.2), and also a marked sensitivity, with MAXENT accurately predicting 88.7 % of the actual records of *Z. marina*.

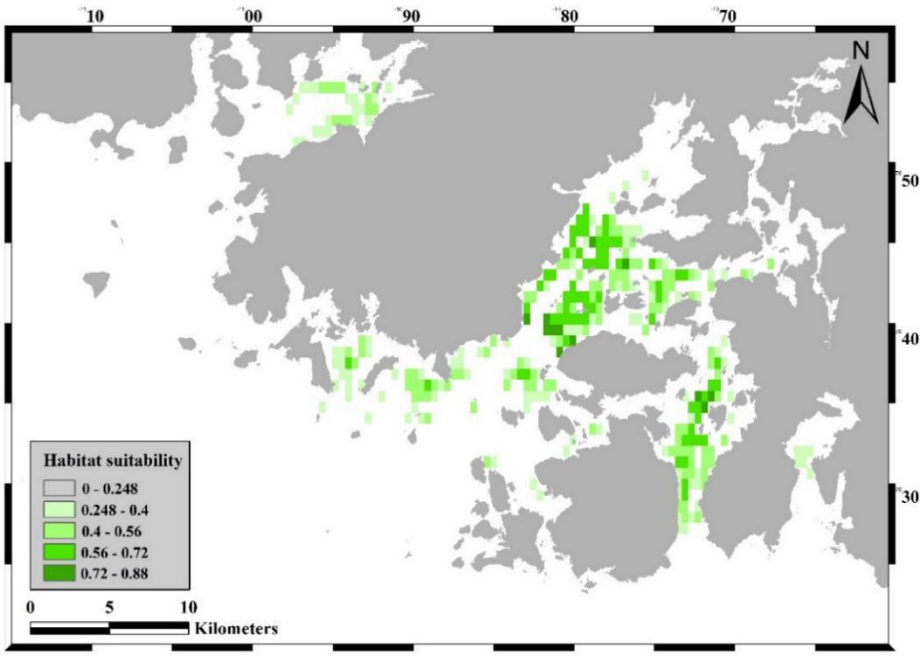

Figure 4.3. *Zostera marina* habitat suitability predicted by MAXENT model.

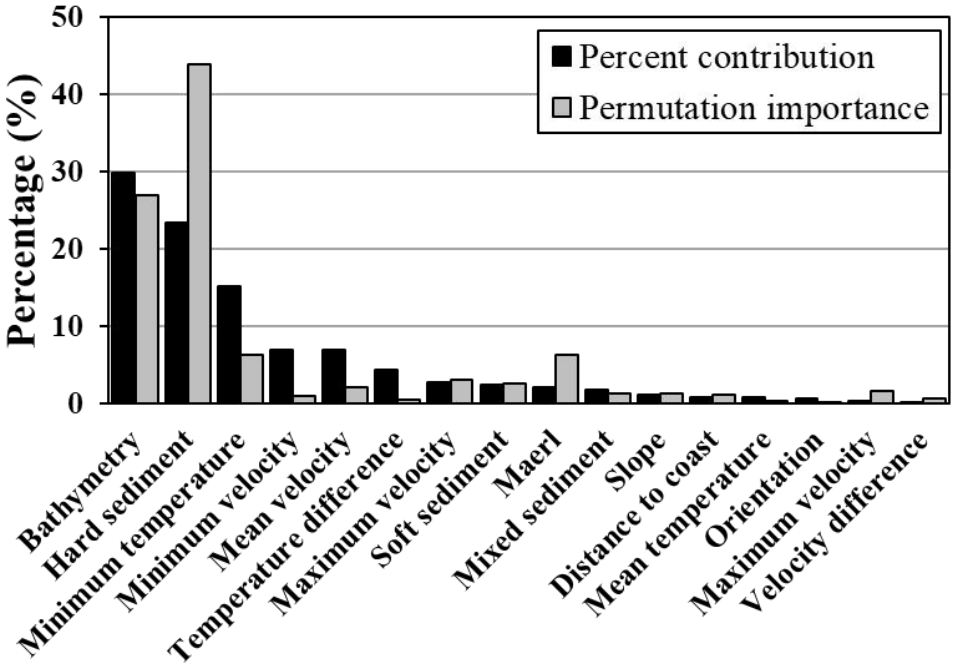

Figure 4.4. Scores (%) of the importance of the environmental factors predicting the habitat suitability of *Zostera. marina* in the study site obtained from MAXENT model.

Table 4.1. Summary of the environmental requirements of the *Zostera marina.* Max = maximum, Min = minimum and Dif = difference.

	Bathymetry (m)	Orientation (°)	Slope (°)	Distance to Coast (m)
Mean	2.3	205.2	0.4	165.3
Maximum	10.4	325.9	1.1	211.8
Minimum	0.1	104.8	0.1	0.1
	Mean Velocity ($m\ s^{-1}$)	**Max. Velocity ($m\ s^{-1}$)**	**Min. Velocity ($m\ s^{-1}$)**	**Dif. Velocity ($m\ s^{-1}$)**
Mean	0.11	0.29	0.002	0.39
Maximum	0.17	0.52	0.004	0.52
Minimum	0.04	0.11	0.001	0.29
	Mean Temperature (°C)	**Max. Temperature (°C)**	**Min. Temperature (°C)**	**Dif. Temperature (°C)**
Mean	12.0	19.5	5.31	14.2
Maximum	12.1	20.9	5.9	16.5
Minimum	11.9	18.5	4.44	12.7
	Soft Sediment	**Mixed Sediment**	**Hard Sediment**	**Maerl**
Mean	0.1	0.6	0.0	0.3
Maximum	1.0	1.0	0.0	1.0
Minimum	0.0	0.0	0.0	0.0

Table 4.2. MAXENT model characteristics

Model Characteristics	
Regularized training gain	1.402
Unregularized training gain	1.711
Training AUC	0.938
Logistic threshold	0.248
Number of interactions	500
Records used for training	46
Points used to determine the Maxent distribution	1584
Output format	Logistic
Regularization multiplier	1
Max number of backgrounds points	10 000
Replicates	1
Replicate run type	Crossvalidate
Maximum interactions	500
Convergence threshold	0.00001
Adjust sample radius	0
Log file	maxent.log
Default prevalence	0.5

4.3.2. Predictor variables

Among the 16 variables chosen to perform the MAXENT model, bathymetry was the most important variable, with a contribution of 29.9 %. This was followed by hard sediment (23.4 %) and minimum temperature (15.1 %) and, finally, minimum and mean velocity which contributed 7.1% and 7%, respectively (Fig. 4.4). These five factors explained 82.5% of the distribution of *Z. marina*, while the sum of the other 11 remaining variables accounted for 17.5%. Results of the permutation importance indicated that the hard sediment descriptor encompassed the highest information with a value of 43.9 %, followed by the bathymetry (26.9 %), and finally minimum temperature and maerl sediment, with 7 % each (Fig. 4.4).

In the study area, *Z. marina* was distributed across an average depth range of 2.3 meters, and occurred at a maximum depth of 10.44 m. Also, the species had a higher affinity for mixed sediments, while there were no records of seagrasses on hard substrata such as rocks or reefs (Table 4.1). In addition, the eelgrass meadows were predominantly distributed at intermediate average current velocities of 0.11 m s^{-1}, whereas they were also occasionally observed at high (0.526 m s^{-1}) and low (0.002 m s^{-1}) current velocities (Table 4.1).

4.3.3. Remote sensing map

The remote sensing map predicted an area of 8,916,639.2 m^2 occupied by *Z. marina*, representing a 5.4 % of the total area modelled (Table 4.4). Again, with this approach we forecasted the presence of the target species in previously unmapped areas, such as in Bertraghboy and Chasla Bay, and also in some regions from Kilkieran Bay (Fig. 4.6).

4.3.4. Integrated map

The map derived from the integrated approach estimated a potential seagrass coverage of 5.033,282.4 m^2, representing 3.6 % of the studied region (Table 4.5).

The largest area was predicted for Kilkieran Bay (4,057,543.1 m^2), followed by Bertraghboy Bay (939,951.2 m^2) and finally Chasla (35,788.0 m^2) (Fig. 4.7).

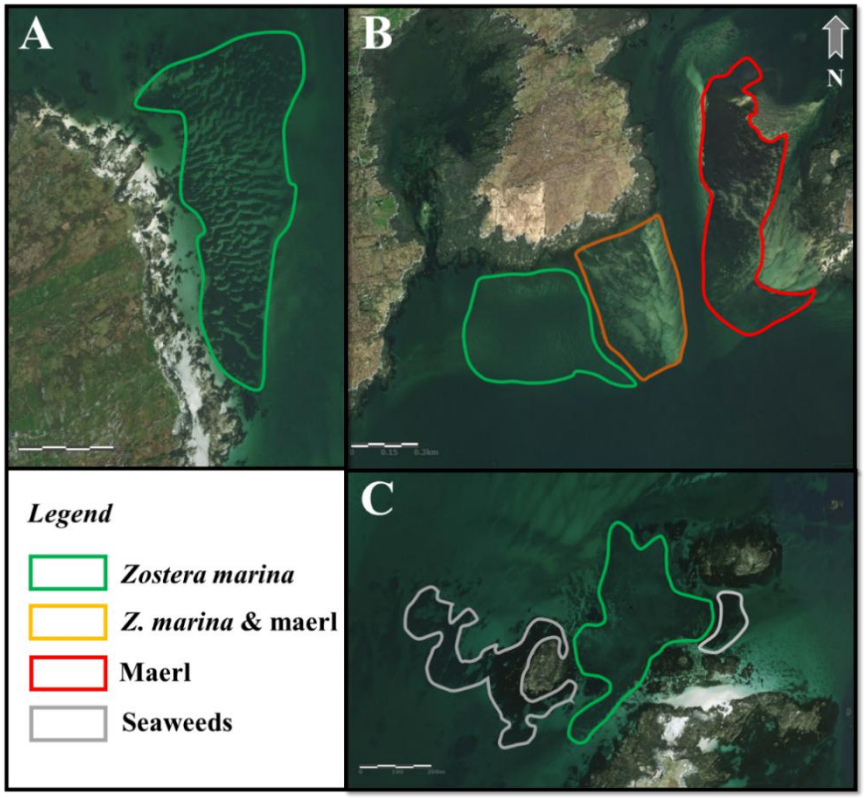

Figure 4.5 Aerial images of the study area indicating the presence of different ecosystems. Green lines represent the seagrass meadows, orange lines represent the interaction of seagrass and maerl, red lines represent the maerl and the grey lines represent seaweed.

4.3.5. Integrated map evaluation

In total, we evaluated 20 new locations where the integrated map suggested the presence of the seagrasses (Table 4.4). For 16 sites we confirmed the presence of the eelgrass, while in 4 locations no seagrass was detected. This field evaluation method suggests an accuracy of 80 % of the final integrated map. Of the sites where we did not find (but predicted), *Z. marina*, 3 were occupied by maerl, and one by *Himanthalia elongata* (Fucales, Phaeophyceae); of these sites, two were located in Bertraghboy Bay and two in Kilkieran Bay. Furthermore, we confirmed *Zostera* spp. meadows in five additional sites (Table 4.3, ID 14, 17, 19, 20 and 21), where seagrass had not been predicted by the integrated approach, although these were

relatively small areas (< 4,000 m^2) representing 0.34 % of the total newly mapped seagrass areas (Fig. 4.8, Table 4.3 and 4.4).

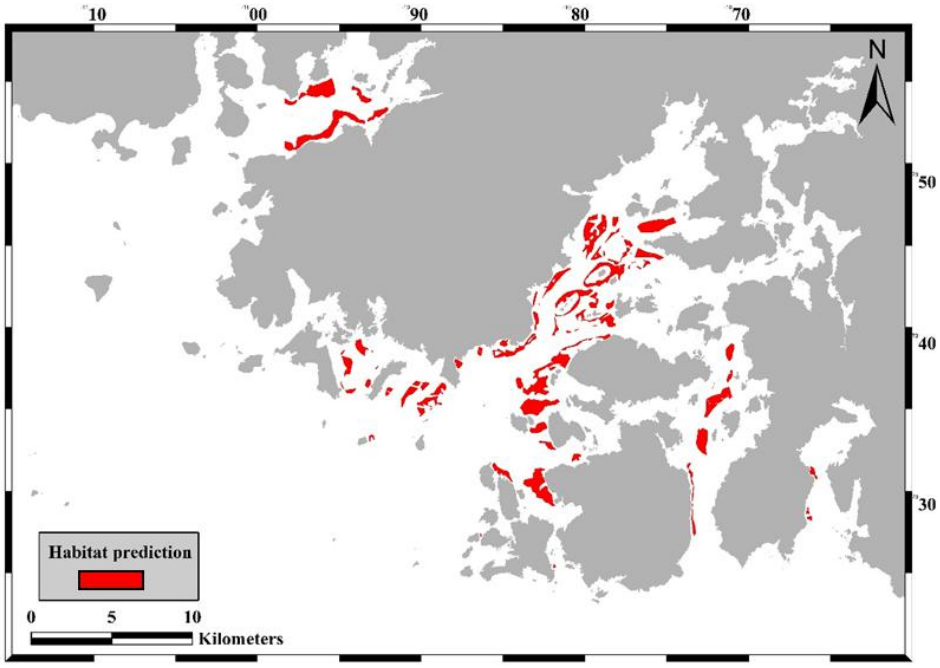

Figure 4.6. Predicted habitat distribution of *Zostera marina* using satellite-derived images from the map base of the world imagery from ArcGIS 10.2 software (ESRI ®)

4.3.6. New estimated *Z. marina* distribution and estimated economic value

The distribution of newly mapped areas of seagrasses encompassed 2,682,977 m^2 which represents 45.6 % of its known distribution previously documented in the literature (Table 4.4, Fig 4.8). In Kilkieran Bay the extent of 1,409,981 m^2, including 15 new locations, was mapped, in addition to four previously unrecorded sites in Bertraghboy Bay (1,235,368 m^2), and finally, two new areas in Chasla Bay (37,628 m^2) (Table 4.3). The newly mapped distribution of seagrass when was confirmed to be occupied by *Z. marina* (2,679,499 m^2), except in one location where *Z. noltii* was present (3,478 m^2). Moreover, in 8 of the newly categorized areas, seagrass and maerl co-occured, mainly in deep *Z. marina* populations (Table 4.3) which were characterised

by low seagrass shoot density and long plants with high biomass (Beca-Carretero et al., *under review a* (Chapter 2)).

Using the data for *Z. marina* distribution derived from this study, the estimated the annual economic value is € 6,578,385 using Eq. 4.1 provided by Constanza (2014) in addition to values based on previously reported seagrass distribution from the available literature is estimated to be € 14,430,002. Hence, the estimated combined sum of both records is € 21,008,187.

4.3.7. Projected *Z. marina* extension

Considering the level of accuracy of our integrated map (80 %), and extrapolating this accuracy to the unchecked areas projected by the integrated approach (3,620,984 m^2), we projected the existence of a larger area covered by seagrass of 2.896,788.1 m^2 (Table 4), with an estimated annual economic value of 6,981,258 €. This projected area represents 49 % of the current seagrass distribution mapped in the literature (Table 4.4, Fig. 4.8).

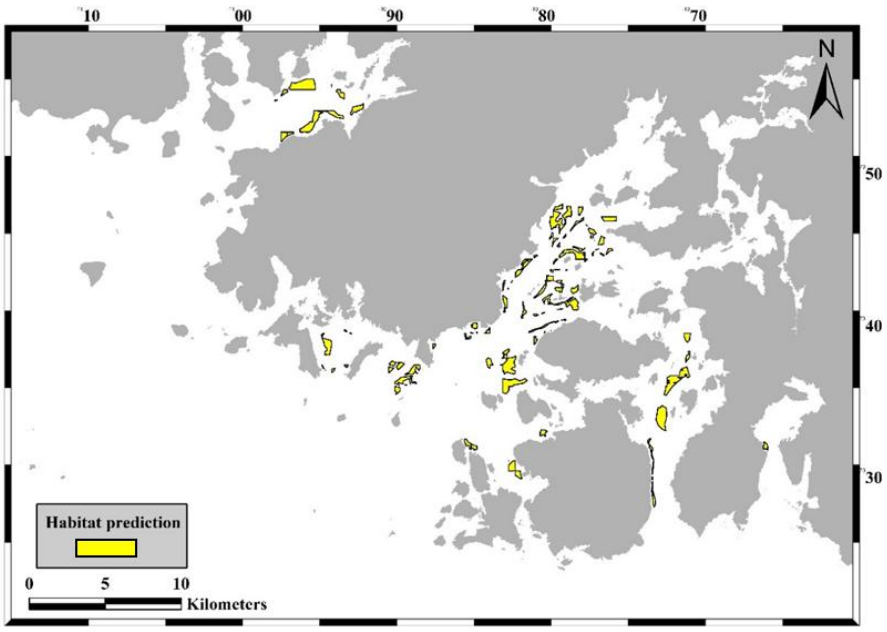

Figure 4.7. Final map of habitat suitability of *Zostera marina* using the spatial information of MAXENT model and satellite-derived images.

Table 4.3. Characteristics of the new mapped seagrass meadows including location, sampling date, area (m^2), depth (m), interaction with maerl, eelgrass coverage, prediction by the integrated map and the seagrass species.

ID	Location	Sampling Date	Area (m^2)	Depth (m)	Interaction with maerl	Eelgrass coverage	Meadow completed	Predicted	Seagrass species
1	Bertraghboy Bay	07/09/2016	319,722	2-6	Partial	Moderate	Yes	Yes	*Z. marina*
2	Bertraghboy Bay	17/7/2017	160,581	2-5	Yes	Moderate	Yes	Yes	*Z. marina*
3	Bertraghboy Bay	17/7/2017	686,769	2-4	Partial	Sparse	Yes	Yes	*Z. marina*
4	Bertraghboy Bay	17/7/2017	68,296	1-10	Partial	Dense	Yes	Yes	*Z. marina*
5	Kilkieran Bay	11/05/2015	143,383	2-4	No	Dense	Yes	Yes	*Z. marina*
6	Kilkieran Bay	08/07/2017	353,268	2-6	No	Moderate	Yes	Yes	*Z. marina*
7	Kilkieran Bay	10/07/2017	10,250	2-8	No	Dense	No	Yes	*Z. marina*
8	Kilkieran Bay	09/07/2017	139,840	2-10	Partial	Dense	No	Yes	*Z. marina*
9	Kilkieran Bay	18/04/2015	111,412	2-5	No	Dense	No	Yes	*Z. marina*
10	Kilkieran Bay	16/04/2015	232,469	2-8	Partial	Dense	Yes	Yes	*Z. marina*
11	Kilkieran Bay	07/05/2016	121,265	2-8	No	Dense	No	Yes	*Z. marina*
12	Kilkieran Bay	11/06/2016	231,806	2-8	Partial	Dense	No	Yes	*Z. marina*
13	Kilkieran Bay	23/07/2016	1,148	2-3	No	Sparse	Yes	Yes	*Z. marina*
14	Kilkieran Bay	14/06/2015	3,221	2-4	No	Dense	Yes	No	*Z. marina*
15	Kilkieran Bay	20/03/2017	47,352	2-5	No	Dense	Yes	Yes	*Z. marina*
16	Kilkieran Bay	23/09/2016	11,877	2-3	No	Moderate	Yes	No	*Z. marina*
17	Chasla Bay	24/03/2017	3,478	3-6	No	Dense	No	No	*Z. marina*
18	Chasla Bay	23/04/2016	34,150	1-2	No	Sparse	Yes	No	*Z. marina*
19	Kilkieran Bay	06/05/2016	1,592	1	No	Moderate	Yes	No	*Z. noltii and Z. marina*
20	Kilkieran Bay	11/010/2016	753	3-4	Partial	Moderate	Yes	No	*Z. marina*
21	Kilkieran Bay	09/07/2017	345	1	No	Moderate	Yes	No	*Z. marina*

4.4. Discussion

This section has been modified from the original article

4.4.1. MAXENT performance and variables affecting *Z. marina* distribution

Results of MAXENT showed a great discriminatory capacity and sensitivity indicating the adequate selection of the SDM, which is a key step to achieve the stated goals of the research (Frankiln 2010). Previous studies successfully applied MAXENT models to predict the habitat suitability of submerged seagrass populations in unmapped regions and to forecast potential areas for seagrass restoration (i.e. Valle et al., 2013; Downie et al., 2013; Chefaoui et al., 2015). MAXENT model predicted a larger area of habitat suitability in the studied area for *Z. marina* than has already been reported in the literature, highlighting the potential presence of the seagrasses in non-surveyed regions, such as, in Bertraghboy Bay and in Chasla Bay. In Kilkieran Bay, which is one of the most studied bays in Ireland, MAXENT model also predicted the presence of *Z. marina* in new regions. Particularly, Bertraghboy Bay and Kilkieran Bay were the locations with the larger areas of potential habitat suitability for the target species predicted by MAXENT model (Fig. 4.3). These outcomes may be explained by the heterogeneous coastal morphology characterized by the presence of islands, channels, bays and coves, which generate large gradients of environmental variables, included current velocity, temperature and type of substrata, thus promoting a high diversity of biological systems in a relative small area (S4.1) (NPWS 2014). By contrast, MAXENT model forecasted lower areas of potential habitability for *Z. marina* in Chasla Bay, potentially associated with its homogeneous coastal geography characterized by the absence of marked ambient gradients (S4.1).

Overall, our results depicted *Z. marina* as a specialist species within a defined ecological niche, distributed from shallow to intermediate photic areas, with preferences for mixed and soft sediments and intermediate temperatures and current velocities (Table 4.1, S4.2). Results from MAXENT model highlighted that landscape metrics, such as, bathymetry and hard sediment, were more important

variables driving the presence of the target species than environmental variables, such as, temperature or current velocity. In the study area, *Z. marina* populations inhabited depths ranging from 2.3 to 10.4 m (mean value = 2.3 m), and with an average orientation of 205º (south-west), indicating the relevance of light availability in controlling its presence. Depth-related levels of irradiance control the photosynthetic performance and metabolism and thus the growth, productivity and distribution of seagrasses (i.e. Ralph et al., 2007; Lee et al., 2007). Prior investigations in the Baltic Sea using SDMs observed that depth was also one of the most influential environmental factors constraining the occurrence of *Z. marina* with higher preferences to colonise a depth range from 0.5 – 10 m (Downie et al., 2013; Bekky et al., 2008). The presence of hard sediment, such as rocks or reefs, also explain robustly the absent of the target species in the study area. The results demonstrated that *Z. marina* can colonize different gradients in sediment size, including mud, sand and coarse sediment, with a habitat predilection for medium grain size substrata (Table 1). *Zostera marina*, as other marine plants, is characterized by the presence of a root-rhizome system adapted to colonize soft and intermediate sediments (i.e. Fonseca et al., 1998; Hemminga 1998).

Among the environmental variables, minimum temperature and current velocity and intermediate current velocity were the most influential parameters explaining the absence or presence of our species (Fig. 4.4). Particularly, both minimum temperature and current velocity determined the absence of the *Z. marina* populations. In the study area, lowest values of both environmental variables are observed in intertidal areas in the innermost and shallowest parts of the bay, which were influenced by the entrance of freshwater and sediment runoff (NPWS 2010, 2013, 2014). Low levels of salinity alongside with depletions in water clarity may be the causes limiting the presence of the *Z. marina* (Van Katwijk et al., 2000). Also, the prolonged dissection of these intertidal ambient may cause a great physiological stress in marine plants which may restrict the colonization of *Z. marina* (Shafer et al., 1987). Finally, our results indicate that optimal hydrodynamic conditions of *Z. marina* (0.108 m s^{-1}) were associated with intermediate to moderate current velocities. Intermediate hydrodynamic conditions improve water oxygenation and nutrient supply and also do not compromise the seagrass attachment to the sediment

(Hemminga & Durate 2000). Lowest current velocities (0.01- 0.02 m s^{-1}) were present at the most sheltered sites of the study area, while highest velocities (0.52 m s^{-1}) were appreciated in the most exposed sites; in both cases the presence of the *Z. marina* was absence or minimal. Our findings were in line with previous observations where *Zostera* spp. displayed a higher affinity for colonising intermediate current velocities (Peralta et al., 2005, 2006; Valle et al., 2014; Downie et al., 2013).

Furthermore, in line with our study, landscape metrics, such as bathymetry or coastal morphology, better defined the distribution or absence of seagrass species at local scales (Valle et al., 2013; Boscutti et al., 2015). On the other hand, studies investigating seagrass species throughout all their distribution range on a global scale found that environmental variables such as sea surface temperature (SST) were the most influential parameters delimiting their distribution (Chefaoui et al., 2015). It is noteworthy that SST define the latitudinal distribution of marine plants in both northern and southern limits (Lee et al., 2007). Hence, as thermal limits are only encompassed at global scales, our temperature data may not reflect the entire effect of sea temperature in the extent of our study area, which thus explains its lower importance in our SDM.

Here, to perform the SDM, we used fine-scale variables specifically developed for our study area such as current velocity, sea water temperature or LIDAR data which are highly representative of the environmental characteristics of the coastal region (Jackson et al., 2012). Hence, regarding a potential extrapolation of this model to larger area may be constrained by a lack of such data for other regions of Ireland.

4.4.2. Remote sensing for mapping seagrasses

The satellite-derived images allowed us to adequately ascertain the presence of uncovered submerged seagrass meadows in the studied bays (Fig. 4.6), which also consistently matched with the maps obtained by applying SDM. The two most important meadow characteristics which facilitated distinction between the seagrass from other vegetation type communities, such as maerl or kelps, were the

striped shape and the colour of the meadows (Fig. 4.5). The fine spatial resolution of the remote sensing image map (3 m) enabled us to detect from medium (~ 10,000 m^2) to large size meadows (> 600,000 m^2) (Table 4.3). Thus, we probably were not able to map small patchy meadows in the monitored area (< 4,000 m^2). Particularly, we found that the greatest challenge was to differentiate between co-occurrence of *Z. marina* and maerl from mono-specific maerl systems as both communities developed similar striped forms. It is possible that this co-occurrence in the developed remote sensing map resulted in an underestimation of seagrass as in some regions striped and dark forms were identified as mono-specific maerl communities, although low densities of seagrass may also have been present. Secondly, we also experienced constraints in ascertaining the presence of deep seagrass populations because of reductions in water clarity at greater depths.

Furthermore, the main differences in the prediction of the distribution of the *Z. marina* between the map of habitat suitability developed with MAXENT and the map created using remote sensing images were that (i) MAXENT model predicted its presence in deeper areas where remote sensing images did not suggest the presence of the *Z. mairna* using, (ii) MAXENT model sometimes forecasted the presence of *Z. marina* in places where we visually estimated maerl coverage, and (iii) finally, remote sensing images could be used to ascertain with a high precision the size and shape of the target species beds, while MAXENT model only gives a probability of habitat prediction.

Table 4.4 Area (m^2) and cover in relation with the total modelled area (%) of different maps of the presence or the habitat suitability of the *Zostera* spp. in the area studied.

	Area (m^2)	**Cover (%)**
MAXENT model	31.667,855	19.3
Remote sensing (RS)	8.916,639	5.4
MAXENT & RS	5.033,282	3.1
Known seagrass (literature)	5.988,294	3.6
Discovered seagrass	2.728,948	1.7
Projected seagrass	2.650,561	1.6

4.4.3. Final map of *Z. marina*

The newly integrated method is highly robust (accuracy over 80%) which led us to map a new distribution of 2,729,942 m^2 which represents 45.8% of the current seagrass cover in the study area (Table 4.4, Fig. 4.8), however, a large distribution may still remain unmapped. Potentially, the main reason a large proportion of submerged seagrass populations in the study area remain unmapped at present is because these bays are under-surveyed. Most new seagrass areas corresponded to Kilkieran Bay and Bertraghboy Bay, which accounted for the 95.6 % of total seagrass in the study area, while Chasla Bay (37,628 m^2) represented only 0.43 %. This may have been related with the larger diversity of environments observed in the former than the latter bays. Nevertheless, water clarity in Chasla Bay was lower, which may be another factor limiting the presence of seagrass.

Here we validated 20 new regions, however, most previous studies using SDMs have overlooked this step and limit their *in-situ* model validation to check 2-4 points in the field (Heide et al., 2009). In the four locations where there were no seagrasses we found maerl (three sites) and *Himanthalia elongata* (one site). Most of the newly mapped areas of seagrass corresponded to *Z. marina,* while *Z. noltii* was present in only one site. The low presence of *Z. noltii* was expected because this species is commonly found in extensive sandy intertidal areas which are scarce in the modelled bays (Hemmiga et al., 2000).

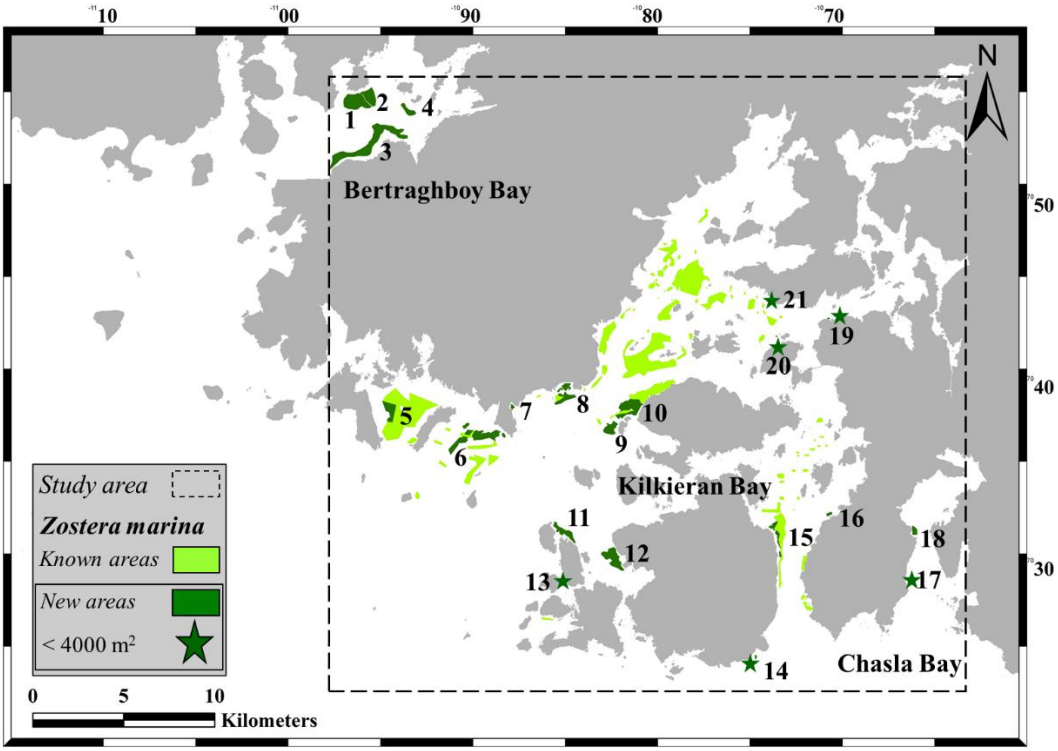

Figure 4.8. Map of the distribution of the known *Zostera marina* provided by the literature (light green), and the distribution of the new discovered locations (dark green) in the study area. The green stars represent the newly discovered meadows with an area less than 4,000 m^2.

In the newly mapped areas we observed a common co-occurrence between *Z. marina* and maerl. Previous studies in western Ireland (De Grave 1999; NPWS 2014) and France (Martin et al., 2005) outlined this exceptional co-occurrence. Maerl beds form a three-dimensional structure of nodular coralline algae which is considered a hot-spot of biodiversity and productivity (Foster 2001; Wilson et al., 2004). In Bertraghboy Bay and Kilkieran Bay this interaction covers large areas, being in some cases the predominant state of *Z. marina*. On the other hand, this co-occurrence was not observed in Chasla Bay, nor was it previously reported in the literature for this site. To our knowledge, the co-occurrence in the study area represents the largest scuh area reported worldwide. Interestingly, areas were MAXENT model were forecasting a high habitat suitability for *Z. marina*, were covered by maerl. Hence, more studies are necessary to assess if this interaction may exclude certain some species, or if there is a mutual relationship which favours their presence.

Furthermore, previous studies of submerged *Z. marina* populations in western Ireland observed significant spatial and temporal fluctuations along the edges of the meadows, with larger extensions observed in summer rather than in colder periods (Dawes et al., 1992; Dale et al., 2007). Also, the exposure to extreme climatic conditions such as storms or hurricanes (Ophelia, October 2017; Emma, March 2018) significantly damaged the *Zostera* spp. meadows reducing their coverage (*pers. obs.*), highlighting that temporal variations in the total extension of the mapped areas may occur.

The integrated map lacks associated characteristics at population or individual level, such as vegetative or reproductive shoot density, above- and below-ground biomass or size and weight of the marine plants; methods described by Winters et al., (2016) or Roelfsema et al., (2009) may be used to maps with relevant specific information.

The new seagrass area mapped in this study along with the known covered area provided by the literature have an estimated economical annual value of € 8,717,242 based on the ecological goods and services that they provide. This economic relevance alongside its conservational and biological value in terms of high productivity and biodiversity (Boyle et al., 2012; NPWS 2013, 2014), render this study area an exceptional location for further research. Hence, we propose to use these mapped seagrass locations as a baseline for developing long-term monitoring programs to assess the potential effects of habitat degradation or climate change. In this basis, the European Water Framework Directive (WFD, European Union, 2000/60/EC) reports that seagrasses, among other marine primary producers, are key bioindicators to assess the ecological status of a transitional marine coastal areas (Foden et al., 2007). Finally, this study represents an important contribution to the international efforts in the conservation of these precious ecosystems and, in particular, within the European Framework. Since 1992, *Zostera* species have been recognized as habitats with special protection by the European Union (EU Habitats Directive) and since 2004 are included in the Initial List of Threatened and Declining Species and Habitats by the OSPAR Commission.

Chapter 5

Implementation of diverse approaches to assess the seagrass distribution in Ireland

5.1. Introduction

Seagrasses rank among the most valuable natural habitats worldwide, providing numerous ecosystem services comparable to tropical forests or saltmarshes (Nordlund et al., 2017). For instance, marine plants represent one of the most efficient ecosystems capturing atmospheric CO_2 and storing it in the sediment, trapping approximately 10-12 % of global emissions (Kennedy et al., 2010; Fourqueran et al., 2012). Seagrasses also contribute with other relevant functions, such as, shelter and nursery habitat, shoreline protection or as a readily available food source (Barbier et al., 2011). However, in spite of its ecological importance, seagrasses are currently disappearing at a rate of 1.2 % annually, and it was reported that between 30 to 50 % of their total distribution has disappeared in recent decades (Waycott et al., 2009). Seagrasses are distributed in sheltered and shallow locations, and thus, they are especially vulnerable to anthropogenic disturbances, which represent the main cause of seagrass habitat loss (Orth et al., 2006; Waycott et al., 2009). However, seagrasses do not receive the same recognition as other charismatic natural habitats such as coral reefs (Nordlund et al., 2018). For these reasons, the mapping and updating their spatial information represent vital steps in developing adequate policies to better manage and conserve these precious systems.

The mapping of observable objectives, such as, biological habitats or vegetation communities in terrestrial ecosystems and intertidal to shallow marine areas, has been widely developed over recent decades, mainly related to the use of novel remote sensing techniques and derived products (Aswani et al., 2006). These methods allow the easy recognition of the targets and detection of changes in their distribution over time. For instance, aerial satellite and airborne images were implemented to identify and to quantify long-term monitoring changes in intertidal and shallow seagrass populations in response to temporal changes or to habitat degradation (Macleod & Congalton 1998). On the contrary, in deeper areas, the implementation of satellite tools are not as effective because they cannot clearly distinguish and identify specific objectives. Therefore, assessing the distribution and potential changes of subtidal distributed marine species, including seagrasses, has presented a major challenge for researches over the last number of decades

(Roelfsema et al., 2013). In prior studies, *in-situ* data collection for mapping submerged to deep distributed seagrass populations has been carried out using different methods such as diver-operated field surveys (Komatsu et al., 2003), multi-beam sonar techniques (Komatsu et al., 2003) or autonomous underwater vehicles (Amstrong et al., 2016). These approaches enable the mapping and assessment of variations in seagrass spatial distribution, abundance, density and percentage cover and even seagrass species succession (Lathrop et al., 2001). However, most of these operational systems have significant logistic and economic costs and may require highly qualified workers. Hence, the development of new strategies is a key step in optimising and expediting the mapping of submerged uncovered vegetation communities. Alternativity, species distribution models (SDMs) allow for the prediction of habitat suitability for species with a relatively low-cost effort. Prior studies of marine ecosystems applied these techniques to identify areas with potential habitability for target organisms in deeper areas, including marine plants (Silva et al., 2008). The implementation of these tools may require the *in-situ* verification of the success and robustness of their spatial predictions (Elith et al., 2009). Interestingly, different strategies to map submerged vegetation habitats have emerged recently. Indeed, a recent study assessing the Portuguese kelp status developed valuable maps from a team of independent volunteers made up of scuba divers and marine coastal zone users. The participants involved reported georeferenced spatial information of the target species which was later used to generate maps of kelp distribution along the Portuguese coast (Assis et al., 2009).

In Ireland, intertidal seagrass meadows, mainly *Zostera noltii* and occasionally some *Z. marina* or *Ruppia* spp. have been thoroughly mapped (i.e. NPWS 2011, 2014, 2015) However, intermediate to deep-distributed seagrass populations may be under-surveyed, and as a result spatial information is far from complete. Therefore, we hypothesized that a potentially larger distribution of subtidal seagrass populations may remain unmapped. Based on these assumptions, we aimed to obtain more detailed present-time seagrass spatial maps in Ireland. To achieve this, we (i) combined the available data on spatial distribution of seagrass in Ireland, (ii) created a volunteer project to gather information from the local community, (iii) developed SDM to predict potential areas of seagrass habitats at a regional scale, (iv) and *in-situ*

validated all the information obtained from the community project and the SDM by undertaking field surveys. Finally, with the compiled information from Irish sources and the verified spatial information, we constructed a new and more complete map of seagrass distribution around the Irish coast.

5.2. Methodology

5.2.1. Data source of species distribution

The study was conducted in Ireland. Maps of spatial information of Irish seagrasses were collected from three sources; (i) the MESH Atlantic project (http://www.emodnet-seabedhabitats.eu/map), (ii) the Site-Specific Conservation Objectives Marine Community Types project (ttps://data.gov.ie), and (iii) the OSPAR Habitats project (http://www.ospar.org/) (For further details see Chapter 4, Section 4.2.2). This spatial information outlined the areas of seagrass meadows. Hereafter, we combined the information of the three sources, creating a single distribution layer.

5.2.2. Collection of additional spatial seagrass information from independent sources

We compiled spatial information of seagrasses in Ireland from different sources such as, specialized websites of biodiversity worldwide, https://www.gbif.org/ or https://biodiversitymapping.org . These sources only provided spatial information of the specific location but not of the extent of the seagrass meadow, hence we did not include these data in Section 5.2.2 “Data source of species distribution”.

In parallel, we created an editable Google map (Fig. 5.2) for local volunteers to add information regarding seagrass presence on the coast of Ireland. This project was entitled “Searching for Seagrasses” and was conducted from June to September 2018. We sent this Google map to community groups such as research centres, conservation associations, professional and recreational divers and local fishermen.

(https://www.google.com/maps/d/edit?mid=1o_7UqE4p4zO28k2xYX1ruW2Qg4U&ll=53.34186759892063%2C-9.018466450000005&z=7).

Alongside with this map, we sent an information box (Table 5.1) to be complete by the local volunteers, containing information regarding seagrass location, type of species, habitat distribution and confidence level. In addition, we provided the participants with an identification guide to potentially distinguish seagrasses from other vegetative marine communities, and also between specific seagrass such as *Z. marina* and *Z. noltii*. Also, we requested the addition of a recent photograph of the meadow, where possible.

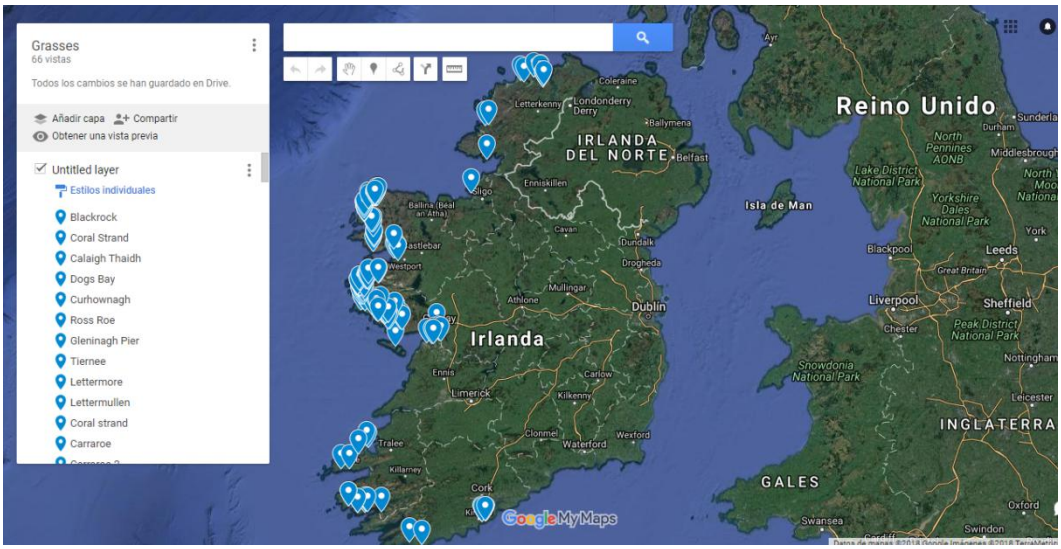

Fig. 5.1 Image of the editable Google map with the potential locations with seagrass from Ireland reported by the volunteers.

Table 5.1 Example of the information box (Example Pedro Beca) filled by the participants in the project "Searching for Seagrasses".

Name and organisation/ person	Pedro Beca – NUIGalway
Name of the location	Coral Beach (Kilikieran Bay)
Date of observation	July - 2016
Species (if possible)	*Zostera marina*
Subtidal or Intertidal	Subtidal
Confidence level (of identification): from 1 (confident), to 3 (not sure):	1
Picture of the meadow	Yes

5.2.3. Predictor variables and pre-processing GIS layers for the regional model

To perform the SDM at a regional scale (Ireland), we used a set of eight environmental variables of which seven were continuous variables (temperature, current energy, fetch, bathymetry, slope, orientation and distance from the coast). The other variable, sediment type, was designated as categorical, which consisted of three different raster layers, soft sediment, mixed sediment and hard sediment.

We derived temperature (°C) data from the bioclimatic layers of the WorldClim – Global Climate Data (http://www.worldclim.org/bioclim); specifically, we sourced the BIO = Annual Mean Temperature with a resolution of 0.5°. Bathymetry (m) data was obtained from the European Marine Observation and Data Network (http://portal.emodnet-bathymetry.eu/), with a resolution of 0.2°. From this layer we created the orientation (°) and the slope (°) layers using the tools *aspect and slope* of the Spatial Analyst extension from ArcGIS 10.3 software (ESRI ®), respectively. Also, we calculated the distance from the shoreline of the Irish shapefile applying the *Euclidean distance* tool of the Spatial Analyst extension. The raster layer of tidal current energy was derived from the National Oceanographic Centre (NOC) as part of the EMODnet Seabed Habitats project (2010) (http://www.emodnet-seabedhabitats.eu/default.aspx). This layer was created using hydrodynamic NOC models developed at 1 m above the seabed in the Celtic Sea. The energy has been expressed in terms of peak kinetic energy (J/m^3). The fetch layer was created using a method described in Finlayson (2005) designed for ArcGIS (ESRI ®). This tool calculates effective fetch for multiple wind directions based upon a text file listing individual compass direction, applying the recommended procedure of the Shore Protection Manual (https://www.umesc.usgs.gov) (USACE 1984). Finally, sedimentological data was sourced from the EUNIS seabed habitat map for the North Sea and Celtic Sea (http://www.emodnet-seabedhabitats.eu/) (Vasquez et al., 2015; Cameron & Askew 2011) which is categorised following the EUNIS classification system (Galparsoro et al., 2012). Following the methodology described in the Chapter 4, Section 4.2.3, we obtained three sedimentological layers (i) soft sediment, (ii) mixed sediment and (iii) hard sediment. All the predictable variables were transformed into raster files ensuring the same resolution extent and projection (WGS_98).

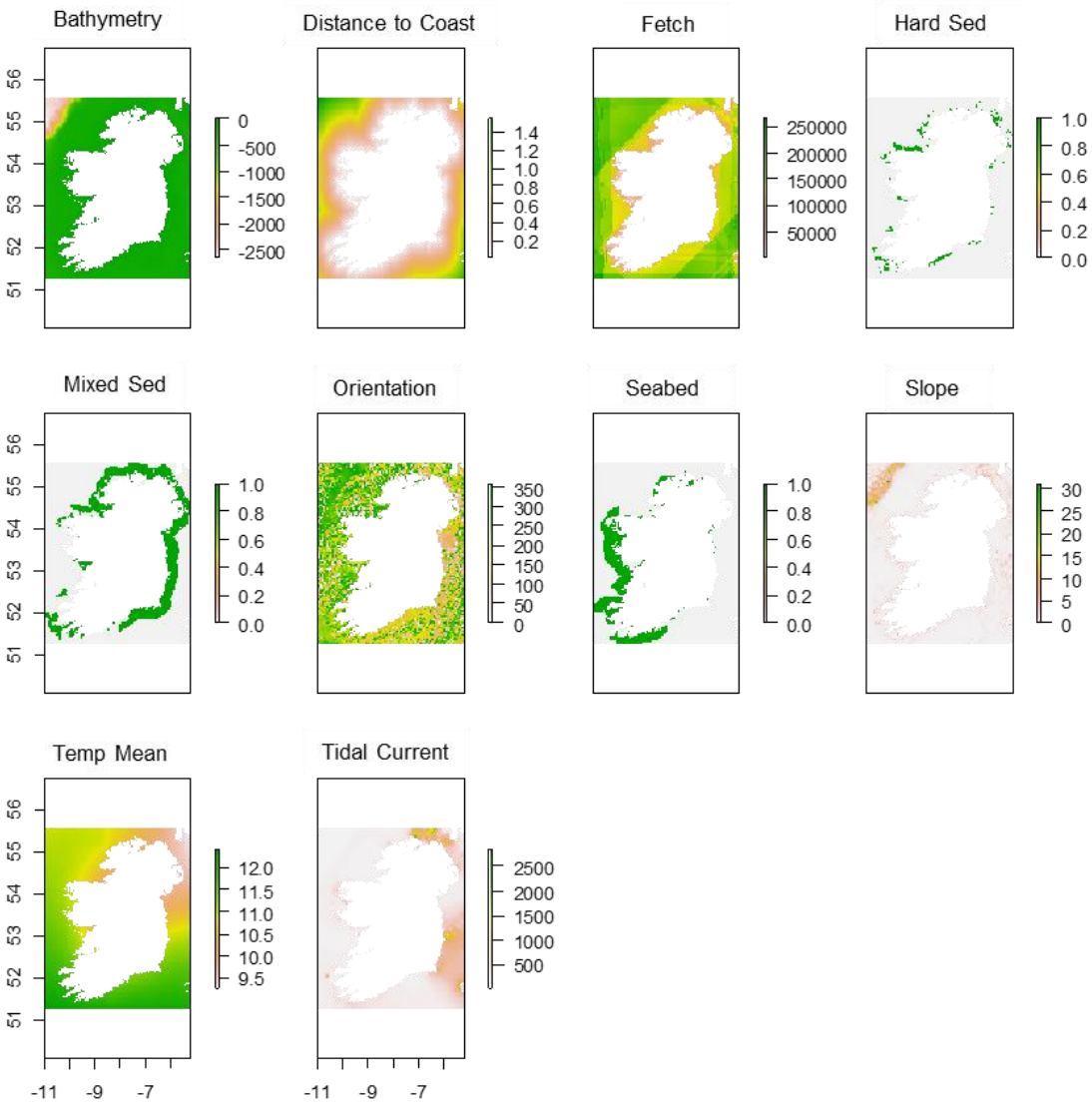

Fig. 5.2 Predictor variables used for performing the *Z. marina* distribution model (MAXENT) for the Irish coast.

5.2.4. SDMs and model evaluation

The MAXENT model was applied to assess which environmental factors best explained the seagrass distribution and to predict its potential habitat suitability along the Irish coast. We ran the MAXENT model with the default response settings (Phillips et al., 2006). For computing the model, we assumed that (i) the seagrass depth distribution must be equal to or below 0 meters, (ii) the distribution of the eelgrass has remained constant since being mapped from 1997 to 2011, (iii) the

distribution of the target species is in equilibrium with the current environmental conditions, (iv) and there is no exclusion through competition with other species. MAXENT generated a continuous raster file with a pixel value ranging from 0 to 1, with 1 representing the highest probability for potential habitat suitability, and 0 the absence of the target species. The model evaluation was performed by applying the threshold- independent metric AUC - area under the "receiver operating characteristic" (ROC) curve following the methodology described in Chapter 4, Section 4.2.5.

5.2.5. Validation of SDM and compiled information from independent sources

We evaluated and verified the spatial information from the MAXENT model and from local volunteers by carrying out several field surveys over 4 years (October 2014 – July 2018). The field surveys were undertaken following the methodology described in Chapter 4, Section 2.8, by georeferencing the presence and edges of the seagrass meadows. In total, we verified the information obtained for more than 300 locations around the coast of Ireland.

5.2.6. Final distribution map

We created a final map of the distribution of seagrass with the available spatial information (Section 5.2.2) and with the validated information from Section 5.2.5. In this new map we included information regarding previously known distribution and the newly discovered distribution, and also information regarding specific seagrass species (*Z. marina*, *Z. noltii* and *Ruppia* sp). For details of the process, see Fig. 5.3.

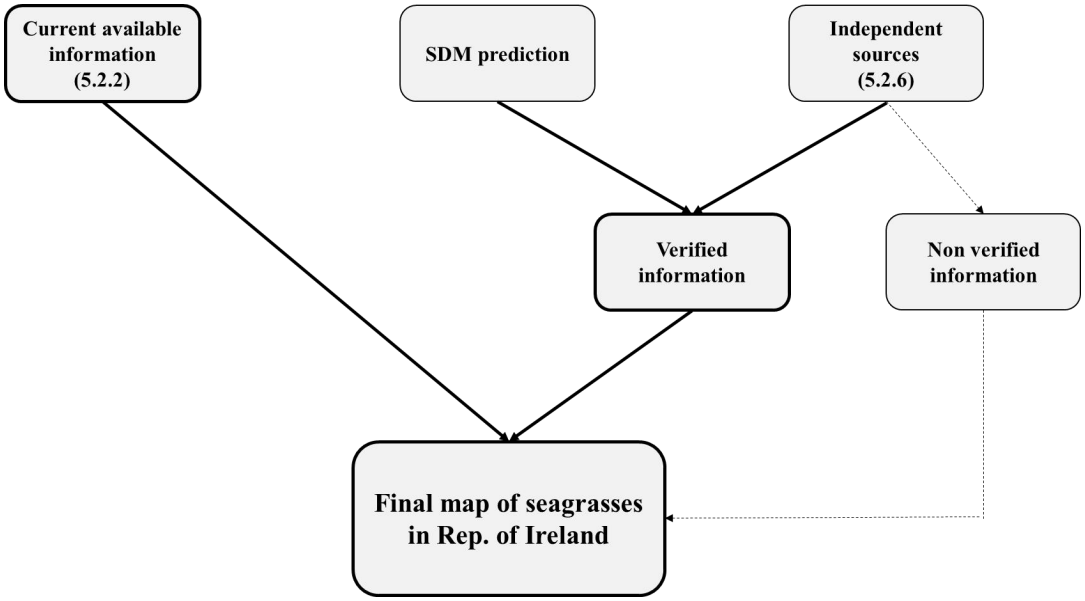

Fig. 5.3 Work flow performed to map the seagrass distribution in Ireland integrating different approaches. Section 5.2.2. is referred to the collaborative project.

5.2.7. Seagrass meadow economic value

We used the formula proposed by Costanza (2014) which estimates the value of seagrass meadows in € 2.41 m^2 yr^{-1} regarding the ecological services they provide and the area they cover (Eq. 5.1). We converted the value of US $ to EU € (based on 1.20 $ = 1.00 €) (June 2018).

Seagrass meadow value = Cover area (m^2) × Seagrass value (2.41 € m^{-2} yr^{-1}) (Eq. 5.1)

5.2.8. Biomass collection for future comparisons

We collected biomass from several parts of Ireland, including eastern, southern, western and northern regions, and from the different seagrass species, *Z. marina*, *Z. noltii* and *Ruppia* spp. These biomasses were kept freeze-dried to preserve the biomass for potential future biochemical analysis.

5.3. Results

5.3.1. Modelling at regional scale

The performance of the model reported a high discriminatory capacity within an AUC value of 0.903 (Fig. 5.4). The MAXENT model predicted a potentially larger area of habitat suitability for *Z. marina* than has previously been mapped in Ireland (Fig. 5.5). The results of the model indicated that, among the environmental descriptors, fetch (39.5 %) and bathymetry (35.7 %) were the most important parameters explaining the distribution of the seagrass followed by distance to coast (19.0 %) and mean temperature (4.2 %). On the contrary, other environmental variables, such as tidal velocity, slope, orientation, and sediment type, did not have a significant importance in explaining the seagrass presence.

Table 5.2 MAXENT model characteristics at regional scale.

Model Characteristics	
Regularized training gain	3.863
Unregularized training gain	4.003
Training AUC	0.993
Logistic threshold	0.058
Number of interactions	500
Records used for training	10126
Points used to determine the Maxent distribution	1584
Output format	Logistic
Regularization multiplier	1
Max number of backgrounds points	10 000
Replicates	1
Replicate run type	Crossvalidate
Maximum interactions	500
Convergence threshold	0.00001
Adjust sample radius	0
Log file	maxent.log
Default prevalence	0.5

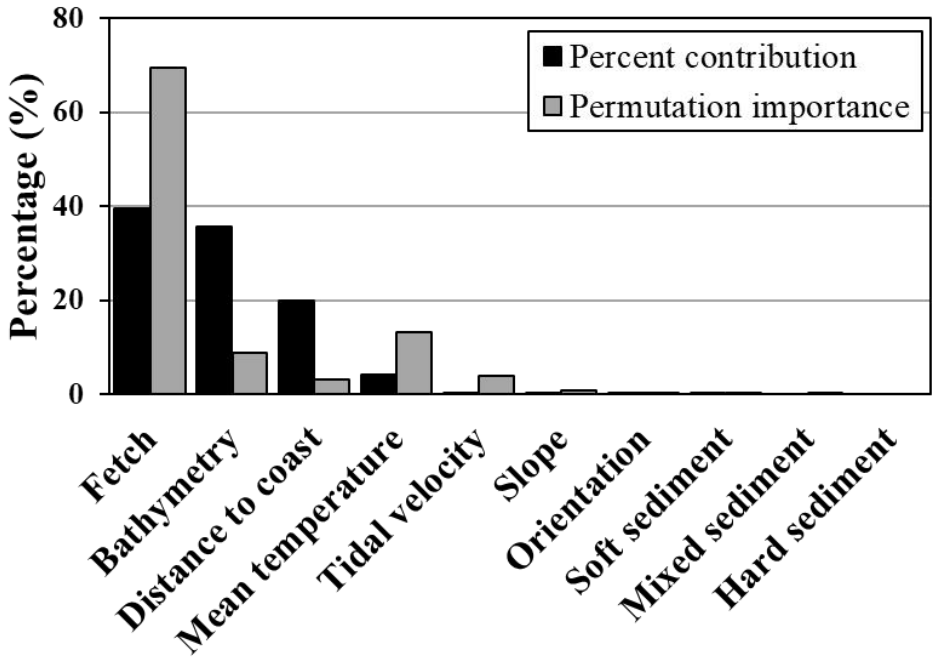

Fig. 5.4 Scores (%) of the importance of the environmental factors predicting the habitat suitability of *Zostera marina* in the study site obtained from MAXENT model.

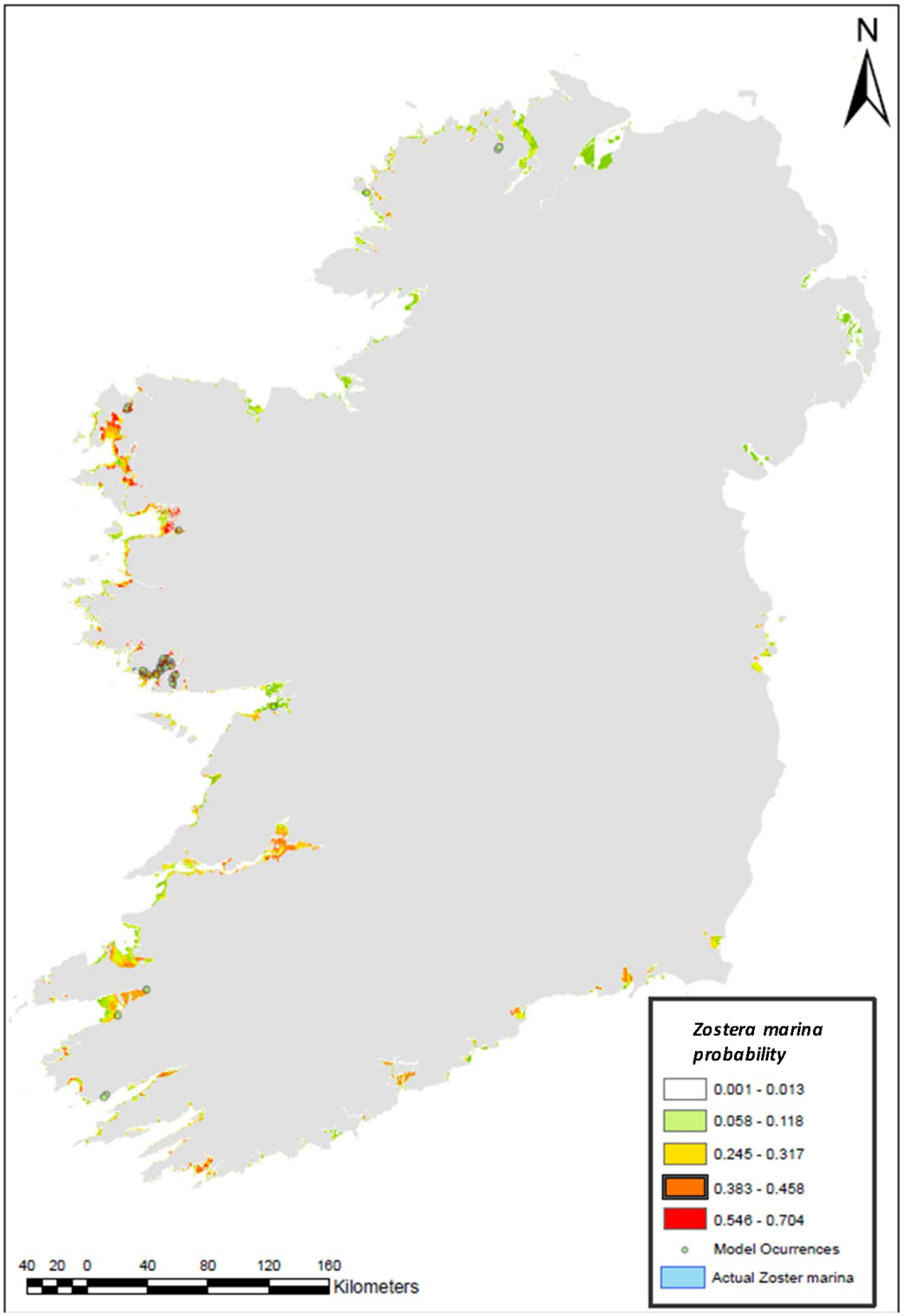

Fig. 5.5 *Zostera marina* habitat suitability predicted by MAXENT model.

5.3.2. Collection of additional spatial seagrass information from independent sources

From the independent sources we compiled a total of 107 locations, on which 37 sites were not previously reported (Section 5.2.2). This information was mainly regarding subtidal *Z. marina* populations and occasionally intertidal *Z. noltti* and *Ruppia* spp meadows. *Ruppia marina* and *R. cirrhossa* have a similar structure and morphology making it highly difficult to differentiate one to each other unless they develop flowers. Most of the information collected are based on observations from thesouth-west of Ireland.

5.3.3. Validation of SDM and spatial information of seagrasses from independent sources

We validated a total of 206 locations modelled by the SDM; of these, we found seagrass in 182, which represented a success of 83.4 %. From the 107 locations provided by independent users, we validated 32, and we found seagrass in 30 sites. This represented a success of 93.1 %.

5.3.4. Final map of distribution and estimated economic value

The validated locations accounted for a total of 36.3 km^2, which represent 25.0 % of there was previously mapped for Irish waters (146.1 km^2). Using the Eq. 5.1 provided by Constanza (2014), we estimated that the annual ecological value of the previously known seagrass distribution to be € 352,101,000 and the newly discovered locations as € 87,483,000.

Table 5.3 Summary of the environmental requirements of the *Zostera marina.* Max = maximum, Min = minimum and Dif = difference

	Bathymetry (m)	**Orientation (˚)**	**Slope (˚)**	**Distance to Coast (m)**
Mean	-2.62	190.3	0.60	10.0
Maximum	-12.2	358.2	2.16	300.0
Minimum	0.00	0.00	0.05	0.0
	Tidal Velocity (m sg^{-1})	**Fetch (m)**	**Temp Mean (˚C)**	
Mean	15.9	467.6	11.1	
Maximum	49.6	5068.7	12.1	
Minimum	0.7	50.2	10.3	
	Soft Sediment	**Hard Sediment**	**Mixed Sediment**	
Mean	0.8	0.0	0.1	
Maximum	1.0	0.0	1.0	
Minimum	0.0	0.0	0.0	

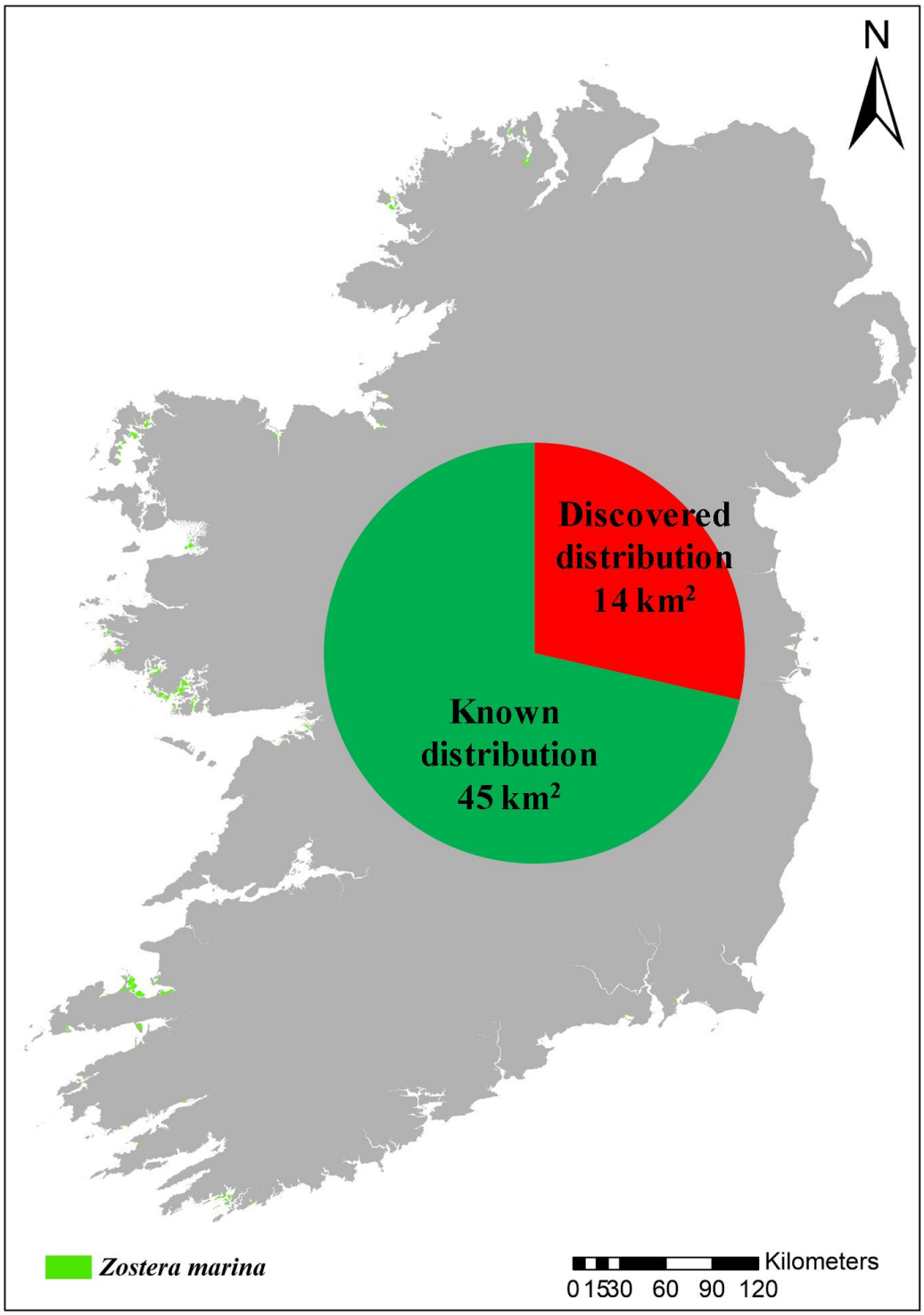

Fig. 5.6 Seagrass (green colour) distribution in Ireland from compiled information and from recent discovered distribution in this study

5.4. Discussion

Seagrasses are marine key ecosystem engineers which provide essential benefits to marine and terrestrial habitats (Fonseca et al., 2002). However, their rapid global habitat destruction over the last few decades represents a major concern (Waycott et al., 2007). To date, substantial scientific and economic efforts have been undertaken to restore these ecologically important ecosystems (e.g. Van Katwijk et al., 2009, 2016). More optimistic findings were recently reported from different studies which estimated the distribution of seagrasses worldwide than is currently mapped (Jayathilake et al., 2018). On this basis, we created a more complete map of Irish seagrass distribution by implementing different strategies with a total mapped area of 180 km^2, of which 34.6 km^2 were newly discovered during this study. Firstly, we compiled all the spatial seagrass information available in Ireland of maps from 1997 to 2011. For this purpose, we used a set of three different sources, including conservational organizations and Irish environmental state institutions, containing more than 40 maps of different marine primary producer species such as kelps or maerl (Section 5.2.2). This suggested that previous efforts were not consciously coordinated which may explain the difficulties in sourcing detailed and complete maps of seagrasses in Ireland.

The second part of the project consisted of obtaining the maximum amount of spatial information possible where seagrasses may potentially be distributed by coupling data provided by (i) the collaborative data collection and (ii) and the SDM. These results proved to be extremely beneficial as we acquired a large quantity of new potential seagrass locations. The computed model pointed out a high discriminatory ability with AUC values of 0.993, indicating a great capacity to predict suitable areas for seagrasses at a regional scale in Ireland. Our SDM reported a potentially larger habitat suitability for marine plants than is currently mapped, suggesting a broader distribution of seagrasses on the coast of Ireland. Fetch, which is defined as the length of sea surface over which a given wind has blown, represents the most important factor explaining seagrass presence distribution (39.5 %). Hydrodynamic forces, such as, fetch or wave exposure are key physical processes which define the presence or absence of seagrasses and also influence their spatial heterogeneity

(Fonseca et al., 1998). This could explain the relative absence of records of seagrass on the Irish east coast which is characterized by straighter coastlines more exposed to physical forces. More suitable conditions for seagrasses were observed on the west coast of Ireland, which is defined by the presence of bays, estuaries or inlets, which protect the seagrasses from physical stressors. Interestingly, the model forecasted a high probability of finding seagrasses in Kenmare Bay (Co. Kerry), Bantry Bay (Co. Cork) and Abbey Estuary (Co. Limerick) which are areas characterized by the absence or scarcity of seagrass records in the available literature (NPWS, (https://www.npws.ie)). For instance, there were no previous records of marine plants in the Abbey Estuary. Two main causes may explain this absence (i) the presence of sediment runoffs in the water column which may reduce the water clarity, thus hampering the seagrass photosynthesis and limiting their existence, and (ii) the presence of freshwater which may drastically reduce the sea water salinity thus affecting the seagrass biological functions. This condition may be less relevant for seagrasses as they can tolerate and survive in low salinities; for instance, we confirmed a small *Z. marina* meadow inside of a small river in Kenmare Bay. Optimal salinity conditions were observed at 25 ppt, while there are even *Z. marina* populations living at 6 ppt on Finnish coasts (Bostrom et al., 1997). Bathymetry was the second most important variable explaining the potential habitat distribution of seagrasses (35.7 %). This result was expected as light energy controls the photosynthetic processes, productivity, growth and population structure, thus defining the vertical seagrass distribution limits. In Ireland, seagrasses are found at an average depth of 2.6 m, and are observed up to depths of 12.2 m, which is in line with other *Z. marina* meadows at similar latitudes (Olesen et al., 2018). Other studies applying SDM also observed that bathymetry ranked among the most important descriptors explaining seagrass presence (Downie et al., 2013).

The volunteer participation initiative implemented here to obtain spatial information on seagrasses in Ireland was adopted from the "GIS-Based Community Participation Project to Assess Portuguese kelp Conservation Status" (Asis et al., 2009). Over the last number of years, with the application of new technologies, such as, GPS, mobile apps or open GIS software, allow the creation of participative mapping projects, such as, SeagrassSpotter (https://seagrassspotter.org). These collaborative projects

involving citizens and volunteers are highly valuable to disseminate to the public the importance of marine habitats. Globally, concerning seagrass research, a lack of communication of the ecological services and benefits that seagrass communities provide was recently reported (Nordlund et al., 2017). Thus, the existence of research studies involving local communities may represent an effective way to communicate the value of these precious ecosystems. This project highlights the success and importance of enrolling part of the society in research activities, similar to conclusions found by Asis et al. (2009) and Evans (2003).

Based on the map of habitat suitability obtained with MAXENT, we validated a total of 206 locations with a success of 83.4 %. These new areas included 26.8 km^2 of seagrass meadows which represent 18.4 % of the current documented seagrass distribution in Ireland. Regarding the volunteer project, the participants reported 107 seagrass locations, mainly observed in western Ireland. In total, we evaluated 32 sites, in 30 of which we found seagrass covering an extent of 7.8 km^2, with *Z. marina* being the most represented species. Most of these locations were also forecasted by the SDM, pointing out the robustness of the model prediction. In total we mapped an area of 34.6 km^2 which represents an addition 24 % of the current reported distribution.

In this project, we identified potential new sites where seagrass may occur, although, these sites remain unsurveyed to this point. This can be explained by the inaccessibility of the potential meadows, but also, the climatic conditions and the hydrodynamic forces on the western coast of Ireland which made checking the presence of seagrasses highly challenging. Additionally, the presence of wild animals such as dolphins and seals in sites of relevance sometimes restricted the access. Due to these handicaps, we hypothesise that some vast seagrass areas may still remain under surveyed to date.

Interestingly, we found that in 11 locations seagrass was distributed in association with anthropogenic structures such as harbours or seaports. Such marine human constructions may change the physio-chemical conditions and sedimentological characteristic of the habitats locally, thus improving the conditions for seagrasses settlement or other engineering species. Seagrass may play an important role

providing different ecological services to these areas such as coastal protection, sediment and nutrient trapping or new habitats for marine life. The application of SDM may be useful to predict potential areas of habitat suitability where seagrasses may be introduced as a recent addition to the marine flora.

Finally, the results from this study represents an important insight to demonstrate that seagrass distribution may be substantially larger that is already reported in the coast of Ireland. These findings highlight a necessity to develop adequate conservative policies, even in the particular in view of the current scenario of seagrass habitat destruction globally, which is expected to continue over the coming years.

Chapter 6

Responses of Irish Zostera marina populations to experimental warming scenarios

6.1. Introduction

Seagrasses play a key role in the coastal zone in terms of high productivity, stimulation of biodiversity, protection of the coastline, carbon storage and nutrient retention (Hemminga & Duarte 2000; Carruthers et al., 2007). *Zostera marina* is currently distributed from subtropical regions of Mediterranean Sea (Setchell et al., 1929) to the Arctic. As a consequence of this broad distribution, the ecology of eelgrass has received considerable research attention (Duarte 1999). Water temperature is generally considered a key factor affecting the metabolism, biochemical composition and growth of marine plants including eelgrass *Z. marina* (Olesen & Sand-Jensen 1993). Optimal temperatures for growth for eelgrass populations were observed to be between 13 °C to 24 °C, suggesting that this species can readjust its growth rates to *in-situ* temperatures (Lee et al., 2007). Irradiance is the most relevant environmental parameter affecting the vertical distribution of seagrasses, and thereby, their photosynthesis and productivity (Ralph et al., 2007).

Over the last decades, seagrass distribution has been reduced by around 30 %, and particularly *Z. marina* is disappearing at a rate of 1.3 % per year (Waycott et al., 2009). The most likely factors explaining these dramatic losses are direct anthropogenic causes and climate change effects (Orth et al., 2006). Their proximity to the coastline make seagrasses particularly susceptible to human-induced damage (Den Hartog, 1987). Reduction in water clarity, and quality, and mechanical disturbances are reported to drive the main losses of seagrass habitats (Waycott et al., 2009). The present-day greenhouse-induced global warming is occurring at almost twice faster than in the Last Glacial Maximum (Intergovernmental Panel on Climate Change (IPCC, 2014)). The current and projected increases in temperature (IPCC, 2014) are expected to affect terrestrial and marine organisms globally, and sessile species in particular (Poloczanska et al., 2013). Eelgrasses mainly inhabit sheltered and shallow areas which are more responsive to rapid changes in temperature such as daily environmental fluctuations or heat waves (Marba et al., 2010). In the context of climate change, with southern eelgrass populations experiencing die-backs due to anomalously high temperatures (Durako & Moffler 1987; Reusch et al., 2005), and Arctic-distributed populations experiencing

more favourable environmental conditions (Krause-Jensen et al., 2015), the response of centrally distributed populations remains unclear (Beca-Carretero et al., 2018*a*).

Essential fatty acid (EFA) (i.e. α-linolenic acid (ALA) and linoleic acid (LA)) have a relevant role in critical physiological processes and healthy state of consumers within marine food webs, from survival, growth, pigmentation and metamorphosis to favour optimal neural, hormonal, reproductive or visual functions (Sargent et al., 1999; Tocher, 2003). Physiological and biochemical responses of primary producers to climate regimes have been demonstrated to play a key role in the production of these nutritional important compounds (Sanina et al., 2008; Viso et al., 1993). The adjustment of key biochemical components, such as photosynthetic pigments and FA, has been proposed to be a seagrass mechanism to cope with different environmental stresses, including temperature and irradiance (e.g. Beca-Carretero et al., 2018*a* (Chapter 7); Beca-Carretero et al., under review (Chapter 8)). For instance, both *Posidonia oceanica* and *Cymodocea nodosa* depicted reductions in polyunsaturated fatty acids (PUFA) along with a marked increase in saturated fatty acids (SFA) as a response to increments in temperature. Also, *Halophila stipulacea* showed increases in PUFA in plants adapted to low light conditions versus shallower ones receiving higher light energy, which correlates with increases in photosynthetic pigments.

Understanding the eco-physiological responses of these engineering species to future scenarios of climate change is a key step towards detecting early signs of stress and vulnerability. Laboratory-controlled experiments allow the creation of the environmental conditions that may occur in the projected climate scenario. In contrast to natural conditions where several factors influence organism responses, laboratory experiments allow a better control of specific ambient factor such as temperature, irradiance or nutrient levels. Several studies have been applied microcosm-based approaches to understand the thermal tolerance and resilience of eelgrass populations from different latitudinal regions exposed to projected increased in water temperatures (i.e. Winters et al., 2013; Abe et al., 2008). The results allowed the elucidation of optimal temperatures for growth and productivity and also helped to develop early warning morphological and physiological stress indicators, which later may be applied in natural habitats.

The aims of this study were to (i) highlight the optimal temperature for growth of Irish eelgrass populations exposed to saturated irradiance conditions versus irradiance-limited conditions, (ii) to identify different responses among both irradiance treatments, (iii) to assess biochemical responses in fatty acids and photosynthetic pigments after exposure to different temperature treatments (7 – 24 °C), and finally, (iv) to elucidate the potential impact of future warming in the nutritional value of eelgrass leaves from Irish populations.

6.2 Materials and Methods

6.2.1. Plant collection

Shoots of *Zostera marina* L. were harvested in an eelgrass meadow situated in western Ireland at Finavarra (FV) (53°8'50" N, -9°07'43"W), southern Galway Bay, which is classified as a Special Area of Conservation (SAC) (NPWS, 2014). For more information of the *Z. marina* meadow of Finavarra see Beca-Carretero et al., *under review* (Chapter 2). Apical shoots were manually harvested by SCUBA diving or snorkeling at a depth of 2-2.5 meters at intervals of 5-10 meters to prevent resampling of the same genotypes. We carefully selected shoots with similar weights (2.7 ± 0.6 g DW shoot^{-1}) and size (37.3 ± 8.3 cm shoot^{-1}). The shoots were transferred to cooling tanks filled with seawater and directly transported to the laboratory in the Ryan Institute building (NUI Galway). The collected samples were carefully cleaned, and all sediment and epiphytic organisms were removed.

6.2.2. Experimental set-up

To carry out the experimental exposure, the collected plants were pre-acclimated to laboratory conditions by storing them in seawater (~25 PSU) at 16 °C (*in-situ* water temperature when at time of collection) for 3 days, at an irradiance of 60 µmol photons m^{-2} s^{-1} in a light (L): dark (D) cycle of 16 h: 8 h (Beca-Carretero et al., 2018). Prior to the start of the experiment, plants were progressively acclimated from 16 °C to the

target temperature by increasing or reducing by 1 °C every 24 h. Once the eelgrass shoots were acclimated to the target temperatures (7, 12, 16, 20, 24 °C) and before starting the experiment, all individual shoots were characterized. We measured: shoot fresh weight (g FW), total length (cm shoot^{-1}) and number of leaves (shoot^{-1}). We adjusted all the shoots to have 2 rhizome segments and 3-4 leaves. To measure growth rates, we performed the punching technique which consisted of piercing two holes, one above the other by a distance of 2 mm at the top of the leaf sheath in the basal meristem (Chapter 1, section 1.3), with a hypodermic needle (Sand-Jensen 1975; Short *&* Duarte 2001). We haphazardly selected a group of 10 eelgrass shoots to assess the ratio of initial fresh weight (FW): dry weight (DW).

For each temperature treatment, 3 cylinders (n = 3) containing three shoots each were incubated under two irradiance conditions of 180 µmol m^{-2} s^{-1} and 60 µmol m^{-2} s^{-1}. The three shoots were incubated in a cylindrical Perspex bottle (ID 10 cm, height 30 cm) with a volume of 3 L. We fixed the shoots to the bottom of the bottles with plastic nets to maintain them in a vertical orientation. We changed the seawater every 5 days (3 times during the experiment) to ensure optimal nutrient conditions. To prevent evaporation, we topped-up all the cylinders each day with distilled water. Saturating irradiance of 180µmol photons m^{-2} s^{-1}; (Olesen *&* Sand-Jensen 1993) below the water surface was provided by Lumilux cool daylight fluorescent lamps (OSRAM L18W/865, Germany) in a 16:8 h light/ dark cycle. We continuously recorded the temperature using HOBO loggers (UA-002-64, Onset) installed in one cylinder per temperature and irradiance treatment. After 15-16 days we measured a set of response parameters (see following).

6.2.3. Morphological descriptors

Firstly, we determined the relative growth rate (RGR, d^{-1}) according to:

$$RGR = -\ Ln(\frac{Bf}{Bi})/t, \qquad \text{(Eq. 6.1)}$$

where B_i is the initial weight and B_f the final weight of the shoot and *t* is the incubation period in days. We quantified the leaf formation rate (leaves shoot^{-1} d^{-1}) identifying those leaves without punched holes and then divided the number of new leaves by the

incubation time (days). Leaf elongation rate (cm shoot^{-1} d^{-1}) was calculated as the length of new leaf material produced during the incubation time (days), measured (i) from the base of the meristem to the punched holes, and (ii) the length of the newly produced leaves (Short & Duarte 2001). Moreover, we evaluated the shoot mortality rate by the following equation:

$$\text{Mortality rate} = -\text{Ln}(\frac{\text{Nf}}{\text{Ni}})/\text{t} \qquad \text{(Eq. 6.2)}$$

Nf, is final shoot population, Ni is the initial shoot population and *t* is the duration of the experiment (in days). Finally, we calculated the necrotic area in the second youngest leaf as the percentage of the total leaf surface area.

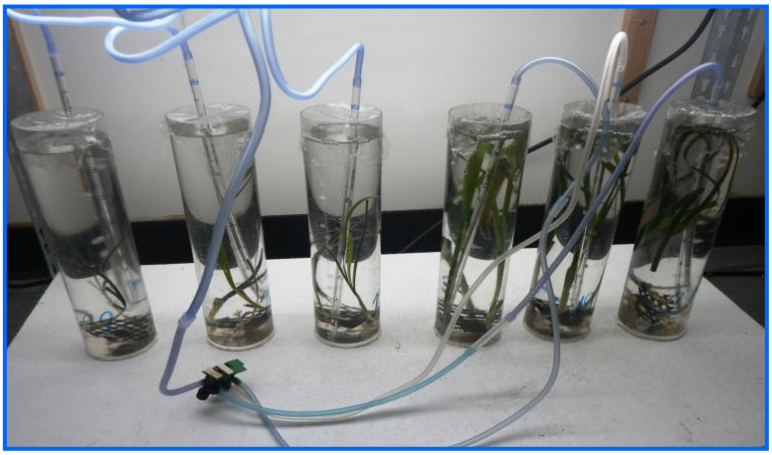

Fig. 6.1 Image of the experimental design of one temperature treatment with shoots from the Irish population of *Zostera marina*. Each cylinder (n = 3) contained 3 shoots and had a volume of 3 L and a height of 35 cm.

6.2.4. Biochemical responses

For performing biochemical analyses, we collected the healthy (avoiding epiphytes or damaged parts) parts of the youngest and second youngest leaves after 14-15 days of incubation under different temperature and irradiances conditions. The selected leaves were cleaned in distilled water and epiphytes were removed if regrow. Fatty acid and pigment content and composition were obtained following the protocol described in Chapter 3, Section 3.2.

6.2.5. Statistics

Differences among temperature and irradiance treatments were analyzed using 2-way Analysis of Variance and Tukey's pairwise test. Data were transformed into Ln and were later checked for homogeneity of variance using Bartlett´s test and for normality. To test potentially different plastic responses regarding morphometric and biochemical variations, we compared the coefficient of variation (CV) between plants incubated at high and low irradiances, which estimates the dispersion of the biological descriptors across the temperature gradients (7, 12, 16, 20, 24 °C). All data treatments and statistical analyses were performed using the IBM SPSS Inc., v.11. Growth rates values are reported as means and standard error (SE); FAs and pigments results are reported as means and standard deviation.

6.3. Results

6.3.1. Morphological descriptors

Overall, *Z. marina* shoots incubated at both high and low irradiances did not show a significant difference in the leaf elongation rates and leaf formation rates during the temperature experiment, whereas, there were significant differences in the relative growth rates (Fig. 6.2, panel A-C) (ANOVA, $p < 0.05$). However, temperature had a significant effect on the morphological descriptors studied in shoots exposed to both light treatments. Optimal temperatures for growth at both light intensities were observed at 16 – 20 °C, and the lowest growth rates were observed at the coolest (6 °C) and warmest (24 °C) conditions (Fig. 6.2). For example, the leaf elongation in plants incubated at saturated irradiance conditions increased steadily from 7 °C (0.0001 ± 0.0001 leaves d^{-1}) until optimum temperature for growth at 20 °C (0.025 ± 0.004 leaves d^{-1}) and later dropped markedly to (0.003 ± 0.001 leaves d^{-1}) at 24 °C (Fig. 6.2, panel C). Particularly, at 6 °C there were no significant differences in the measured growth rates in shoots incubated between irradiances. However, after the optimal temperature for growth (16 - 20 °C), plants incubated at low irradiances displayed a significantly lower

(~70 %) RGR and only ~25 % of leaf elongation rate than shoots acclimated at saturated light conditions.

Table 6.1. Temperature projections for Galway, Ireland. Values are obtained from the Community Climate System Model (CCSM) (Collins et al., 2006).

	Current	SST (°C)	Difference Present (°C)	
	Annual	Summer	Annual	Summer
Present	11.1	15.6	-	-
rpc 26	12.2	16.7	1.1	1.1
rpc 45	12.3	16.8	1.2	1.2
rpc 60	12.6	17.2	1.5	1.6
rpc 85	13.2	18.1	2.1	2.5

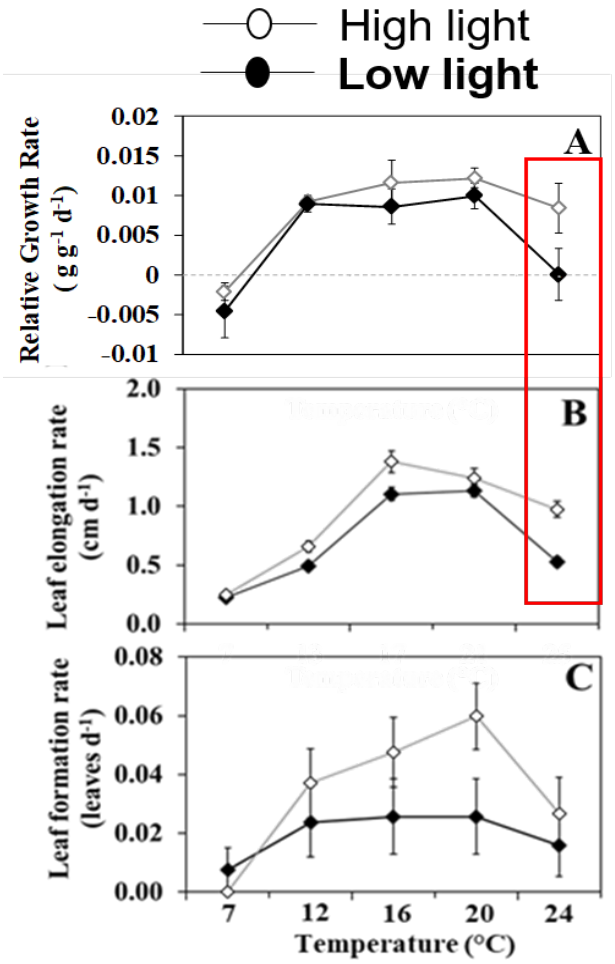

Fig. 6.2 Growth responses of *Zostera marina* shoots incubated under high light (180 µmol photons m^{-2} s^{-1}) and low light (60 µmol photons m^{-2} s^{-1}) to a gradient of temperatures (7, 12, 16, 20, 24°C). All values are reported as means and standard error (SE).

6.3.2. Biochemical descriptors

TFA in plants incubated at different irradiance intensities did not display significant differences; high-light 1.16 ± 0.1 and low-light 1.2 ± 0.1 % of DW. On the other hand, the temperature generated a marked effect in the accumulation of TFA in both light treatments (Table 6.1, Fig. 6.3) (ANOVA, $p < 0.05$). At both irradiance levels, higher levels of TFA were observed at 12 and 16 °C, followed by 20 °C and significantly lower at 7 and 24 °C. Interestingly, plants incubated at high and low light displayed different biochemical profiles. For instance, plants exposed to 180 μmol photons m^{-2} s^{-1} maintained a more homogenous content of TFA, with a lower coefficient of variation (CV) of 7.5 % across the temperature experiment. Plants incubated at 60 μmol photons m^{-2} s^{-1} reported more TFA differences in the temperature experiment with a higher CV of 13.3 %. At optimal temperatures for TFA production (12 and 16 °C), plants at low irradiances (average 1.18 ± 0.02 % DW) accumulated slightly, but markedly, higher contents than plants at higher light levels (1.32 ± 0.02 % DW) (Table 6.1, Fig. 6.2) (ANOVA, $p < 0.01$). By contrast, the opposite trend was observed at the more extreme temperatures (7 and 24 °C) at both irradiance levels where the plants incubated under light limited conditions accumulated markedly fewer TFAs (Table 6.2; Fig. 6.3).

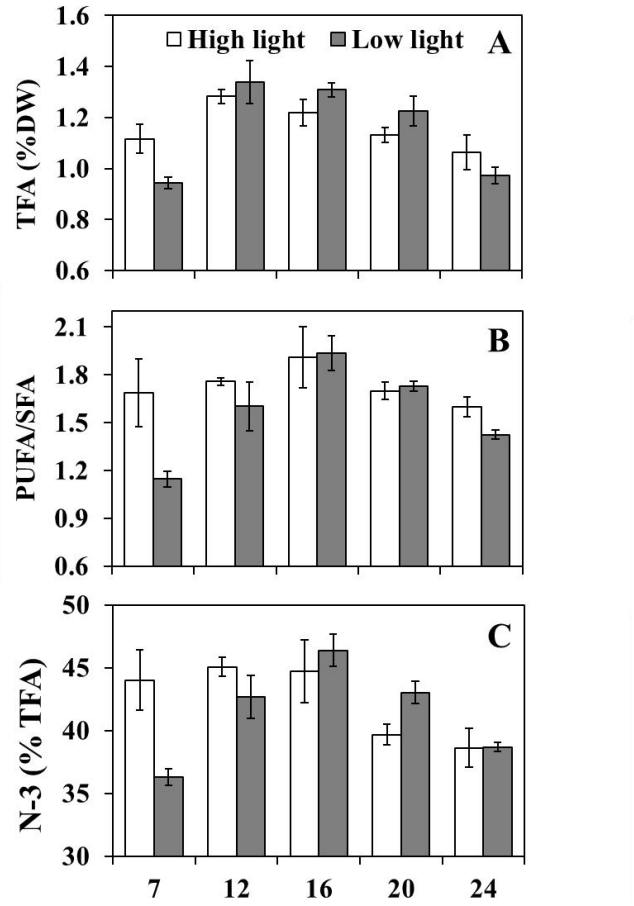

Fig. 6.3 TFA (Panel A), PUFA/SFA ratio (Panel B) and n-3 (% of TFA) (Panel C) responses of *Zostera marina* shoots incubated under high light (180 µmol photons m^{-2} s^{-1}) and low (60 µmol photons m^{-2} s^{-1}) and a gradient of temperature temperatures (7, 12, 16, 20, 24°C). Results are expressed as mean ± SD (n = 5–6).

There were also no marked differences in the PUFA, SFA, PUFA/SFA ratios and n-3 PUFA (% of TFA) levels between plants incubated at different irradiances (Table 6.1; Fig. 6.3 B-C). In both light treatments, temperature levels significantly affected the proportions of different FA groups, such as, SFA, PUFA and MUFA (Fig. 6.4) (ANOVA, $p < 0.01$). Plants incubated at 60 µmol photons m^{-2} s^{-1}displayed a larger CV than plants incubated at irradiance conditions imitating shallow conditions (Table 6.1). These differences were mainly related to higher levels of PUFA and n-3 PUFA (% of TFA) observed under optimal conditions for accumulation of these compounds (16 – 20 °C) in shoots exposed to low light regimes. On the other hand, the opposite pattern was observed in less favourable temperatures for FA production (6, 12 and 24 °C).

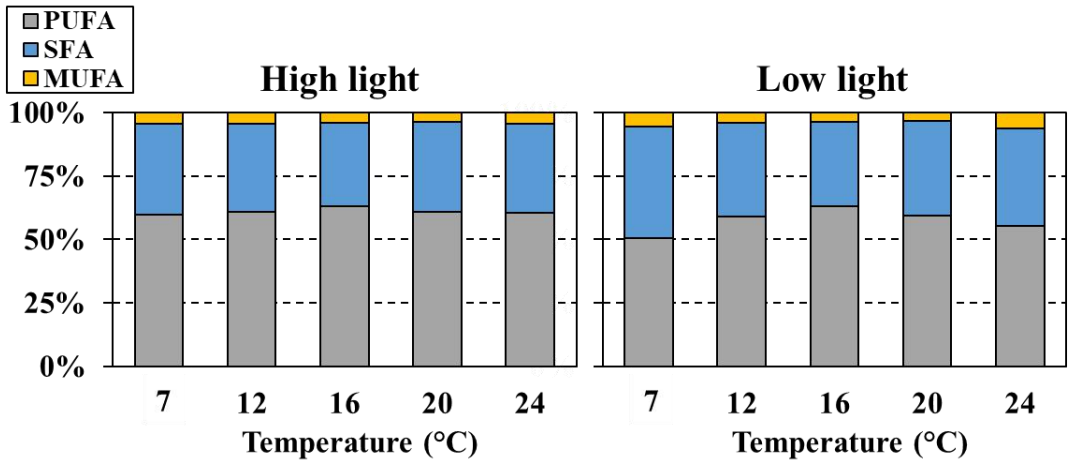

Fig. 6.4 PUFA, SFA and MUFA responses of *Zostera marina* shoots incubated to high light (180 µmol photons m^{-2} s^{-1}) (panel A) and low light (60 µmol photons m^{-2} s^{-1}) (panel B) conditions exposed to a gradient of temperatures (7, 12, 16, 20, 24 °C). Results are expressed as mean ± SD (n = 5–6).

The accumulation of photosynthetic pigments showed marked differences between irradiance treatments, with higher values found in shoots incubated at 60 µmol photons m^{-2} s^{-1} (6.0 ± 0.3 mg g^{-1} DW) than those at 180 µmol photons m^{-2} s^{-1} (5.3 ± 0.3 mg g^{-1} DW) (Fig. 6.5) (ANOVA, $p < 0.05$). The accumulation of pigments varied significantly across the temperature experiment in both irradiance conditions (ANOVA, $p < 0.05$). In both light conditions, significantly higher pigments were found a 20 °C (ANOVA, $p < 0.05$). On the contrary, significantly lower values at high light treatment were reached at 6 °C, while at low irradiances lower contents of pigment were observed at 24 °C (Fig. 6.5) (ANOVA, $p < 0.05$).

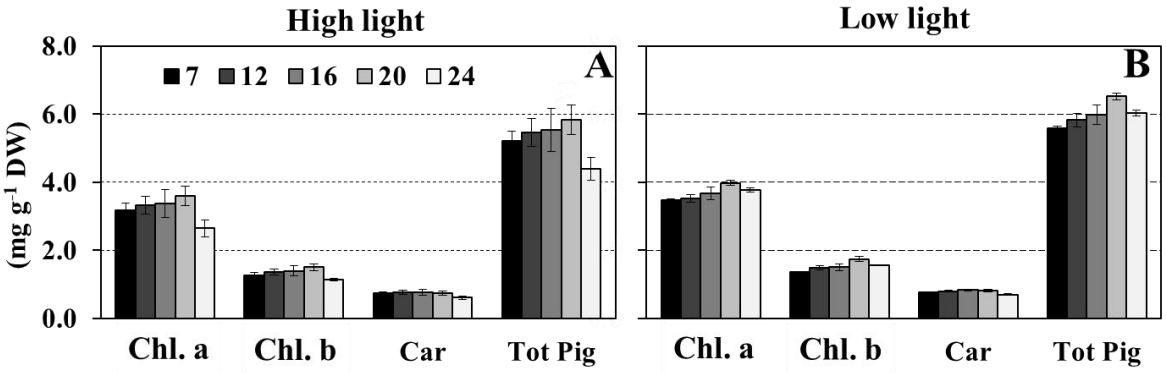

Fig. 6.5 Photosynthetic pigments of leaves of *Zostera marina* plants incubated to high light (180 µmol photons m^{-2} s^{-1}) (Panel A) and low light (60 µmol photons m^{-2} s^{-1}) (Panel B) conditions exposed to a gradient of temperatures (7, 12, 16, 20, 24 °C). Results are expressed as mean ± SD (n = 5–6).

Table. 6.2 Total fatty acid content (% DW) and composition; saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) (% TFA) of *Zostera marina* plants incubated to high light (180 µmol photons m^{-2} s^{-1}) and low light (60 µmol photons m^{-2} s^{-1}) conditions exposed to a gradient of temperatures (7, 12, 16, 20, 24°C). Results are expressed as mean (n = 5–6).

Irradiance treatment	**High**	**Light**					**Low**	**Light**				
Temperature	**7**	**12**	**16**	**20**	**24**	**CV**	**7**	**12**	**16**	**20**	**24**	**CV**
Fatty acids (% DW)	**1.1**	**1.3**	**1.2**	**1.1**	**1.1**	**7.5**	**1.0**	**1.3**	**1.3**	**1.2**	**1.0**	**13.3**
Saturated fatty acids												
14:0	0.4	0.6	0.3	0.3	0.5		0.6	0.6	0.4	1.0	0.4	
16:0	30.7	29.9	28.3	32.0	30.0		37.7	31.5	28.0	29.9	32.0	
18:0	1.7	1.8	1.8	1.8	2.4		2.2	1.7	1.8	2.0	2.9	
23:0	0.7	0.5	0.7	0.2	0.3		0.2	0.7	0.7	0.5	0.6	
Sum of SFA	**33.5**	**32.8**	**31.1**	**34.3**	**33.2**	**3.6**	**40.7**	**34.5**	**30.9**	**33.4**	**35.9**	**10.3**
Monounsaturated fatty acids												
14:1	1.4	1.2	0.9	1.0	1.1		1.9	1.2	1.0	1.4	2.0	
16:1 n-7	0.4	0.6	0.5	0.5	0.6		0.4	0.5	0.5	0.5	0.6	
18:1 n-7	0.7	0.6	0.4	0.3	0.2		1.0	0.6	0.5	0.3	0.6	
18:1 n-9	1.6	1.8	1.6	1.3	2.4		1.7	1.5	1.6	1.4	2.5	
Sum of MUFA	**4.1**	**4.2**	**3.4**	**3.1**	**4.3**	**12.1**	**5.0**	**3.8**	**3.6**	**3.6**	**5.7**	**13.4**
Polyunsaturated fatty acids												
16:2 n-3	0.60	0.3	0.5	0.2	0.4		0.7	0.4	0.4	0.2	0.3	
16:3 n-3	4.20	4.5	4.2	3.0	2.7		3.3	4.3	3.1	3.4	3.0	
16:3 n-4	2.10	1.8	1.4	1.2	1.6		2.9	1.8	2.2	2.0	1.4	
18:2 n-6	12.10	12.5	13.0	16.4	18.7		10.1	12.4	15.2	14.4	12.2	
18:3 n-3	32.40	34.6	37.4	34.0	31.0		26.0	32.6	36.3	34.2	31.5	
20:5 n-3	4.00	3.5	3.1	3.2	2.2		4.3	3.6	2.9	3.1	3.5	
22:5 n-3	2.80	2.1	1.2	1.1	2.2		2.0	1.8	2.4	2.1	1.5	
Sum of PUFA	**58.20**	**59.3**	**60.8**	**59.1**	**58.8**	**1.6**	**49.3**	**56.9**	**62.5**	**59.4**	**53.4**	**8.7**
Others	4.20	3.7	4.7	3.5	3.7		5.0	4.8	3.0	3.6	5.0	
PUFA/SFA	**1.7**	**1.8**	**2.0**	**1.7**	**1.8**	**5.1**	**1.2**	**1.6**	**2.0**	**1.8**	**1.5**	**18.1**
n3/n6	**3.6**	**3.6**	**3.6**	**2.5**	**2.1**	**15.1**	**3.6**	**3.4**	**3.0**	**3.0**	**3.3**	**8.5**
% n3 (TFA)	**44.0**	**45.0**	**46.4**	**41.5**	**38.5**	**4.2**	**36.3**	**42.7**	**45.1**	**43.0**	**39.8**	**8.0**

6.4. Discussion

6.4.1. Growth responses to temperature gradients

Irish eelgrass populations exhibited similar experimental optimal temperatures (16 – 20 °C) for growth to those of other *Z. marina* populations adapted to similar *in-situ* temperature conditions. For example, populations from Denmark (56 °N), Oregon (USA, 44 °N) or Japan (35 °N) exhibited optimal RGR at18 – 20 °C (Beca-Carretero et al., 2018; Höffle et al., 2011; Abe et al., 2008). A previous review of *Z. marina* pointed out that local optimal growth temperatures for temperate eelgrasses across its latitudinal range varied between 15 °C and 20 °C (Lee et al., 2007). At temperatures higher than 20 to 25 °C biomass productivity depletions were observed, which agrees with our observations (Lee et al., 2007). Differences in the optimum growth temperatures may be explained by the adaptation of seagrasses to *in-situ* local thermal conditions. Recent latitudinal laboratory experiments observed that southern distributed *Z. marina* populations can healthily better acclimate to higher experimental temperatures than northern plants (Bergmann et al., 2010; Winters et al., 2011). On the other hand, in our study, the lowest values were reached after incubation at 7 and 24 °C. Plants exposed to 7 °C, which is a similar temperature to winter season in western Ireland, appeared to grow healthily, but this was accompanied by a slow leaf turnover, probably induced by decreases in plant metabolism in response to low temperatures. In temperate regions, *Z. marina* productivity is strongly controlled by seasons, with low productivity observed in winter due to lower temperatures and daily irradiance (Lee et al., 2007). By contrast, at 24 °C the reported low growth rates were potentially related to thermal stress conditions as Irish plants incubated at 24 °C showed signs of physiological stress, with several plants affected by leaf necrosis and also with high mortality rates of 66 %. Previous controlled experiments correlated increases in respiration, growth inhibition and high mortality rates with anomalously high temperatures (Marsh et al., 1986). In this context, field observation in temperate eelgrass systems reported several shoot mortalities after water temperatures exceeding 25 – 28 °C (Greve et al., 2003; Pedersen et al., 2014). However, in Ireland there are no records of any die-

backs of *Z. marina* populations due to anomalous warming conditions. Currently, Irish eelgrass populations are living at *in-situ* summer averages SST of 16 - 17 °C (Chapter 2, Section 2.3.2), thus expected warming of 2-3 °C (IPCC, 2014) may generate increases in plant biomass productivity of 22.5 % and a potential habitat expansion by the end of this century.

This study was conducted over 14-15 days; thus, potential diverse effects of high temperature in the growth and physiological responses may expected with increases in the incubation time. Previous studies from different regions worldwide with plants incubated between 44 and 60 days observed that optimal temperatures for growth were between 10 °C and 20 °C, while the experiments over 2 to 4 days revealed optimum temperatures for growth between 24 °C to 35 °C (Biebl & McRoy 1971; Staehr & Borum 2011). These differences may be explained by longer exposure periods which may induce a consumption in of energetic reserve such as carbohydrates or fatty acids which, in turns, may negatively affect the performance of seagrasses under stress conditions (Lee et al., 2007). Consequently, the length of the incubated period under high (stress) temperature conditions is a critical factor for the observed tolerance ranges and temperature optima. Other factors related to the experimental setup, such as, nutrient content, salinity or shoot density may affect photosynthetic responses, hence influencing the growth responses. Given these circumstances, it may be interesting to standardise future experimental designs to make the results obtained more comparable.

6.4.2. Effect of light reduction in Irish eelgrass growth

Surprisingly, growth results revealed that plants incubated at low irradiance conditions 60 μmol photons m^{-2} s^{-1} had a similar optimum temperature for growth and similar growth rates to plants incubated at 180 photons m^{-2} s^{-1}. Moreover, plants incubated at low light levels did not display significant differences across temperatures from 7 °C to those optimal for growth (16 - 20 °C) when compared to different irradiance regimes. These results were unexpected and indicated that a potential acclimatizing mechanism may compensate for the lower light energy

received by plants when incubated at 60 μmol photons m^{-2} s^{-1}. Such a mechanism may be related to the use of reserve energy compounds. For instance, the use of carbohydrates allocated in the rhizome was reported to be a strategy of deep-adapted seagrass populations in less favourable environmental conditions (Ralph et al., 2007). Previous studies with *Z. marina* indicated that optimum temperatures for growth of plants receiving less irradiance to be 5 to 15 °C lower than of plants adapted to higher irradiance levels (Bulthuis 1987; Masini et al., 1995). Remarkably, after the optimal temperature (20 °C) for growth, plants incubated at 60 μmol photons m^{-2} s^{-1} showed a significantly higher reduction in growth rates compared with plants receiving higher light energy (180 μmol photons m^{-2} s^{-1}). This may suggest that (i) populations living at greater depths may be more vulnerable to expected warming, and also (ii) southern adapted populations living close to their thermal limits may be more susceptible than northern eelgrass populations to increases in temperature and future sea level rises. In this regard, depletion in water quality due to anthropogenic pressures, such as runoff of sediments or water eutrophication may compromise the survival of plants living in the deepest edge of the meadow (Krause-Jensen et al., 2005). Recent observations found that extreme summer heat waves provoked a great mortality in deep populations of *Posidonia oceanica* which were not able to recover during the following years, whereas shallow populations were less affected, and were able to recover fully (Marba et al., 2010).

6.4.3. Biochemical adjustments to temperature and irradiance conditions

Over the last number of years, most of the research studies have targeted temperature and irradiance impacts on *Z. marina* responses to assess the potential effects of climate change, while only a few studies have focussed on studying the role of these stresses in the nutritional value of seagrasses (e.g. Hernan et al., 2017). Here, for the first time, we addressed how projected scenarios of warming may affect the composition of essential fatty acids (EFA) in eelgrass leaves.

Optimum temperatures for TFA, and particularly n-3 PUFA accumulation, in eelgrass leaves were observed at 12 – 16 °C; these were 5-10 °C lower than optimum temperatures for growth at both irradiance conditions. Field observation of Irish *Z. marina* populations reported similar patterns with higher levels of TFA and PUFA observed in April and lower in July 2016, which depicted higher growth rates (Chapter 3). The larger production of these key compounds may be associated with the necessity of the plants to promote higher unsaturation levels in the membrane of the thylakoids under colder conditions (12 – 16 °C). Moreover, at 20 or 24 °C higher levels of SFA and the lowest PUFA/SFA ratios were observed. Increases in SFA at the optimal growth temperature were previously related as a mechanism to store the excess of energy produced during the photosynthesis in seagrasses and other primary producers (e.g. Pal et al., 2011). SFA are mainly partitioned into triacylglycerols (TAGs), which are stored as energy reserves (e.g. Bravo et al., 2001; Solovchenko 2012). Furthermore, plants receiving lower light energy increased their levels of TFA, PUFA and n-3 PUFA significantly in optimal temperatures for growth (16 – 20 °C). In particular, PUFAs promote the fluidity of the thylakoid membranes of the chloroplasts, enhancing the electron transport in the photosystems and, therefore, optimising the photosynthetic activity (Gombos et al., 1994; Sanina et al., 2004, 2008). Previous experimental studies in culture conditions with microalgae revealed increases in the TFA and PUFA contents in response to changing light regimes (Guihéneuf & Stengel 2013). Taken together, these studies suggest that the regulation of the PUFA content in photosynthetic structures is an essential mechanism of primary producers to ensure their optimal physiological functioning under different irradiance gradients. Thus, expected global warming may favour Irish seagrass growth and dispersion, however, these changes may be accompanied by reductions in the production of n-3 PUFA levels, and increases in SFA in the eelgrass leaves. As *Z. marina* represents an important food source for different marine species (Thormar et al., 2016), changes in their nutritional value may have an important effect on consumers and the next trophic levels. Prior studies indicated that changes in temperature and acidification expected to increase the metabolic demands of herbivores (Burnell et al., 2013) and thus affect their nutritional preferences (Hernán et al., 2016; Pagès et al., 2017).

Interestingly, this research identified the laboratory-controlled conditions driving the responses of specific fatty acid such as n-3 PUFA or SFA (16:0), thus regulating the lipid nutritional value of eelgrass leaves. Is noteworthy to highlight that other nutritional components of the seagrass leaves, such as carbohydrates, phenols or vitamins have different synthetic pathways and responses. These outcomes can be applied incontrolled herbivorous experiments to understand the potential effects of diets withreduced PUFA content, imitating future warming conditions. However, results obtained from culture or experiments under lab-controlled conditions cannot bedirectly related or extrapolated to field situations in natural habitats (De Boeck et al.,2010). Findings observed from mesocosms experiment may represent short time effects such as marine heat waves which are characterized by reduced duration with ahigher intensity and evolution and are easier reproduced in controlled conditions (Hobday et al., 2016). The, this experiment conducted here (14 – 15 days) can beused as a proxy to understand the impact that anomalously increased temperature may generate in Irish seagrass communities. Finally, we propose that future research efforts should address the effect of simulating climate change on the life stages of different seagrass consumers, such as, growth, survival or reproduction.

Chapter 7

Effects of an experimental heat wave on fatty acid composition in two Mediterranean seagrass species

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Author contribution

Juan M. Ruiz developed the experimental design; Lázaro Marín-Guirao, Jaime Bernardeau-Esteller and Rocío García-Muñoz conducted the experiment in Murcia (IEO, Spain); Pedro Beca-Carretero and Freddy Guihéneuf carried out the fatty acid analysis and prepared a first draft of the manuscript; Pedro Beca-Carretero performed the statistical analysis; Dagmar B. Stengel and Juan M. Ruiz provided resources and funding for fatty acid analysis; Dagmar B. Stengel and Juan M. Ruiz contributed to the interpretation of the data and the writing of the manuscript; All co-authors commented on and provided edits to finalize the original manuscript.

Abstract from original article

Global warming is emerging as one of the most critical threats to terrestrial and marine species worldwide. This study assessed the effects of simulated warming events in culture on two seagrass species, *Posidonia oceanica* and *Cymodocea nodosa*, which play a key role in coastal ecosystems of the Mediterranean Sea. Changes in fatty acids as key metabolic indicators were assessed in specimens from two geographical populations of each species adapted to different *in situ* temperature regimes. Total fatty acid (TFA) content and composition were compared in *C. nodosa* and *P. oceanica* from natural populations and following exposure to heat stress in culture. After heat exposure, individuals of *C. nodosa* and *P. oceanica* adapted to colder temperatures *in situ* accumulated significantly more TFA than controls. For both species, the proportion of polyunsaturated fatty acids (PUFA) decreased, and the percentage of saturated fatty acids (SFA) increased significantly after the heat treatment. These results highlight that populations of both species living at warmest temperatures *in situ* were more thermo-tolerant and exhibited a greater capacity to cope with heat stress by readjusting their lipid composition faster. Finally, exposure of seagrasses to warmer conditions may induce a decrease in PUFA/SFA ratio which could negatively affect their nutritional value and generate important consequences in the healthy state of next trophic levels.

Key words: Mediterranean Sea, *Posidonia oceanica*, *Cymodoceanodosa*, Climate change, Heat wave, Fatty acid plasticity, Laboratory experiment, Acclimatization, Temperature.

7.1. Introduction

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Anthropogenic activity has rapidly increased greenhouse gasses concentration in the atmosphere over the last decades. Global warming is a direct consequence of such gas emissions and has emerged as a threat for terrestrial and marine species worldwide (Cheung et al., 2009; Pounds et al., 2006). Over recent years, anomalous summer heat waves have been recorded from the Mediterranean Sea, with sea water temperatures exceeding 28 °C in 2003 and 2006 (Marba et al., 2010). Marine heat waves are generaly defined as prolonged discrete anomalously warm water events that can be described by their duration, intensity, rate of evolution, and spatial extent (Hobday et al., 2016). Projected environmental changes predict an increase in such extreme events in number and intensity over this century, with anticipated increases in average summer seawater temperatures by 4-5 °C (IPCC, 2014). These potential scenarios may alter the metabolism, growth and life cycle of marine foundation species (Pörter and Farrell, 2008; Hoegh-Guldberg and Bruno, 2010) and also the ecological interactions among associated communities, including seagrass systems (Post and Pedersen et al., 2008). With some native primary producers already living at their thermal upper limit, future climate change may compromise their survival, with dramatic effects on Mediterranean ecosystem functioning (Meehl & Tebaldi 2004). Future projections estimate that Mediterranean ecosystems will experience the largest change in biodiversity worldwide resulting in conditions less favourable for native seagrasses and but more favourable for tropical species (Parry, 2000; Sala et al., 2000; Moschella, 2008).

Posidonia oceanica and *Cymodocea nodosa* are the dominant seagrass species in coastal Mediterranean ecosystems with contrasting biological attributes and ecological strategies. The endemic *P. oceanica* is the most dominant species in infralittoral bottoms to a maximum depth of 25-40 m (Procaccini et al., 2003). It is a large, long-lived (Arnauld-Haond et al., 2012) seagrass with low growth and recovery rates (Duarte et al., 2006), making it particularly vulnerable to environmental disturbances. Declines in the extent of *P. oceanica* meadows have been reported elsewhere mainly caused by anthropogenic impacts of coastal

development (Marba et al., 2005; Boudouresque et al., 2009; Díaz-Almela et al., 2009). *C. nodosa* is the second most abundant seagrass in Mediterranean Sea, and its distribution ranges from the Iberian Peninsula to Senegal and Cape Verde. It is a species adapted to a wide range of coastal habitats with contrasting regimes of salinity, temperature and nutrients, from the open sea to semi-enclosed coastal lagoons. Compared to *P. oceanica*, it is smaller in size, with higher growth rates, high phenotypic plasticity and rapid recovery such as from temperature disturbances (Olesen et al., 2002; Duarte et al., 2006; Sandoval-Gil et al., 2014). In the summer of 2003, Mediterranean seagrass meadows were exposed to an extreme heat wave episode leading to reduced recruitment rates and hence increased mortality (Balearic Islands, Marba et al., 2010) and inducing massive flowering (Díaz-Almela et al., 2007). Responses of *C. nodosa* to heat stress are less well studied but are rather variable and less severe than those of *P. oceanica* (Olsen et al., 2012; Marín-Guirao et al., 2016; Tutar et al., 2017).

At the base of the marine food web, primary producers represent the main source of food, as well as essential PUFA (Iken et al., 1998). Since seagrasses constitute an essential part of herbivores diet, future warming may affect their nutritional value, and therefore the health of potential grazers via dietary intake (Havelange et al., 1997). Recent studies showed that future scenarios of climate change may induce changes in seagrass leaf composition, such as pigments concentration, and generate changes in the herbivore diet preferences (Hernan et al., 2017; Beca-Carretero et al., 2017). Previous studies in marine primary producers observed an increase of PUFA levels with a decrease of temperature (Gosch et al., 2015; Schmid et al. 2017a). Hence, decreases in PUFA levels and in PUFA/SFA ratios are anticipated under future warming. Therefore, further detailed analyses of the role of fatty acid metabolism as potential adaptive mechanism of marine plants to heat stress and hence, to future climate change scenarios, are required. However, potential effects of stress caused by heat waves on lipid metabolism in seagrasses have not been evaluated experimentally.

In this study, the effects of simulated warming events were investigated in a mesocosm system to assess changes in key metabolic indicators, such as fatty acids, in specimens from two geographical (northern and southern) populations of *P.*

oceanica and *C. nodosa* adapted to different in-situ temperature regimes. Total fatty acid content and composition were determined after exposure to heat treatment for six weeks, and again following six subsequent weeks of recovery. Moreover, the variation in the proportion of PUFA and PUFA/SFA ratios in seagrasses from across their geographical distribution range in relation to in-situ annual seawater surface temperatures (SST) were assessed. Finally, the ecological implications of changes in fatty acid composition under potential future warming scenarios in higher trophic levels are discussed.

7.2. Methods

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7.2.1. Experimental Setup

In June 2016, large fragments of rooted *Posidonia oceanica* and (Linnaeus) Delile, and *Cymodocea nodosa* (Ucria) Ascherson bearing apical growth meristems and a large number of connected shoots were collected by divers in two Mediterranean regions of Spain at 5-7 m depth: the northern populations were located in Catalonia (42º 06'23''N, 3º 10' 16''E), such as *P. oceanica* in Cala Montgó and *C. nodosa* in Ebro Delta, and the southern populations in the Murcia region (37º34'20.8''N, 1º 12'28.1''W). These two regions are about 700 km apart, displaying substantial differences in SST of about 6 ºC in summer and up to 10 ºC in autumn (Vargas-Yáñez 2007, IEO data base, http://www.ieo-santander.net; pers. obs.; Fig. 1). Such temperature differences are sufficiently large to expect local adaptions of different populations. In the case of *P. oceanica*, the southern population was representing the warm-adapted population. By contrast, for *C. nodosa*, the (assumed) warm-adapted population was located in the northern region since the seagrass population was present at a shallower and more sheltered site (Ebro Delta) where temperatures in summer can reach 33 ºC (southern population: 27-28 ºC).

Plant fragments of similar size (20-25 cm length) with 15-20 interconnected shoots were transplanted into the mesocosm system based at the Oceanography Centre of

Murcia (see Marín-Guirao et al., 2011 and Sandova-Gil et al., 2014 for details) within 36 h after up-rooting. For each species, plant fragments were placed in plastic pots filled with coarse, cleaned of sediment and six plants from each of the two regions (Fig. 2) were randomly placed in one of twelve 500 L tanks: for each population (colder vs warmer site), three of the six tanks were randomly assigned to heat treatment (H) and the other three remained as controls (C). Four pots were positioned in each individual tank (n=3).

Plants were acclimated for two weeks with the mean ambient conditions recorded at the sampling sites: salinity of 37.5 PSU and irradiance of 300-340 µmol photons m^{-2} s^{-1} under a 14h:10h light:dark photoperiod using 500 W metal halide lamps. Mean ambient temperature was 23 ºC for *P. oceanica* from the northern sites and 25 ºC for southern *P. oceanica* and *C. nodosa* populations. After this acclimation period (T1), temperature was gradually increased (by 0.5 ºC per day) in tanks with H treatment, to reach 4 ºC above the control temperature. The duration of this exposition phase (T2) was six weeks, after which temperatures in H treatments were gradually returned to control values to allow plants to recover from heat stress for another six weeks (T3, recovery phase). The temperature treatment simulated the heat wave reported in Mediterranean Sea in 2003 (Marbá et al., 2010) and a heat wave recorded during the same year of the experiment using underwater sensors (data not available, *pers. obs*.). At the end of T2 and T3, samples were collected for fatty acid analysis.

On each sampling occasion (T2 and T3), fresh leaf biomass was collected from mature and healthy leaves of *P. oceanica* and *C. nodosa* for fatty acid analysis. Selected leaves were manually cleaned in distilled water and epiphytes or grazing marks removed. Results are expressed as the mean values of 5-6 replicates (n=5-6) for each treatment combination.

7.2.2. Fatty acid analysis

Fatty analysis was performed using gas chromatography (GC). For description see Methods in Chapter 3, Section 3.2.2.

7.2.3. Latitudinal and in situ annual SST comparison

Data obtained from plants incubated at control conditions (T2) were used to compare % PUFA and PUFA/SFA ratios with previous findings of seagrasses across their latitudinal distribution range in relation to site-specific annual seawater surface temperatures. SST data for the latitudinal comparison were derived from the Bio-ORACLE database (http:// http://www.bio-oracle.org) (Tyberghein et al., 2012) with a resolution of 0.5 km^2.

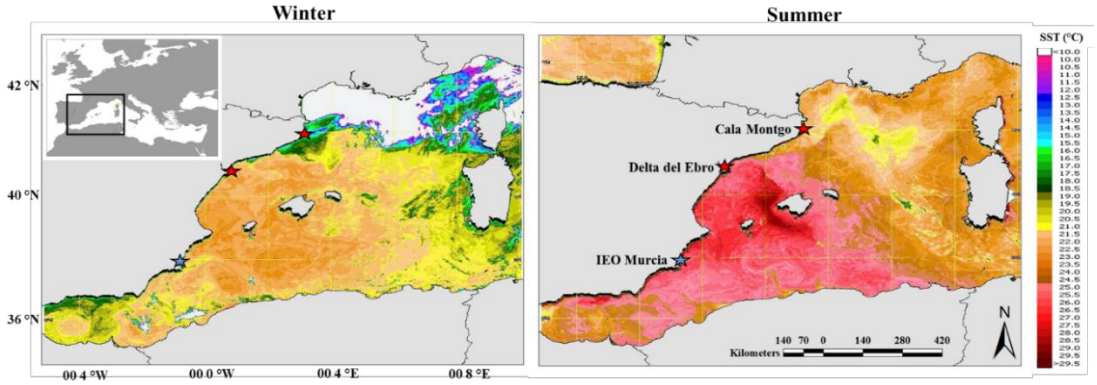

Figure 7.1 Map of satellite-derived data of winter and summer sea surface temperatures (SST) (http://www.ieo-santander.net) in the western Mediterranean Sea with the location of the two sites where the samples were collected (red stars), two Catalonia, Cala Montgo and Delta del Ebro. IEO Murcia is the location where the experiments were conducted (blue star).

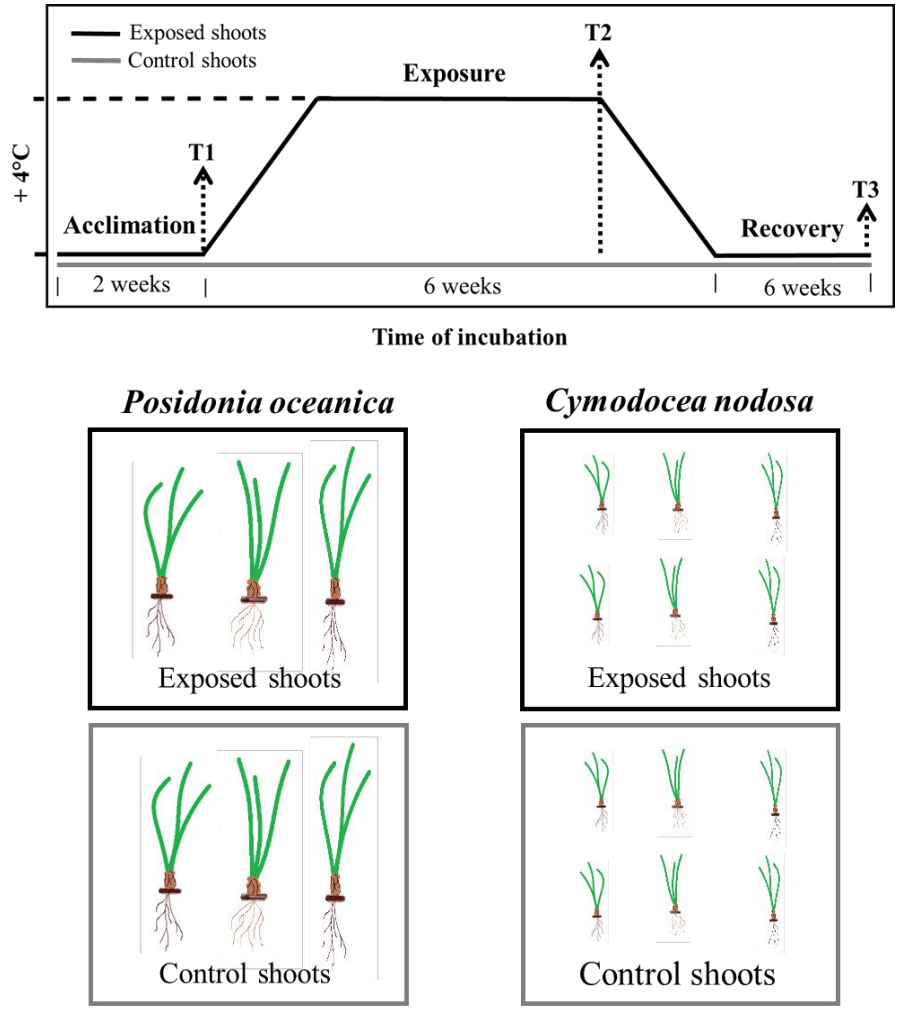

Figure 7.2 Schematic of the experimental design. Exposed (black line) and control (grey line) of plants of *C. nodosa* and *P. oceanica*. T2 (exposed) and T3 (recovery) represent the time period when plants were collected for fatty acid (FA) analysis.

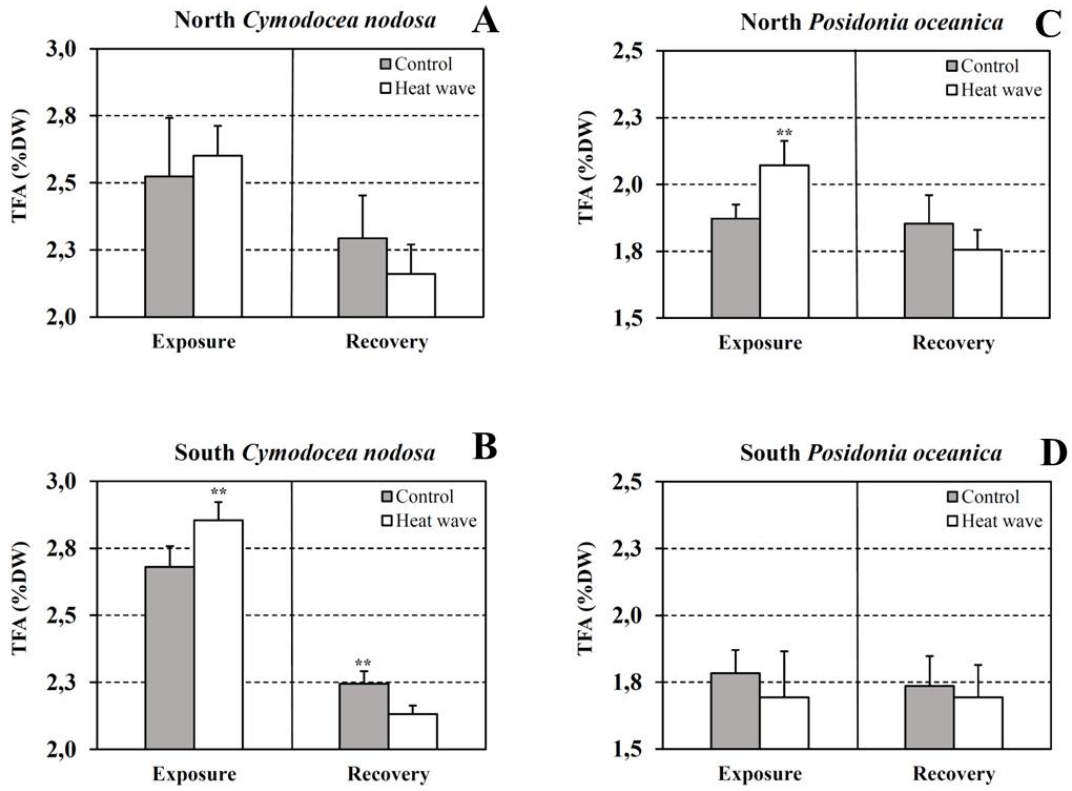

Figure 7.3 Total fatty acid contents (% DW) of northern and southern populations of *C. nodosa* and *P. oceanica* exposed to control and heat wave temperatures, and their recovery. Differences between temperature treatments were evaluated performing t-student (** represent $p<0.01$ and * represent $p<0.05$-0.01).

7.2.4. Statistical analyses

Prior to performing statistical analyses data were tested for normal distribution using the Kolmogorov-Smirnov test (Sokal & Rohlf 1981) and homogeneity using Levene's test. Data that did not pass the test were transformed into Ln to ensure the assumption of normality (Draper & Smith 1981), and homogeneity. To report responses to different temperature treatments *t*-test was applied to assess significant effects ($p<0.05$). A one-way ANOVA and *post hoc* Tukey's pairwise test was conducted to evaluate differences between northern and southern populations, and between *C. nodosa* and *P. oceanica*. All data treatments and statistical analyses were performed using IBM SPSS Inc., v.13.

Table 7.1 Total fatty acid content (% DW) and composition (% TFA) in northern and southern populations of *C. nodosa* exposed to control and heat wave temperatures, and their recovery. Results are expressed as mean± SD (n=5-6).

Cymodocea nodosa								
Location	**Northern population**				**Southern population**			
Treatment	**Exposure**		**Recovery**		**Exposure**		**Recovery**	
Time	**CNT**	**HW**	**CNT**	**HW**	**CNT**	**HW**	**CNT**	**HW**
Fatty acids (% TFA)								
Saturated fatty acids								
14:0	1.1 ± 0.1	1.1 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	1.1 ± 0.04	1.2 ± 0.1	0.4 ± 0.03	0.4 ± 0.02
16:0	20.3 ± 0.6	23.4 ± 0.3	21.2 ± 0.4	21.7 ± 0.6	19.7 ±0.3	22.0 ± 0.5	21.2 ± 0.4	21.2 ± 0.4
18:0	1.0 ± 0.04	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.1	1.0 ± 0.04	1.2 ± 0.1	1.2 ± 0.04
Sum of SFA	**22.4 ± 0.5**	**25.4 ± 0.2**	**22.7 ± 0.5**	**23.4 ± 0.8**	**22.0 ± 0.3**	**24.2 ± 0.4**	**22.9 ± 0.5**	**22.6 ± 0.4**
Monounsaturated fatty acids								
14.1	0.5 ± 0.1	0.5 ± 0.1	1.0 ± 0.02	1.0 ± 0.1	0.8 ± 0.4	0.4 ± 0.1	0.9 ± 0.04	0.9 ± 0.04
16:1 n-7	0.4 ± 0.02	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.1	0.4 ± 0.03	0.2 ± 0.02	0.4 ± 0.04	0.4 ± 0.1
18:1 n-7	0.2 ± 0.03	0.3 ± 0.02	0.3 ± 0.04	0.3 ± 0.02	0.3 ± 0.02	0.2 ± 0.03	0.3 ± 0.04	0.3 ± 0.03
18:1 n-9	2.0 ± 0.3	2.6 ± 0.3	1.6 ± 0.1	1.9 ± 0.2	2.9 ± 1.1	2.4 ± 0.3	2.0 ± 0.1	2.2 ± 0.1
Sum of MUFA	**3.2 ± 0.3**	**3.9 ± 0.3**	**3.1 ± 0.2**	**3.4 ± 0.3**	**4.4 ± 1.6**	**3.3 ± 0.3**	**2.9 ± 0.2**	**3.1 ± 0.3**
Polyunsaturated fatty acids								
18:2 n-6	18.8 ± 0.3	19.5 ± 1.5	21.2 ± 0.4	18.0 ± 0.9	19.5 ± 0.9	21.4 ± 1.3	22.3 ± 0.9	22.1 ± 0.6
18:3 n-3	47.4 ± 0.5	41.8 ± 1.7	46.2 ± 1.6	47.2 ± 0.4	45.4 ± 2.0	43.0 ± 0.9	43.4 ± 0.9	43.4 ± 1.2
20:4 n-6	0.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.6	0.02 ± 0.1	0.1 ± 0.1	0.05 ± 0.1
20:5 n-3	1.2 ± 0.1	1.8 ± 0.7	1.3 ± 0.5	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.5	1.0 ± 0.1	1.1 ± 0.1
Sum of PUFA	**67.6 ± 0.2**	**64..0 ± 0.4**	**67.6 ± 0.3**	**66.6 ± 0.5**	**66.5 ± 1.5**	**65.4 ± 0.6**	**66.8 ± 0.7**	**66.8 ± 0.6**
n-3/n-6	2.6 ± 0.2	2.2 ± 0.1	2.2 ± 0.1	2.7 ± 0.2	2.3 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.2
PUFA/SFA	3.0 ± 0.2	2.5 ± 0.3	3.0 ± 0.3	2.8 ± 0.2	3.0 ± 0.3	2.7 ± 0.2	2.9 ± 0.2	3.0 ± 0.3
Others	**6.7 ± 0.3**	**6.7 ± 0.2**	**6.6 ± 0.3**	**6.6 ± 0.2**	**7.2 ± 0.2**	**7.2 ± 0.2**	**7.4 ± 0.3**	**7.5 ± 0.1**
Fatty acids (%DW)	**2.52 ± 0.2**	**2.60 ± 0.1**	**2.29 ± 0.2**	**2.16 ± 0.1**	**2.68 ± 0.1**	**2.85 ± 0.1**	**2.24 ± 0.05**	**2.13 ± 0.03**

Table 7.2 Total fatty acid content (% DW) and composition (% TFA) in northern and southern populations of *P. oceanica* exposed to control and heat wave temperatures and their recovery. Results are expressed as mean± SD (n=5-6).

Posidonia oceanica								
Location	**Northern population**				**Southern population**			
Treatment	**Exposure**		**Recovery**		**Exposure**		**Recovery**	
Time	**CNT**	**HW**	**CNT**	**HW**	**CNT**	**HW**	**CNT**	**HW**
Fatty acids (% TFA)								
Saturated fatty acids								
14:0	0.6 ± 0.1	0.7 ± 0.04	0.6 ± 0.03	0.6 ± 0.2	0.5 ± 0.04	0.6 ± 0.03	0.6 ± 0.1	0.6 ± 0.1
16:0	19.8 ± 0.6	20.4 ± 0.6	20.2 ± 0.1	20.3 ± 0.8	19.8 ± 0.4	21.3 ± 0.7	19.6 ± 0.05	19.7 ± 0.5
18:0	2.4 ±0.1	2.6 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	2.4 ± 0.2	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.3
Sum of SFA	**22.7 ± 0.8**	**23.8 ± 0.8**	**22.8 ± 0.7**	**22.9 ± 0.8**	**22.7 ± 0.6**	**23.9 ± 0.7**	**22.2 ± 0.6**	**22.2 ± 0.8**
Monounsaturated fatty acids								
14:1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.04	0.4 ± 0.1	0.5 ± 0.04	0.5 ± 0.1	0.4 ± 0.05	0.4 ± 0.1
16:1 n-7	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.03	0.3 ± 0.04	0.4 ± 0.1
18:1 n-7	0.2 ± 0.01	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.02	0.1 ± 0.1	0.3 ± 0.03	0.3 ± 0.05	0.3 ± 0.02
18:1 n-9	4.4 ± 0.4	5.5 ± 0.4	4.7 ± 0.3	4.7 ± 0.5	4.4 ± 1.1	6.0 ± 1.1	5.8 ± 0.7	7.0 ± 1.9
Sum of MUFA	**5.5 ± 0.3**	**6.5 ± 0.5**	**5.6 ± 0.4**	**5.7 ± 0.5**	**5.3 ± 1.1**	**7.0 ± 1.1**	**6.8 ± 0.8**	**8.0 ± 1.9**
Polyunsaturated fatty acids								
18:2 n-6	18.3 ± 1.0	15.0 ± 0.7	18.6 ± 0.5	19.3 ± 1.3	17.7 ± 1.5	24.9 ± 7.8	20.1 ± 1.1	22.5 ± 1.9
18:3 n-3	47.4 ± 0.7	47.9 ± 1.7	47.2 ± 0.8	46.4 ± 1.4	48.3 ± 2.0	38.8 ± 8.0	45.0 ± 1.2	41.8 ± 1.3
Sum of PUFA	**65.7 ± 0.7**	**62.9 ± 1.2**	**65.8 ± 0.9**	**65.7 ± 0.7**	**66.0 ± 0.6**	**63.7 ± 1.3**	**65.2 ± 0.6**	**64.3 ± 1.5**
n-3/n-6	2.6 ± 0.1	3.2 ± 0.3	2.5 ± 0.1	2.4 ± 0.1	2.7 ± 0.2	1.6 ± 0.1	2.2 ± 0.1	1..9 ± 0.1
PUFA/SFA	2.9 ± 0.1	2.6 ± 0.1	2.9 ± 0.1	2.9 ± 0.2	2.9 ± 0.1	2.7 ± 0.2	2.9 ± 0.3	3.2 ± 0.2
Others	**6.0 ± 0.2**	**6.8 ± 0.1**	**5.8 ± 0.1**	**5.6 ± 0.1**	**6.0 ± 0.3**	**5.4 ± 0.5**	**5.8 ± 0.2**	**5.5 ± 0.2**
Fatty acids (%DW)	**1.87 ± 0.05**	**2.07 ± 0.1**	**1.85 ± 0.1**	**1.76 ± 0.1**	**1.78 ± 0.1**	**1.69 ± 0.2**	**1.74 ± 0.1**	**1.69 ± 0.1**

7.3. Results and Discussion

This section has been modified from the original article

7.3.1. TFA content and composition in *Cymodocea nodosa* and *Posidonia oceanica*

Overall, the average total fatty acid (TFA) content of *C. nodosa* (2.45±0.3 mg g^{-1} DW) was always significantly higher than the one observed in *P. oceanica* (1.80±0.3 mg g^{-1} DW) (t-test, $p<0.001$). This could be explained by the ability of different species to store excess energy in the form of carbon, such as fatty acids partitioned and accumulated into TAG, as observed in some seaweed species. For example, species-specific acclimation potential was observed between two important Irish intertidal macroalgae, summer-acclimated *Fucus serratus* diverted excess energy into storage lipids (TAG), while *Palmaria palmata* was more sensitive and susceptible to degradation of its chloroplast membranes, resulting in a decrease in TFA, PUFA-rich polar lipids and pigments, and a release of free fatty acids (Schmid et al., 2017b). Of interest, in previous studies, Sandoval-Gil et al. (2012) reported that *C. nodosa* had higher concentrations of soluble sugars than *P. oceanica* when exposed to an increase in salinity; this could be interpreted as an adaptive trait of *C. nodosa* to cope with environmental stress. The higher carbohydrate content supports the very high capacity of *C. nodosa* to uptake carbon via photosynthesis, as indicated by the ^{13}C isotopic signal (Sandoval-Gil et al., 2014). Indeed, *C. nodosa* appears to be more tolerant than *P. oceanica* to environmental stress, in particular to changes in temperature and salinity (Marba et al., 1996; Sandoval-Gil et al., 2014; Marín-Guirao et al., 2016). Differences in TFA could also be associated with variation in leave structure and morphology of both species, with *P. oceanica* having large and thick leaves with high contents of cellulose and lignin (Romero et al., 1992), low nutrient content (Sandoval-Gil et al., 2014), and slow leaf turnover. By contrast, *C. nodosa* has smaller and thinner leaves with fast turnover rates (Perez el al., 1989). Therefore, a greater leaf surface per biomass in *C. nodosa* may be associated with higher photosynthetic activity in comparison to *P. oceanica* (Olsen et al., 2012), inducing a greater carbon fixation capacity and therefore a higher TFA content.

The TFA composition of both seagrasses is displayed in Tables 1 and 2, indicating only slight variations in profiles. The main, but only slight, difference was observed regarding the overall level of polyunsaturated fatty acids (PUFA), which was 66.4±1.1% of TFA in *C. nodosa* and 64.9±1.1% in *P. oceanica* (*t*-test, $p < 0.05$). For both species, the most abundant PUFA in leaves was α-linolenic acid (ALA, 18:3 n-3). Previous studies also reported high levels of ALA in *P. oceanica*, with significantly higher percentages in leaves than in rhizomes (Viso et al., 1993), supporting the role of this n-3 PUFA in photosynthetic performance. In addition, PUFA in *C. nodosa* and *P. oceanica* contained substantial levels of linoleic acid (LA, 18:2 n-6) ranging, respectively, from 18.8 to 22.3% and 15.0 to 24.9% of TFA. Significant differences in the overall percentages of monounsaturated fatty acids (MUFA) were also observed between both species, which ranged from 3.4±0.5% in *C. nodosa* to 6.3±0.9% in *P. oceanica* (t-test, $p<0.001$). The main difference was observed in the levels of oleic acid (OLE, 18:1 n-9) which was 55% higher in *P. oceanica* than in *C. nodosa*. No significant differences in overall saturated fatty acid (SFA) levels were observed between each species (23.0±1.0% of TFA in *C. nodosa* and 22.9±0.6% in *P. oceanica*), palmitic acid (PAL, 16:0) being the main SFA. Also, noteworthy, *C. nodosa* contained low levels of long-chain (LC)-PUFA, such as arachidonic (ARA, 20:4 n-6) and eicosapentaenoic (EPA, 20:5 n-3) acids, which never exceeded 2% of TFA (Table 1). The presence of such LC-PUFA was also previously reported from some other seagrasses, as represented in Table 3, including *Posidonia sinuosa*, *Zostera* spp., *Thallassia hemprichii* and *Halphila ovalis*.

LA and ALA are the most abundant PUFA previously reported from leaves of a range of seagrass species (Nichols et al., 1982; Khotimchenko, 1993). Both PUFA are precursors for synthetizing essential fatty acids for the next trophic levels (Veloza et al., 2006; Richoux et al., 2008). Essential fatty acids (EFA) are fatty acids not biosynthesized effectively by animals (Arts et al., 2001), such as ALA and LA, which are both PUFA, and precursors for the synthesis of biologically important LC-PUFA (e.g. EPA and DHA). The four main fatty acids (PAL, OLE, LA and ALA) accounted for 80-90% of the TFA composition in both species analysed here. In the case of *P. oceanica,* these findings are in accordance with previous studies conducted in different regions of the Mediterranean Sea (Viso et al., 1993), but our study

represents the first detailed investigation of the fatty acid composition of *C. nodosa*. Moreover, as shown in Table 3, previous analyses of seagrass leaves have demonstrated similar fatty acid profiles similar to results here with the four mentioned major fatty acids ranging from 62 to 90% of TFA (i.e. Jeffries, 1972; Viso et al., 1993; Kharlamenko et al., 2001). The presence of n-6 and n-3 LC-PUFA (>20 carbon) such as ARA, EPA and docosahexaenoic (DHA, 22:6 n-3) acids as traces reported for some seagrass species (Table 3) are potentially due to algal epiphytes colonizing their leaf surfaces which are able to synthetize and accumulate LC-PUFA (Khozin-Goldberg et al., 2011; Schmid et al., 2014).

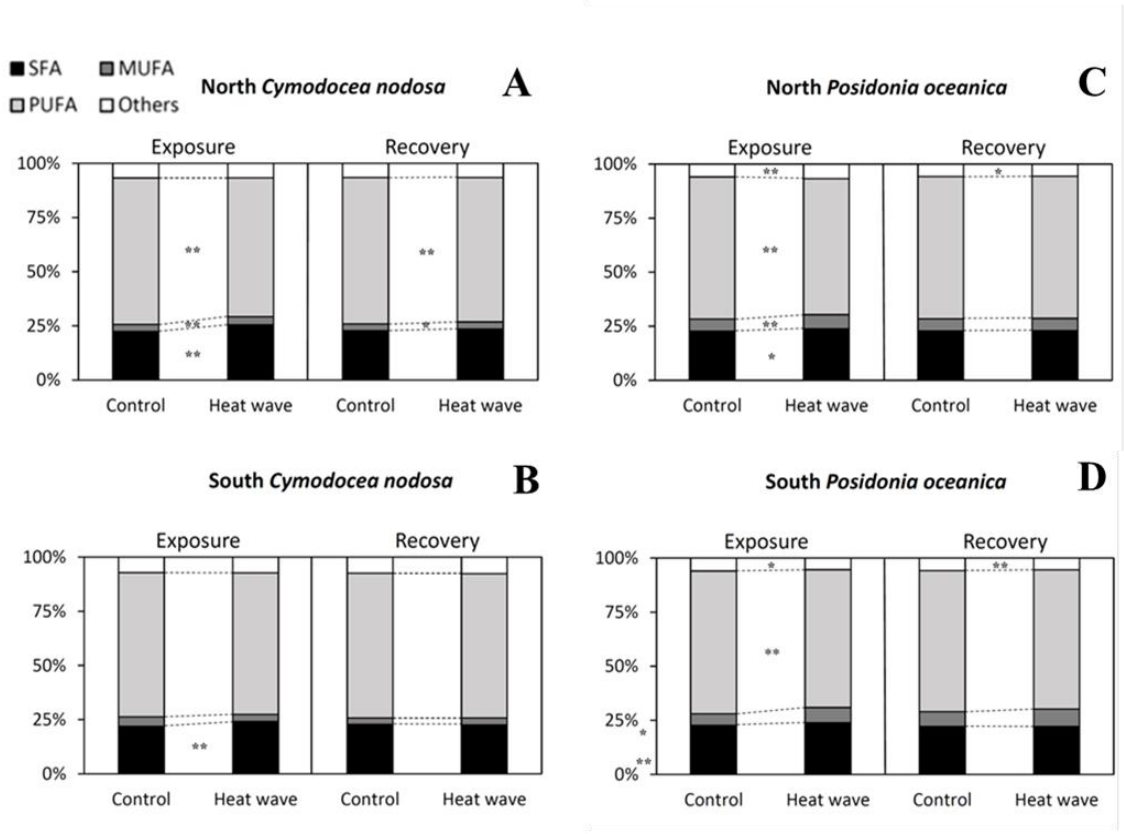

Figure 7.4 Proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and non-identified (Others) fatty acids (% TFA). Differences between temperature treatments were evaluated performing t-student (** represent $p<0.01$ and * represent $p<0.05$-0.01).

7.3.2. Regulation of FA metabolism exposed to experimental warming

The result indicated a species-specific metabolic response of fatty acids to experimental warming. After exposure to heat stress for 6 weeks, *C. nodosa* from the southern population, considered to be adapted to colder temperatures *in-situ*, demonstrated a significantly increase of 9% in TFA content in comparison to control plants (t–test, $p < 0.001$) (Fig. 7.3B). A similar pattern was observed for the cold-adapted (northern) population of *P. oceanica*, where plants exposed to warmer temperatures produced 4.7% more TFA content than controls (*t*–test, $p < 0.001$). By contrast, seagrass populations adapted to warmer temperatures, i.e. northern population of *C. nodosa* and southern population from *P. oceanica*, did not reveal any significant differences in TFA contents after heat exposure.

For both species, the proportion of PUFA (i.e., LA and ALA) decreased, while SFA (i.e., PAL) increased after the heat treatment (Fig. 7.4). As PUFA are mainly partitioned to structural lipids promoting membrane fluidity, electron transport rate and photosynthetic activity, our results may be explained by the reduced requirement for PUFA to maintain membrane fluidity under elevated temperatures (Marr 1962).These findings support recent evidence regarding the transcriptomic modulation of lipids biosynthesis in experimentally heat-stressed *P. oceanica* plants (Marín-Guiraoet al., 2017); they are thus in accordance with previous studies on seagrasses, and unicellular algae, seaweeds and terrestrial plants, where a decrease in PUFA was related to an increase in SFA when exposed to higher temperatures *in-situ* or during lab-experiments (Cohen., et al 1988; Sanina et al., 2004).

Heat-stressed seaweeds and terrestrial plants typically respond to abiotic stress by remodelling membrane fluidity by realising PUFA (i.e. ALA) from membrane lipids (Gosch et al., 2015; Upchurch 2008), inducing therefore an increase in the proportion of SFA. In parallel, fatty acid desaturases activities regulate the unsaturated fatty acid levels and mediated changes in membrane fluidity. In algae, accumulation of storage lipids, such as TAG containing mostly SFA, is also a common mechanism to store excess of energy generated by photosynthesis, and to cope with stressors such as increased temperatures, light or nutrient deficiency (Goncharova et al., 2004; Pal et

al., 2011; Solovchenko 2012). Moreover, under heat stress conditions (T2), % of MUFA increased significantly in populations of *P. oceanica* adapted to lower temperatures *in-situ*. These results are in agreement with previous studies on other marine primary producers where, under temperature stress, the % of MUFA increased more in thermo-intolerant than in thermo-tolerant species (Roush et al., 2003). Such changes may be associated with a reduced synthesis of PUFA under heat stress (Sukenik et al., 1993). Overall, our results highlight that seagrasses living at warmest temperatures *in situ* were more thermo-tolerant, with a higher capacity to adjust their fatty acid composition, than populations inhabiting colder regions.

7.3.3. Recovery from experimental heat wave events

After six weeks of exposure to the heat treatment, both species were incubated for a further six weeks at their original *in-situ* temperatures, in an attempt to test their ability to recover after an anomalous heat wave event.

C. nodosa appeared to recover fully, but TFA of the two populations displayed two distinct patterns, with a slightly lower content in the southern population at T3 (end of recovery) which was equivalent to the control in the northern population (Figure 1, A and B). Heat-exposed plants and controls from both populations had lower TFA contents at T2 (end of heat exposure) and T3 – with significant decreases of 15-20% (ANOVA, $p < 0.001$).

The TFA content of *P. oceanica* reached initial levels within the recovery period. In contrast to *C. nodosa*, TFA content of northern and southern populations of *P. oceanica* did not change significantly between exposure (T2) and recovery (T3) (Fig. 3, C and D). The fact that the sampling date of *C. nodosa* and *P. oceanica* shoots harvested at T3 occurred in late September may explain these different species-specific performances; *C. nodosa* typically can maintain high productivity rates until the end of the summer, while the productivity of *P. oceanica* may decrease in mid-summer (Buia et al., 1992; Marba et al., 1996).

Mediterranean seagrasses are pre-adapted to seasonal temperature cycles and may be additionally influenced by their internal biological clock (Buia et al., 1992; Marba et

al., 1996). Rather than acclimating to short-term ambient environmental factors, the growth pattern of *P. oceanica* is relatively independent of seasonality. By contrast, growth of *C. nodosa* is highly sensitive to environmental conditions, displaying marked seasonal patterns (Marba et al., 1996). Here, after recovery (T3), the TFA compositions of both *C. nodosa* and *P. oceanica* populations were similar to controls (Fig. 4), suggesting full recovery from heat stress by readjusting their lipid composition and membrane fluidity to control conditions.

Moreover, data displayed in Tables 2 and 3 suggest that plants adapted to warmer *in situ* conditions, which performed better after the heat treatment, showed higher ratio of n-3/n-6 PUFAs after the exposure (T2) than after the recovery (T3). As suggested by Sanina et al. (2004), these results may be related to the important role of n-3 PUFAs within lipids of photosynthetic membranes; on the other hand, n-6 PUFAs are mostly linked to polar lipids of extra-plastidial membranes (Schmid et al., 2017*b*). In general, *C. nodosa* displayed a greater plasticity with regard to fatty acid composition than *P. oceanica*, resulting in a fast adjustment in composition and lipid structure in response to environmental changes.

Table 7.3 Comparison of the fatty acid profiles (% TFA) of *C. nodosa* and *P. oceanica* determined here in comparison with data from other studies. Data are expressed as ranges in % TFA. References 1 to 9 represent data compiled from published literature shown in Table 4.

Species	***Posidonia oceanica***	***Posidonia sinuaosa***	***Cymodocea nodosa***	***Zostera marina***	***Zostera noltii***	***Zostera capensis***	***Phyllospadix iwatensis***	***Halophila ovalis***	***Ruppia maritima***
References	**Our study, 9**	**6**	**Our study**	**1,2 and 5**	**3**	**7**	**2**	**6**	**8**
Fatty acids (% TFA)									
Saturated fatty acids									
14:0	[0.5-0.7]	0.2	[0.4-1.1]	0.4	0.8			0.89	
16:0	[19.7-21.3]	22.5	[19.7-23.4]	[16.5- 19.9]	12.99	31	12.4	32.42	18.1
17:0		0.3		0.2	0.51	0.9		0.26	
18:0	[2.0-2.6]	6.7	[1.0-1.3]	[1 -1.5]	2.64	2.1	0.8	4	2
20:0		0.4		[0.7-0.9]	2.65		1.0		
22:0		0.9		[1.9-2.1]	4.86	0.3	2.5	0.04	
24:0		1.6		[0.6-0.7]	5.71		1.6	1.59	
28:0		7.7						0.61	
30:0		10.0						0.03	
Sum of SFA	**[22.0-25.4]**	**50.2**	**[22.0-25.4]**	**[17.5-25.7]**	**30.2**	**34.3**	**18.3**	**39.8**	**20.1**
Monounsaturated fatty acids									
14:1	[0.4-0.6]		[0.5-1.0]	0.1					
16:1									3.4
16:1 n-7	[0.3-0.5]	2.1	[0.2-0.4]	[1 - 2.4]	0.24	1.8	1.2	1.94	
17:1									4.1
18:1									5.3
18:1 n-7	[0.1-0.3]		[0.2-0.3]	0.5	1.17	0.8			
18:1 n-9	[4.4-7.0]	4.1	[1.6-2.9]	[1.5 - 1.8]	0.62	1.6	0.7	3.04	
20:1 n-9		0.1							

22:1 n-9								0.42	
24:1 n-9		0.1			1.03			0.06	
Sum of MUFA	**[2.9-4.4]**	**6.4**	**[2.9-4.4]**	**[2.6-4.3]**	**3.1**	**4.2**	**1.9**	**5.5**	**12.8**
Polyunsaturated fatty acids									
16:2 n-4		0.09							
16:3 n-3				[3.5-7.1]		2.9	8.4	0.05	
16:3 n-4					2.28				
18:2 n-6	[15.0-22.5]	12.23	[18.0-22.3]	[15.7-21.2]	14.82	28	3.0	13.96	16.6
18:3									41.9
18:3 n-3	[38.8-48.3]	23.02	[41.8-47.4]	[41.3-49.3]	46.38	28.8	60.6	31.26	
18:3 n-6		0.08						0.56	
18:4 n-6		0.26							
20:4 n-6		0.33	[0.02-0.5]		0.49	0.3		0.38	
20:5 n-3		0.4	[0.9-1.8]	[0.1 -0.3]	0.17		0.2	1.88	
22:2 n-6				0.4					
22:4 n-6									
22:5 n-3									
22:6 n-3		0.05			0.7			0.03	
Sum of PUFA	**[62.9-66.0]**	**36.5**	**[64.0-67.6]**	**[60.6-78.3]**	**64.8**	**60.0**	**72.2**	**48.1**	**58.5**
Others	**[6.6-7.5]**	**7.3**	**[6.6-7.5]**	**[1.8-3.2]**	**1.96**	**1.5**	**7.6**	**6.6**	**8.6**

Table 7.4 Data compiled from published literature used to assess differences of fatty acids profiles of different seagrass species across world

Identification	References	Species	Location	Latitude	Longitude	PUFA	PUFA/SFA	Mean SST
Our data	This study	*Posidonia oceanica*	Spain, Cala Montgo	40.73	0.87	65.70	2.89	19.05
Our data	This study	*Posidonia oceanica*	Spain, Delta del Ebro	42.11	3.17	66.00	2.91	17.18
Our data	This study	*Cymodocea nodosa*	Spain, Cala Montgo	40.73	0.87	67.60	3.02	19.05
Our data	This study	*Cymodocea nodosa*	Spain, Delta del Ebro	42.11	3.17	66.50	3.02	17.18
1	Sanina et al (2004)	*Zostera marina*	Russia, Sea of Japan	43.92	135.57	78.30	4.47	8.24
2	Vaskovsy et al (1996)	*Zostera marina*	China, Yellow Sea	35.03	125.81	72.20	3.84	15.17
2	Vaskovsy et al (1997)	*Phyllospadix iwatensis*	China, Yellow Sea	35.03	125.81	72.20	3.95	15.17
3	Coelho et al (2011)	*Zostera noltii*	Portugal, Ria Aveiro	40.63	-8.75	64.84	2.15	16.08
4	Nicholst et al (1985)	*Thallassia hemprichii*	USA, Lizard Island	-16.90	145.77	40.50	1.23	26.10
5	Kharlamenko et al (2001)	*Zostera marina*	Russia, Sea of Japan	42.54	132.12	66.40	2.61	9.33
6	Hanson et al (2010)	*Halophila ovalis*	Australia, Bay Marine Park	-30.51	115.04	48.12	1.21	21.53
6	Hanson et al (2011)	*Posidonia sinuosa*	Australia, Bay Marine Park	-30.51	115.04	50.21	1.38	21.50
7	Richoux et al (2008)	*Zostera capensis*	South Africa, Kariega estuary	-33.69	26.68	60.00	1.75	20.38
8	Jeffries et al (1972)	*Ruppia maritima*	USA, Rodhe Island	41.51	71.10	58.50	2.91	12.60
9	Viso et al (1993)	*Posidonia oceanica*	Greece, Athens	37.94	23.68	51.40	1.28	20.19
9	Viso et al (1994)	*Posidonia oceanica*	France, Villefrnache-sur-mer	43.69	7.27	70.00	3.14	18.50

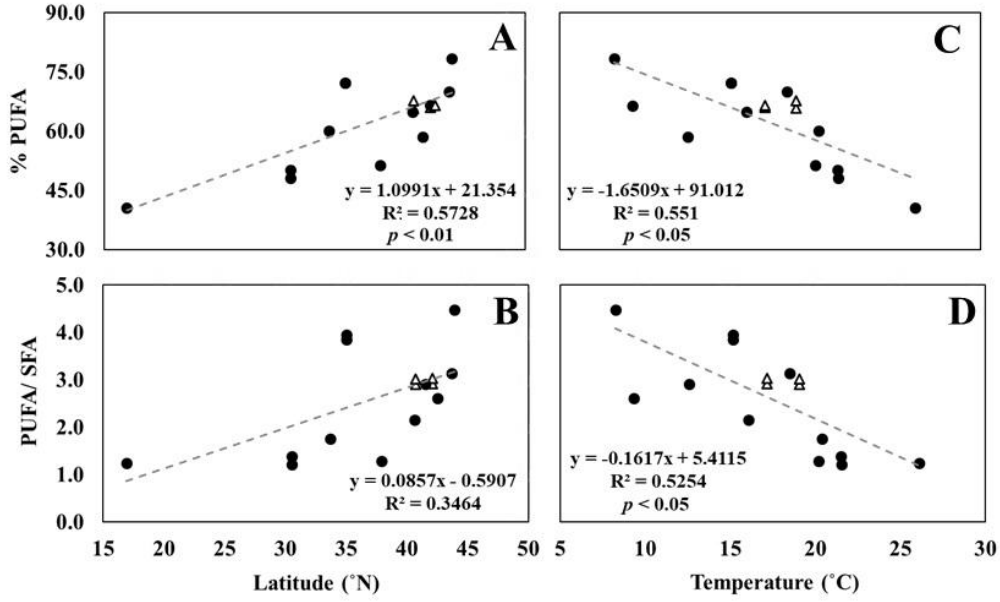

Figure 7.5 Percentage of PUFA and ratio between PUFA: SFA and corresponding geographical latitudinal distribution range (Panel A and B) and annual sea surface water temperature (Panel C and D) with results from the literature (circles) and from the present study (triangles) (Table 3 and 4). Grey lines represent the linear regression line.

7.3.4. Latitudinal and *in-situ* annual SST comparison

Both % of PUFA and PUFA/SFA ratio were correlated with latitude, with an average of 1.4 and 0.12 increase per degree latitude, respectively (Fig. 5, A and B). In addition, % of PUFA and PUFA/SFA ratio were correlated with *in-situ* annual SST, with an average of 2.12 and 0.18 decrease per increase of 1 °C, respectively (Fig. 5, C and D). Lowest proportions of PUFA (40.5 - 48.12 %) and of PUFA/SFA ratios (1.23 - 1.21) were observed in two tropical species *Thallassia hemprichii* and *Halophila ovalis* adapted to warmest annual SST (Table 3 and 4). By contrast, larger proportions of PUFA (78.3 %) and of PUFA/SFA ratios (4.47) were observed in the subarctic *Zostera marina* populations adapted to annual SST of 8.24 °C. In line with these results, previous studies highlighted that leaf production in eelgrass was also correlated with temperature, and productivity increased with temperature (Lee et al., 2007; Olesen et al., 2015; Beca-Carretero et al., *under review* (Chapter 2)). These findings demonstrate that fatty acid composition changes in accordance with *in situ* temperature, suggesting a physiological adaptation to large-scale factors such as

annual seawater temperature. In macroalgae, changes in PUFA levels to changes in temperature (Viso et al., 1993; Gosch et al., 2015) were previously related with the capacity of acclimatization of photosynthetic organisms to adjust their membrane lipid structures to support adequate membrane fluidity tin response to different environmental conditions (Falkowski & Raven 2013).

7.3.5. Implications of fatty acid responses to heat waves for the next trophic level

Mediterranean seagrasses systems represent an important food source for keystone herbivores such as the fish *Salpa salpa* (Sparidae) and the sea urchin *Paracentrotus lividus* (Echinidae) and for other consumers such as *Idotea baltica* Pall. (Idoteidae) or herbivores belonging to Palaemonidae (Ackman et al., 1968; Kirkman and Young, 1981; Velimirov, 1984) Overall, these present findings suggest that future warming could significantly affect the nutritional value of seagrasses, with a remarkable depletion in the synthesis EFA (i.e., ALA and LA), and thus potential consequences in the fatty acid composition of higher trophic consumers and in their physiological functions. Warming is also expected to increase the metabolic requirements of herbivores (Burnell et al., 2013), which may fulfil their nutritional requirements by increasing grazing pressure on seagrasses or changing preferences for other dominant macrophytes (Hernan et al., 2016; Pagès et al., 2017). Climate-driven changes in the strength of herbivory may have important consequences for Mediterranean coastal ecosystems due to the important role of grazing in altering seagrass biomass, productivity and in modulating species composition (Kirsch et al. 2002; Valentine and Duffy 2006). Future warming is therefore expected to directly, and indirectly, modify productivity and distribution of seagrasses that will cascade throughout the food web and affect the overall functioning of Mediterranean coastal ecosystems.

Chapter8

Depth-induced adjustments of fatty acids and pigment composition suggest a high biochemical plasticity in the tropical seagrass Halophila stipulacea

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Author contribution

Pedro Beca-Carretero and Gidon Winters developed the experimental design and collected the samples in Gulf of Aqava (Israel); Pedro Beca-Carretero and Freddy Guihéneuf carried out the fatty acid analysis and photosynthetic pigments extraction; Pedro Beca-Carretero performed the statistical analysis; Pedro Beca-Carretero prepared a first draft of the manuscript; Dagmar B. Stengel and Gidon Winters provided resources and funding for research; All co-authors commented on andprovided edits to finalize the original manuscript.

Abstract from original article

Halophila stipulacea is the dominant, subtidal meadow-forming seagrass in the tropical Gulf of Aqaba (GoA; northern Red Sea), characterized by warm and oligotrophic waters. This species occurs across a wide range of environmental conditions,and is considered the most deep-adapted seagrasses worldwide. For the first time, this investigation assessed the morphological and biochemical responses of *H. stipulacea* populations adapted to different depths (6 – 21 m), focussing on total fatty acid (TFA) content and composition, and photosynthetic pigments. *H. stipulacea* leaves (collected July 2016) from greater depths contained 25% more TFA and 22% more photosynthetic pigments than plants from shallow locations. Increases in TFA were mainly related to higher levels of polyunsaturated fatty acids (PUFA) and a lower production of saturated fatty acids (SFA). As PUFA promote the fluidity of the membranes of the chloroplasts, as well as the electron transport in the photosystems, their observed increase with depth may favour optimal photosynthetic responses under less favourable (e.g., low-irradiance) conditions. Moreover, cluster analyses of fatty acid compositions from the literature for other species and sites, highlights the fact that PUFA levels in *H. stipulacea* leaves are more similar to those found in seagrass species inhabiting higher latitudes, and thus colder regions, than in tropical or subtropical species. With *H. stipulacea* successfully spreading into non-native areas, such as the eastern Mediterranean and Caribbean Seas, it is critical to understand the eco-physiological mechanisms that allow this species to acclimate to a wide range of environmental conditions.

Keywords: Biochemical plasticity; Depth acclimatization; Essential fatty acids (EFA); Polyunsaturated fatty acids (PUFA); Total fatty acids (TFA); *Halophila stipulacea*; Gulf of Aqaba (GoA); invasive species

8.1. Introduction

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In tropical and subtropical marine areas, seagrasses are marine ecosystem engineer species which provide several ecological services to coastal marine ecosystems such as shelter and nursey habitat, protection of the coastline and carbon storage (Duarte et al., 2006; Norlund et al., 2017). The tropical Gulf of Aqaba (GoA) is characterized by warm (21-27 °C) and oligotrophic waters (Winters et al., 2017). In this region, *Halophila stipulacea* (Forsk.) Aschers is the most common seagrass species, representing a key component of adjacent coral reef systems (Edwards & Head 1987). This species is a small, dioecious tropical species (Neguyen et al., 2018), native to the Red Sea, Persian Gulf and Indian Ocean (El Shaffai 2011; Winters et al., 2017). In the northern GoA, *H. stipulacea* can be found growing from shallow (2 m) to light limited deep (> 50 m; Sharon et al., 2011; Winters et al., 2017) environments, and this species is considered the deepest seagrass reported worldwide (Sharon et al., 2011; Lipkin et al., 2003; Short et al., 2007).

Previous studies have highlighted the marked phenotypic plasticity of *H. stipulacea* to acclimate to a wide range of environmental conditions including temperature, irradiance, salinity and nutrient concentrations (Sharon et al., 2009, 2011; Mejia et al., 2016; Oscar et al., 2018). Variations in irradiance and spectral composition have been shown to affect the performance of seagrasses such as *Halophila* spp., controlling metabolic processes such as photosynthetic and respiration rates, resulting in local morphological and physiological adaptations (e.g. Beer et al., 2000; Sharon et al., 2009; 2011, Rotini et al., 2017). Also, differences in light regimes affect the reproductive effort and seed germination, processes which are associated with the settlement and colonization capacity of seagrass species (Diaz-Almela et al., 2007).Previous studies observed a high biometric plasticity in *H. stipulacea*, with the development of larger leaves and the production of significantly higher photosynthetic pigment levels at greater depths, but a reduction in percentage cover of the species (Lipkin 1979; Rotini et al., 2017; Winters et al., 2017). Sharon et al (2011) documented *H. stipulacea* growing at a depth of 48 m where it received less than 5% of surface photosynthetically active radiation (PAR), demonstrating that this species may extend to depths with less than 11% of surface PAR,

which was previously considered the PAR limit for seagrass survival (Duarte 1991). Mechanisms related to the exceptional capacity of *H. stipulacea* to tolerate strong gradients of light availability are not completely understood.

Seagrasses, like other marine primary producers, accumulate many essential fatty acids (EFA) (Behrens et al., 1996; Dalsgaard et al., 2003) which are not biosynthesised by higher trophic levels (Arts et al., 2001). In photosynthetic organisms, lipid metabolism and fatty acid synthesis play an important role in the formation of structural membranes and energy storage capability (Rabbani et al., 1998; Mendoza et al., 1999; Klyachko-Gurvich et al., 1999). Polyunsaturated fatty acids (PUFA) are mainly partitioned into structural lipids (glycolipids and phospholipids) constituting the cellular membranes. In particular, PUFA promote the fluidity of the thylakoid membranes of the chloroplasts, enhance the electron transport in the photosystems, and therefore optimise the photosynthetic activity (Gombos et al., 1994; Sanina et al., 2004, 2008). On the other hand, saturated fatty acids (SFA) are mainly partitioned into triacylglycerols (TAGs) which are used as reserve energy compounds (e.g. Bravo et al., 2001; Pal et al., 2011; Solovchenko 2012). In aquatic plants, fatty acid metabolism is strongly regulated by environmental factors such as temperature (Viso et al., 1993; Beca-Carretero et al., 2018*a*; Chapter 7).

Here, we hypothesize that both morphological and biochemical (photosynthetic pigments, fatty acids) adaptations of *H. stipulacea* occur in response to decreasing PAR along an increasing depth profile. To test this, we selected two independent transects along a depth gradient within a meadow of *H. stipulacea.* The two transects were exposed to otherwise similar environmental conditions to ensure that other factors, such as, hydrodynamic forces or anthropogenic pressures, did not significantly affect the structural and biochemical responses of the plants. We then assessed (i) changes in the leaves biometry and in TFA production and composition, (ii) shifts in photosynthetic pigment levels, (iii) any potential correlation between fatty acids and pigments with increasing depth, and finally, (iv) compared the contents of PUFA (% DW) and PUFA/SFA ratios in *H. stipulacea* from this study (northern GoA) to other seagrass populations worldwide.

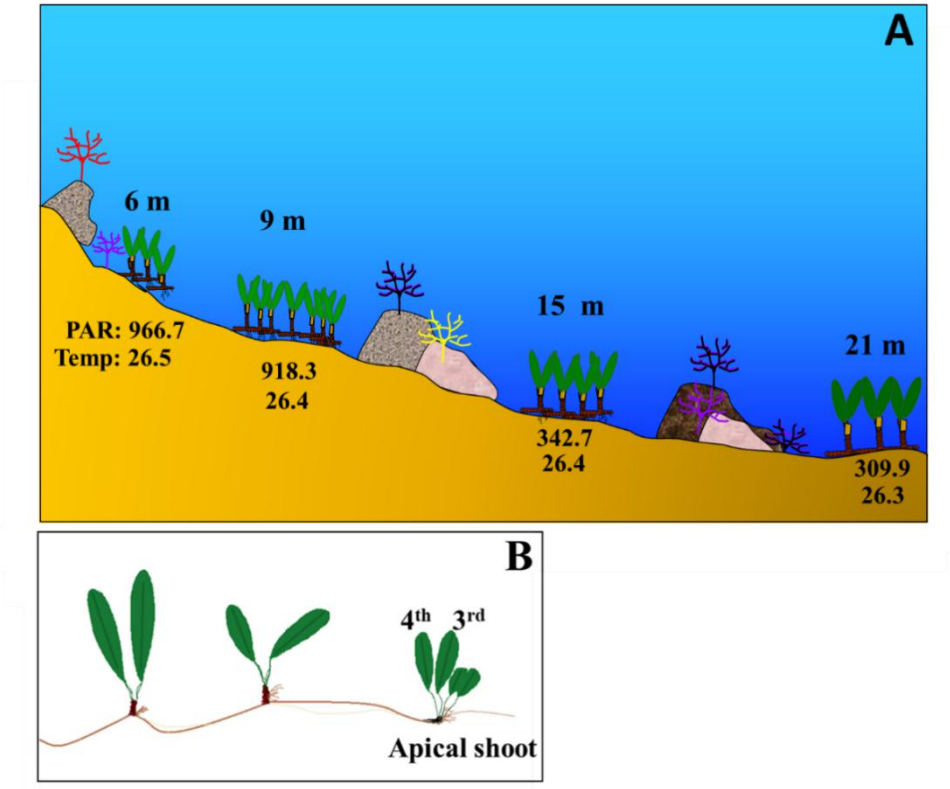

Fig. 8.1 Schematic drawings representing the sampling design at 3 different depth levels (6/9, 15, 21 m) in the *Halophila stipulacea* meadow modified from Rotini et al., (2016). (Panel A). Representation of the apical shoot highlighting the 3rd and 4th youngest leaves used for biochemical and morphological analysis (Panel B).

8.2. Methods

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8.2.1. Environmental variables

Long term (2011-2015) salinity, Secchi depth (Sd [m]), sea surface temperature (SST), surface water contents of NH_4, NO_2 and NO_3 measured at 1 m depth were obtained from Israel's National Monitoring of the Gulf of Eilat (September 2016; NMP; http://iui-eilat.ac.il/Research/NMPMeteoData.aspx). An estimation of the maximal underwater photosynthetically active radiation (PAR) at different depths was calculated following Beer-Lambert:

$$I_D=I_0e^{-Kd} \quad \text{(Eq. 8.1)}$$

With I_D= Light Intensity at depth (measured in Einsteins); I_0= Light Intensity at the Surface; K_d= is the light attenuation coefficient; D= Depth. K_d was estimated according to the Duarte (1991) equation: K_d= 1.7 Sd. *in-situ* data of temperature (˚C) and PAR (µmol photon m^{-2} s^{-1}) in adjacent waters (29.493 N, 34.909 E) (~ 200 - 400 m from T-1 and T-2, respectively) collected in July 2016 along a depth gradient (0 to 50 m) from Israel's National Monitoring of the Gulf of Eilat (S8.1) (http://www.meteo-tech.co.il).

8.2.2. Sample collection

The study area was located on the western shore of the northern tip of the GoA, northern Red Sea, Israel (Eilat; Fig. S8.1). The meadows studied belong to a medium-sized (61,900 m^2) seagrass area characterized by high light penetration, a pronounced slope (18^o), coarse-grained sediment with high hydrodynamics and low anthropogenic pressures (Mejia et al., 2016). *H. stipulacea* in this meadow co-occurs with hermatypic corals (Winters et al., 2017; Rotini et al., 2017). This study area was chosen because of its relative low human presence compared to other regions in the northern GoA (Mejia et al., 2016; Winters et al., 2017).

*H. stipulacea s*hoots were collected at noon from two different transects, T-1 (29.497 N, 34.911 E) and T-2 (29.500 N, 34.915 E; Fig. S8.1) at three different depths (6 – 9, 15 and 21 m) by SCUBA diving on 6th July 2016. Depths of 6 and 9m represented the shallowest part of the meadow in T-1 and T-2, respectively. Differences in depths in the shallowest edges of the *H. stipulacea* meadow were related to the larger widespread presence of coral reefs in T-2 (9 m) than in T-1 (6 m). In both transects, biomass was harvested at intervals of 5-10 m along each depth to potentially prevent resampling of the same genotypes as this marine plant is known as a clonal species (Kenworthy et al., 2000). We only selected apical shoots to ensure that the selected leaves were recently produced. *H. stipulacea* has a formation rate of new leaves at intervals of 4-12 d, depending on the season, with an average production of one leaf every 8.1 d (Wahbeh et al., 1984). Healthy shoots were transported to the laboratory where the third and fourth youngest leaves per apical shoot were selected for biochemical analysis (Fig. 8.1B).

8.2.3. Biometry

Leaf length (mm) of *H. stipulacea* was measured (±1 mm) using a ruler in 10 freeze-dried leaves from each site and depth, haphazardly selected for subsequent biochemical analyses.

8.2.4. Biochemical analyses

Selected healthy green leaves were manually cleaned in distilled water and epiphytes were removed. Fresh biomass was hand-dried on blotting paper before freezing at -80 °C for 6 h. Subsequently, leaves were freeze-dried for 12 h using a freeze-dryer system (Labconco Freezone, Kansas City, MO, USA) before storage at -30 °C (2-3 months), until analyses were conducted. Selected leaf biomass was then ground to a fine powder using a bead mill homogenizer Beadmill 4 (Fisher Scientific, USA) at 5 m s^{-1} for 3 min. Fatty acids analysis and photosynthetic pigments were performed using the gas chromatography (GC). See description in in methods in Chapter 3, Section 3.2.2. For each depth and location, we used three replicates (n = 3).

8.2.5. Biochemical comparison across seagrass populations worldwide

Data of % PUFA and PUFA/SFA ratios of seagrass from different locations worldwide were compiled from Beca-Carretero et al (2018) (Chapter 8) and Sousa et al (2017) and were compared with *H. stipulacea* data presented here. SST data were derived from the Bio-ORACLE database (http://www.bio-oracle.org) (Tyberghein et al., 2012) with a resolution of 0.5 km^2.

8.2.6. Statistical analyses

Prior to performing statistical analyses data were Ln transformed and the assumptions of normality (Kolmogorov–Smirnov test) and homogeneity (Bartlett test) were tested. A

two-way ANOVA and *post hoc* Tukey's pairwise test were conducted to evaluate differences between transects and among depths in the biochemical and morphological descriptors. Data treatments and statistical analyses were performed using IBM SPSS Statistics V13.0 (IBM Corporation, USA). All values are reported as means and standard deviation (SD). Moreover, for conducting cluster and SIMPROF (similarity profile analysis; Clarke et al., 2008) analysis, data of PUFA (% DW) and PUFA/SFA ratios from different seagrass species worldwide (n = 20) were first log-transformed and standardized. Then we resembled the data and performed the analysis using euclidean distance and the group average linkage method. Cluster and SIMPROF analyses were conducted using the statistical programme PRIMER&PERMANOVA 6.

8.3. Results

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8.3.1. Environmental variables

Estimated midday underwater PAR demonstrated the clarity of the coastal waters in the northern GoA, where values in the shallow parts of the meadows reach 1408.9 ± 87 and 969.8 ± 76 μmol photon m^{-2} s^{-1} at T-1 (6 m) and T-2 (9 m), respectively. Mid-day PAR levels in the deeper areas of the meadows (21 m) were of 352.8 ± 32 μmol photon m^{-2} s^{-1} (Table 1). These results were in accordance with *in-situ* measurements of in adjacent waters obtained in July 2016 (Fig. S8.2B, Table 8.1). Temperatures (°C) in July 2016 did not vary significantly from 6 m depth (26.5 °C) to 21 m depth (26.3 °C) (Fig. S8.2A, Table 8.1); particularly, the northern GoA is characterised by a marked summer thermal stratification of the water column (with a maximum during July-September) (Carlson et al., 2014).

Table 8.1 Environmental variables measured in the study area in June (2011 - 2015). Data of turbidity (Secchi depth (m)); estimated PAR at 6 – 9, 15 and 21 m; surface water temperature (˚C); surface water content of NH_4, NO_2, NO_3 (nmol/l). *In-situ* temperature (˚C) and PAR (µmol photon m^{-2} s^{-1}) measurements were taken at sampling depths of 6 – 9, 15, 21 m in adjacent waters in July 2016 (Israel's National Monitoring of the Gulf of Eilat) (http://www.meteo-tech.co.il).

Environmental descriptors in the study area (July 2011- July2015)		
Turbidity	Secchi depth (m)	21.5 ± 3.9
PAR	Estimated PAR at 6 / 9 m	1408.9 ± 87.0 / 969.8 ± 76.3
	Estimated PAR at 15 m	661.28 ± 48.2
	Estimated PAR at 21 m	352.8 ± 32.1
Water temperature	Surface water temperature (°C)	25. 3 ± 0.3
Nutrients	Surface water NH_4 (nmol/l)	50.8 ± 32.8
	Surface water NO_2 (µmol/l)	0.085 ±0.03
	Surface water NO_3 (µmol/l)	0.84 ± 0.2

Environmental descriptors in adjacent waters (July 2016)		
Depth (m)	**Temperature (°C)**	**PAR (µmol photon m^{-2} s^{-1})**
6	26.5	966.7
9	26.4	918.3
15	26.4	342.8
21	26.3	309.9

8.3.2 Biometry

Measurements of leaf lengths (averages of all leaves from all depths at each transect) revealed only small differences between transects (T-1 = 12.2 ± 0.7 mm, T-2 = 12.6 ± 0.7 mm). Leaf length displayed a similar pattern in both transects, with significant increases of 11 % % in T-1 and 12 % in T-2 in leaves from shallow depths compared with their shallower counterparts from the same transect from shallow to greater depths (ANOVA, $p < 0.05$) (Fig. 8.2, Table 8.3).

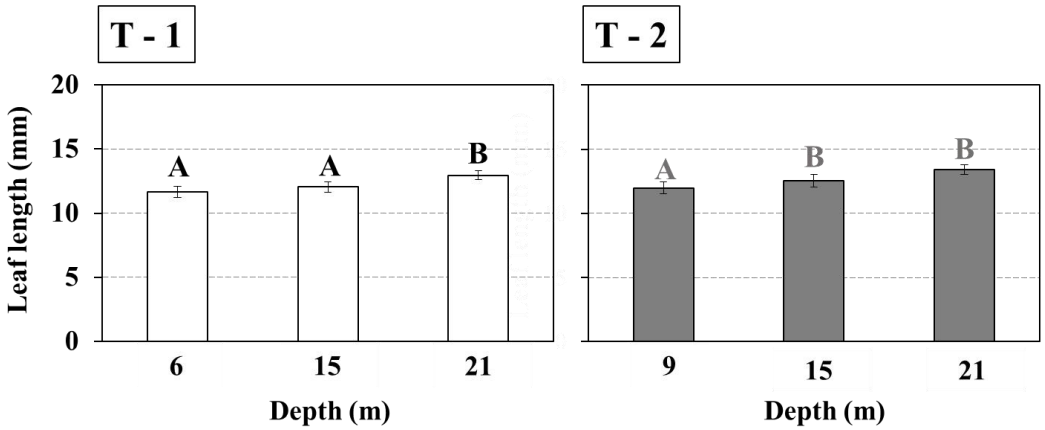


Fig. 8.2 Mean leaf (3rd and 4th youngest leaves (See details in Fig. 8.1)) length of freeze-dried samples of *Halophila stipulacea* at each transect with depth. Letters indicate significant differences between depths (T-1 = lowercase letters; T-2 = capital letters) according to the *post hoc* Tukey's pairwise test. Data are presented as mean ± standard deviation (n=10).

8.3.3. Biochemical content and composition of *Halophila stipulacea*

Details of total fatty acid content and composition of *H. stipulacea* leaves collected from the two transects (T-1 and T-2) across a depth gradient (6/9, 15, 21) are summarized in Table 8.2. Average total fatty acid (TFA) contents in samples T-1 (1.39 ± 0.2 % DW) and T-2 (1.42 ± 0.15 % DW) were similar (ANOVA, $p > 0.05$, Table 8.3, Fig. 8.3). In both transects, PUFA accounted for the larger percentages of FA with 66.0 ± 1.2 %, with α-linolenic acid (ALA, 18:3 n-3, 45.4 ± 0.6 %) and linoleic acid (LA, 18:2 n-6, 13.7 ± 0.4 %) present as the major PUFAs (Fig. 8.4, Table 8.2). Saturated fatty acid (SFA) represented 25.1 ± 1.0 % of TFA; palmitic acid (PAL, 16:0) being the main SFA with 23.4 % of TFA, followed by stearic acid (18:0) which accounted for 2.7 % (Fig. 8.4, Table 8.2). Finally, monounsaturated fatty acids (MUFA) accounted for 4.6 ± 0.3 % of TFA; the most abundant FA being oleic acid (OLE, 18:1 n-9) (2.9 ± 0.2 % of TFA) (Fig. 8.4, Table 8.2).

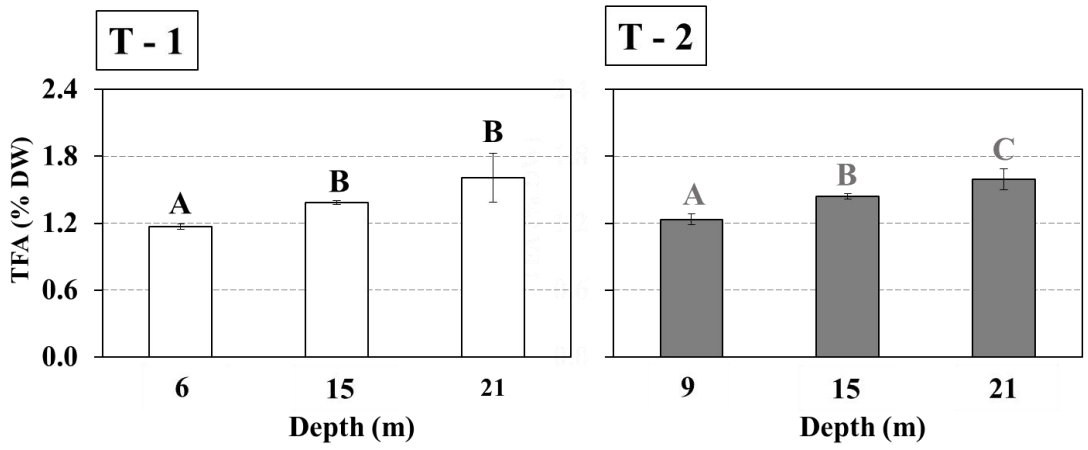


Fig. 8.3 Total fatty acid contents (% DW) of *Halophila stipulacea* collected at (Transect 1) T-1 and (Transect 2) T-2 across a depth gradient. Letters indicate significant differences between depths (T-1 = Black capital letters; T-2 = Grey capital letters) according to the *post hoc* Tukey's pairwise test. Data are presented as mean ± standard deviation (n=3).

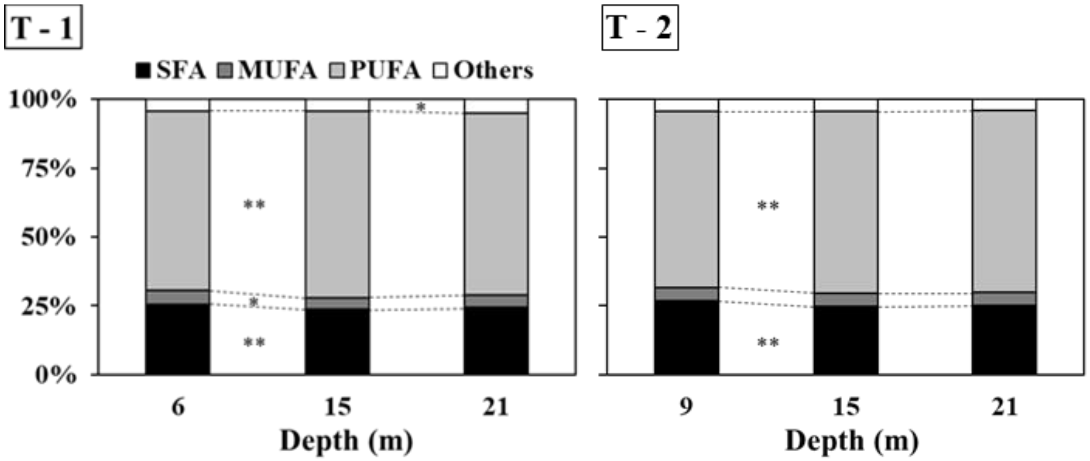


Fig. 8.4 Proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and non-identified (Others) fatty acids (% TFA) of T-1 and T-2 individuals. Differences between depths were evaluated according to the *post hoc* Tukey's pairwise test (** represents $p<0.01$ and * represents $p<0.05$-0.01) (n=3).

TFA content of leaves from both transects displayed similar trends, with significant increases from shallow to deep-adapted plants, while there were no marked significant (ANOVA, $p > 0.05$) differences between transects (Fig. 8.3). On the other hand, there were slight, but significant, differences in the percentages of both FA groups (PUFA and SFA) among transects (ANOVA, $p < 0.01$) (Fig. 8.4, Table 8.3). In both transects,

TFA content increased significantly with depth, from 1.16 ± 0.02 to 1.61 ± 0.2 % DW and from 1.23 ± 0.05 to 1.60 ± 0.1 % DW, respectively (ANOVA, $p < 0.001$) (Fig. 8.3, Tables 8.2 and 8.3). These changes were mainly associated with the markedly higher synthesis of PUFA in plants growing at greater depth (15 and 21 m) (66.6 ± 07 % of TFA) than those from shallow depths (6 and 9 m) (64.6 ± 0.6 % of TFA) (ANOVA, $p < 0.05$) (Fig. 8.4, Table 2). For both transects, there was a significant increase in the ratio n-3/n-6 PUFA from shallow- to deep-adapted plants (T-1 = 21.1 %, T-2 = 11.0 %). Levels of SFA in *H. stipulacea* leaves were, on average, 6 - 7 % higher in plants from shallow depths (6 and 9 meters), compared to those collected at 15 or 21 m (Fig. 8.4, Table 8.2). Finally, the percentages of MUFA also changed with depth, with largest values observed at 6/9 m (4.9 ± 0.1 % of TFA), and lowest at 21 m (4.4 ± 0.1 % of TFA) (ANOVA, $p < 0.01$), although there were no marked differences between plants from T-1 and T-2 (Fig 8.4, Table 8.2).

Photosynthetic pigments displayed slight, but significant, differences between T-1 (3.7 ± 0.3 mg g^{-1} DW) and T-2 (4.0 ± 0.3 mg g^{-1} DW) (Fig. 8.5, Table 8.3). In both transects, Chl. *a* was the most abundant photosynthetic pigment (T-1 = 2.2 ± 0.2 mg g^{-1} DW, T-2 = 2.5 ± 0.2 mg g^{-1} DW), followed by Chl. *b* (T-1 = 1.0 ± 0.1 mg g^{-1} DW, T-2 = 1.0 ± 0.1 mg g^{-1} DW), and finally total carotenoids (T-1 = 0.5 ± 0.1 mg g^{-1} DW, T-2 = 0.5 ± 0.04 mg g^{-1} DW). Overall, total pigment content significantly increased with depth (38 % in T-1, 18 % in T-2) (ANOVA, $p < 0.05$) (Fig. 8.5, Table 8. 3). Specifically, Chl. *a* increased by 27 % on average, Chl. *b* by 30 %, and carotenoids by 23 %.

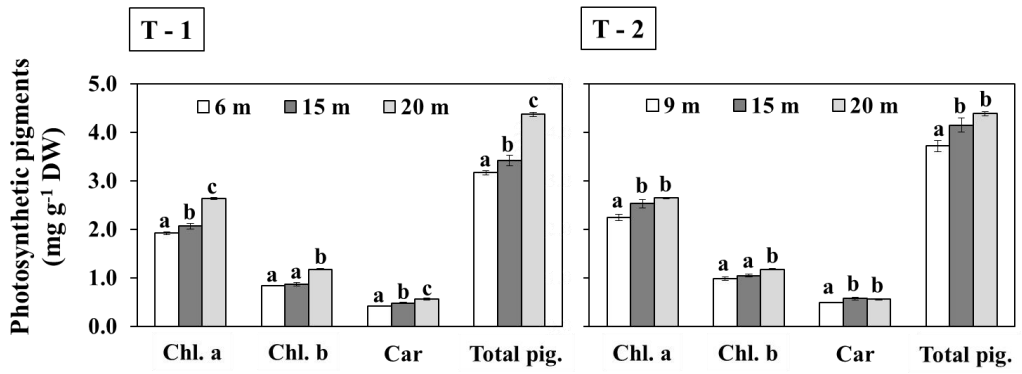


Fig. 8.5 Total pigment contents, chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*) and total carotenoids (Car) (mg g^{-1} DW) of T-1 and T-2 transects. Lowercase letters indicate significant differences between depths according to the *post hoc* Tukey's pairwise test (n=3).

8.3.4. Biochemical comparison across seagrass populations worldwide

Data of fatty acid composition compiled from the literature and unpublished data used to assess similarities between seagrass populations worldwide. Cluster and SIMPROF analysis of fatty acid composition of 18 studies, covering 11 species identified four distinct groups of seagrass species (Table 8.4). One group was characterized by lowest levels of PUFA (40.5 – 50.2 % DW) and low PUFA/SFA ratios (1.2 – 1.4) comprised by *Thalassia hemprichii*, *Halophila ovalis* and *Posidonia sinuosa*; the remaining groups were characterized by larger content of PUFA and higher ratios of PUFA/SFA (Fig. 8.7, Table 8.4). *H. stipulacea* was included in a subgroup with *Posidonia oceanica* (Spain), *Zostera marina* (Russia, Sea of Japan) and *Cymodocea nodosa* (Spain), with intermediate values of PUFA (65.7 – 67.6 %) and PUFA/SFA ratios (2.6 – 3.0). Finally, *Phyllospadix iwatensis* and two populations of *Z. marina* (Russia, Sea of Japan and China, Yellow Sea) formed another group, characterized by highest values of PUFA (72.2 – 74.3 %) and SFA/PUFA ratios (3.8 – 4.1) (Table 8.4).

Table 8.2. Data of fatty acid composition compiled from the literature and unpublished data used to assess similarities between seagrass populations worldwide.

ID	References	Species	Location	Latitude	Mean SST	PUFA (% TFA)	PUFA/SFA
1	Our data	*Zostera marina*	Ireland, Kilkieran Bay	53.2	11.8	70.2	3.3
2	Our data	*Zostera noltii*	Ireland, Kilkieran Bay	53.3	12.0	70.1	3.1
3	This study	*Halophila stipulacea*	Israel, Red Sea	29.5	24.0	66.6	2.8
4	Sousa et al (2017)	*Zostera noltii*	Portugal, Ria Aveiro	40.4	16.1	62.1	2.1
5	Beca-Carretero et al (2018)	*Posidonia oceanica*	Spain, Cala Montgo	40.7	19.1	65.7	2.9
6	Beca-Carretero et al (2018)	*Posidonia oceanica*	Spain, Delta del Ebro	42.1	17.2	66.0	2.9
7	Beca-Carretero et al (2018)	*Cymodocea nodosa*	Spain, Cala Montgo	40.7	19.1	67.6	3.0
8	Beca-Carretero et al (2018)	*Cymodocea nodosa*	Spain, Delta del Ebro	42.1	17.2	66.5	3.0
9	Sanina et al (2004)	*Zostera marina*	Russia, Sea of Japan	43.9	8.2	74.3	4.1
10	Vaskovsy et al (1996)	*Zostera marina*	China, Yellow Sea	35.0	15.2	72.2	3.8
11	Vaskovsy et al (1996)	*Phyllospadix iwatensis*	China, Yellow Sea	35.0	15.2	72.2	3.9
12	Coelho et al (2011)	*Zostera noltii*	Portugal, Ria Aveiro	40.6	16.1	64.8	2.1
13	Nicholst et al (1985)	*Thallassia hemprichii*	USA, Lizard Island	-16.9	26.1	40.5	1.2
14	Kharlamenko et al (2001)	*Zostera marina*	Russia, Sea of Japan	42.5	9.3	66.4	2.6
15	Hanson et al (2010)	*Halophila ovalis*	Australia, Bay Marine Park	-30.5	21.5	48.1	1.2
16	Hanson et al (2010)	*Posidonia sinuosa*	Australia, Bay Marine Park	-30.5	21.5	50.2	1.4
17	Richoux et al (2008)	*Zostera capensis*	South Africa, Kariega Estuary	-33.7	20.4	60.0	1.7
18	Jeffries et al (1972)	*Ruppia maritima*	USA, Rodhe Island	41.5	12.6	58.5	2.9
19	Viso. et al (1993)	*Posidonia oceanica*	Grecce, Athens	37.9	20.2	51.4	1.3
20	Viso. et al (1993)	*Posidonia oceanica*	France, Villefrnache-Sur-Mer	43.7	18.5	70.0	3.1

Table 8.3. Effect of location (Transect 1(T-1) and transect 2 (T-2)) (L) and depth (6/9, 15 and 21 m) (D) on the synthesis of TFA content (% DW) and composition (% TFA), and on the production of total pigment level, chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*) and carotenoids (mg g^{-1} DW) and also in the length (mm $shoot^{-1}$) on leaves of *Halophila stipulacean* F-values of two-way ANOVA are shown along with significance levels (*p < 0.05; **p < 0.01, ***p < 0.001).

		df	*MS*	*F*
PUFA	Transect (T)	1	0.001	25.7**
	Depth (D)	2	0.002	62.7***
	TxD	2	0.001	6.3*
SFA	Transect (T)	1	0.01	21.7**
	Depth (D)	2	0.01	21.4**
	TxD	2	0.00	2.9
MUFA	Transect (T)	1	0.01	1.2
	Depth (D)	2	0.03	5.5*
	TxD	2	0.01	1.6
TFA	Transect (T)	1	0.00	1.0
	Depth (D)	2	0.10	26.0***
	TxD	2	0.02	5.8*
Chl. *a*	Transect (T)	1	0.10	241.1***
	Depth (D)	2	0.09	233.5***
	TxD	2	0.03	69.2***
Chl. *b*	Transect (T)	1	0.08	160.2***
	Depth (D)	2	0.13	256.3***
	TxD	2	0.02	41.4***
Carotenes	Transect (T)	1	0.12	141.7***
	Depth (D)	2	0.07	82.5***
	TxD	2	0.04	44.7***
Total pigment	Transect (T)	1	0.10	213.4***
	Depth (D)	2	0.10	219.8***
	TxD	2	0.03	60.3***
Leaf length	Transect (T)	1	0.02	11.9*
	Depth (D)	2	0.06	45.3***
	TxD	2	0.00	0.1

Table 8.4. Total fatty acid content (% DW) and composition (% TFA) of *H. stipulacea* at transect 1(T-1) and transect 2 (T-2) across a depth gradient (6/9, 15, 21). Results are expressed as mean ± SD (n = 3).

Halophila stipulacea						
Location	**T-1**			**T-2**		
Depth	**6**	**15**	**21**	**9**	**15**	**21**
Fatty acids (% TFA)						
Saturated fatty acids						
14:0	0.5 ± 0.1	0.9 ± 0.01	0.4 ± 0.01	0.6 ± 0.05	0.5 ± 0.1	0.4 ± 0.1
16:0	23.0 ± 0.2	21.4 ± 0.4	22.3 ± 0.2	23.6 ± 0.2	19.8 ± 0.2	22.7 ± 0.3
18:0	2.0 ± 0.3	2.3 ± 0.1	2.0 ± 0.01	2.7 ± 0.05	2.5 ± 0.3	2.2 ± 0.1
Sum SFA	**25.5 ± 0.3**	**24.6 ± 0.3**	**24.5 ± 0.3**	**26.8 ± 0.2**	**25.5 ± 0.3**	**25.3 ± 0.3**
Monounsaturated fatty acids						
14:1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.01	0.6 ± 0.05	0.6 ± 0.1	0.6 ± 0.05
16:1 n-7	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.5 ± 0.3
18:1 n-7	0.5 ± 0.1	0.8 ±0.1	0.4 ± 0.05	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.05
18:1 n-9	3.2 ± 0.1	2.9 ± 0.6	2.4 ± 0.1	2.8 ± 0.05	3.2 ± 0.1	2.9 ± 0.1
Sum MUFA	**5.0 ± 0.1**	**4.2 ± 0.2**	**4.3 ± 0.1**	**4.8 ± 0.1**	**5.0 ± 0.1**	**4.5 ± 0.2**
Polyunsaturated fatty acids						
18:2 n-6	14.9 ± 0.4	12.9 ± 0.4	13 ± 0.2	13.3 ± 0.2	14.8 ± 0.4	13.5 ± 0.2
18:3 n-3	44.6 ± 0.5	47.6 ± 0.4	47.1 ± 0.2	45.1 ± 0.3	44.6 ± 0.4	47.3 ± 0.6
18 :3 n-6	0.3 ± 0.05	0.3 ± 0.02	0.2 ± 0.02	0.2 ± 0.05	0.3 ± 0.05	0.2 ± 0.1
18:4 n-3	0.3 ± 0.03	0.4 ± 0.04	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.01	0.3 ± 0.1
20:4 n-6	0.6 ± 0.1	1.1 ± 0.2	0.3 ± 0.02	0.7 ± 0.04	0.7 ± 0.5	0.6 ± 0.05
20:5 n-3	0.3 ± 0.05	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.05	0.3 ± 0.1	0.4 ± 0.1
Sum PUFA	**65.1 ± 0.4**	**67.8 ± 0.4**	**67.4 ± 0.2**	**64.0 ± 0.2**	**65.2 ± 0.3**	**66.2 ± 0.4**
n-3/n-6	2.9 ± 0.2	3.4 ± 0.1	3.5 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.3 ± 0.2
PUFA/SFA	2.6 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.6 ± 0.3
Others	**4.3 ± 0.2**	**4.2 ± 0.2**	**4.9 ± 0.2**	**4.4 ± 0.2**	**4.3 ± 0.1**	**4.0 ± 0.3**
Fatty acids (% DW)	**1.16 ± 0.02**	**1.39 ± 0.02**	**1.61 ± 0.2**	**1.23 ± 0.05**	**1.44 ± 0.01**	**1.60 ± 0.1**

8.4. Discussion

This section has been modified from the original article

8.4.1. TFA content and composition in *Halophila stipulacea*

Pooled averages of total fatty acid (TFA) content of samples collected from the two transects were similar which could be expected due to their close proximity (less than 500 m apart; Fig. S8.1); they were exposed to similar environmental conditions, and low anthropogenic pressures (Mejia et al., 2016; Winters et al., 2017). Polyunsaturated fatty acids (PUFA) were the dominant FA group present in *H. stipulacea* leaves, with an average of 66.0 ± 1.2 % of TFA, where the nutritionally important α-linolenic acid (ALA) and linoleic acid (LA) were the dominant PUFAs. Most PUFAs are considered essential fatty acids (EFA) being precursors to the LC-PUFA [e.g. eicosapentaenoic (EPA, 20:5 n-3), arachidonic (ARA, 20:4 n-3) and docosahexaenoic (DHA, 22:6 n-3) acids] which play an essential role in the state of health of consumers of the next trophic levels (Brett et al. 1997). For example, ARA and EPA regulate the optimal physiological state in the reproductive and juvenile stages of several marine species (Harel et al., 2002). Indeed, recent investigations in marine aquaculture have observed an optimization of the growth and health of important commercial fish species (*Oreochromis niloticus)* when seaweeds diets were enriched with LC-PUFA (Tian et al. 2014). In GoA *H. stipulacea* biomass represents an important food source for different species including sea urchin species (*Tripneustes gratilla*) (Hulings & Kirkman 1982) and also some charismatic animals such as green turtles (*Chelonia mydas*) (Turkozan & Durmus 2000; Christianen et al., 2014, 2018).

8.4.2. Effects of light attenuation on the synthesis of FA

Our results demonstrate a slight, but significant, increase in leaf length of *H. stipulacea* plants from shallow to deep areas which was likely to be caused by changes in light availability rather than temperature. Previous studies of *H.*

stipulacea also reported this morphological plasticity which coincided with reductions in PAR at increasing depths (Rotini et al., 2017); such morphological changes are associated with enhanced light absorption at increasing depths (Olesen et al., 1993; Lee et al., 2007, Sharon et al. 2011). Results also revealed a high capacity of *H. stipulacea* to acclimate FA levels and composition to environmental conditions at different depths. In both transects, TFA content increased with depth; such changes were mainly associated with the higher synthesis of PUFA in plants growing in deeper/reduced PAR habitats (15 and 21 m). Interestingly, the ratio n-3/n-6 also increased, pointing towards a major production in the content of fatty acids with a higher level of unsaturation. In terrestrial plants, an increment in the synthesis of n-3, mainly 18:3, was associated with decreases in temperature, increments in salt and pathogen stress (Upchurch 2008; Yaeno et al., 2004). Recent investigations with temperate *Zostera marina* also found similar trends with slight but marked increases of 8 % of TFA (% DW) and 7 % of PUFA (% TFA) in plants acclimated to low irradiance (60 μmol m^{-2} s^{-1}) than those incubated at saturating (high) irradiance levels (180 μmol m^{-2} s^{-1}) (Chapter 6). Previous studies on other marine primary producers, such as microalgae and macroalgae, reported a species-specific response of the lipid and fatty acid metabolism to changing light and temperature regimes occurring with season (Schmid et al., 2017a, 2017b) or culture conditions (e.g. Guihéneuf & Stengel 2013). In some species, reduction in light availability induced remodelling of the lipids within membrane structures by increasing the content of TFA, more specifically the proportion of PUFA (e.g. ALA), and reducing the ratio of SFA (Schmid et al., 2014, 2017a, 2017b). Also, under low light conditions, PUFA synthesis and production is induced, and therefore, phytoplankton species developed larger thylakoid membranes than microalgae exposed to higher irradiances (Goss & Wilhelm 2010). This adaptive mechanism can be expected as PUFA control chloroplast membrane fluidity and facilitate photosynthetic electron transport, thus promoting photosynthetic activity (Gombos et al., 1994; Moon et al., 1995). Taken together, these studies suggest that the regulation of the PUFA composition in photosynthetic structures, is an essential mechanism of photosensitive primary producers to ensure their optimal physiological functioning under different irradiance gradients.

In seagrasses, increases in the production of SFA were previously associated with their exposure to different environmental stressors, including increases in salinity and elevated temperatures; for instance, exposure of *Posidonia oceanica* and *Cymodocea nodosa* to artificial heat-waves increased the synthesis and accumulation of SFA (Sousa et al., 2017; Beca-Carretero et al., 2018). The production of TAG, primarily constituted by SFA, is a typical mechanism of primary producers to store the excess energy generated by photosynthesis which increases under unfavourable growth conditions (Goncharova et al., 2004; Pal et al., 2011; Solovchenko 2012). Therefore, as *H. stipulacea* in this part of the northern GoA is distributed at depths ranging from 2 to > 50 m (Sharon et al., 2011; Winters et al., 2017), the results presented here suggest that *H. stipulacea* plants at shallow depths experience some environmental stress which induced a higher proportion of SFA. These observations are supported by previous studies in the same region of GoA (South Beach), which reported a high photoinhibition effect from shallow *H. stipulacea* populations, which negatively affects their performance (Mejia et al., 2016). The synthesis of SFA in seagrass leaves may be considered to be readily accessible energy reserves in above-ground structures as was previously observed in terrestrial plants and other marine primary producers (e.g. Alberdi and Corcuera 1991; Bravo et al., 2001; Pal et al. 2011; Solovchenko 2012).

8.4.3. Correlation between photosynthetic pigments and fatty acids

Recently, Rotini (2017) reported the development of larger surface areas (increase of 19.4 %) and higher pigment contents (Chl. *a* and *b*) as a function of increasing depth. Moreover, field experiments of transplantation of *H. stipulacea* to different depths (8 and 33 m, and *vice versa*) showed a rapid (within less than 7 d) physiological acclimatization that included and adjustment of its photosynthetic response (quantum yield efficiency of photosystem II; F_v/F_m) and pigment production, highlighting the large plasticity of this species to adjust to changes in light availability (Sharon et al. 2009). The synthesis of higher levels of photosynthetic pigments is a well-described

adaptive mechanism of seagrasses and other primary producers to increase photosynthetic efficiency in low-light habitats (Cummings & Zimmerman 2003; Lee et al., 2007).

Both total pigments (mg g^{-1} DW) and Chl. *a* (mg g^{-1} DW) were significantly correlated ($p < 0.001$, $R^2 = 0.7$) with TFA and PUFA contents (% DW), respectively (Fig. 8.6), as both components increased with depth. Collectively, these outcomes highlight the ability of *H. stipulacea* to adjust its photosynthetic apparatus to clearly optimise the use of the environmental light resources.

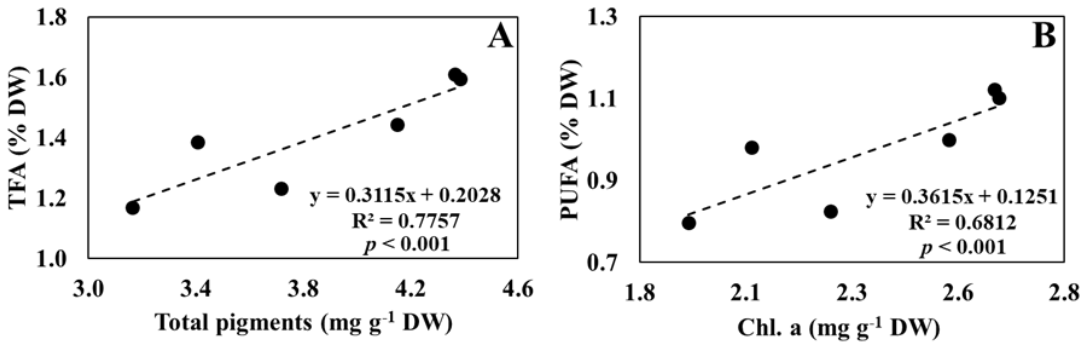


Fig. 8.6 Linear correlations between total fatty acid (TFA) (% DW) and total pigment (mg g^{-1} DW) contents (Panel A), and PUFA (% DW) and Chl. *a* (mg g^{-1} DW) contents (Panel B). Dotted line represents the linear regression line.

Cluster and SIMPROF analysis were used to compare fatty acid composition of seagrass species from different geographic regions. Similar levels of SFA, ranging from 17.5 to 39.8 %, and MUFA from 1.9 % to 6.4 %, were observed in other seagrasses worldwide (e.g. Vaskovsy et al., 1996; Nichols & John 1985; Sanina et al., 2004). Our analysis revealed that other seagrass species adapted to similar ranges of temperature (21-27 ºC; Winters et al. 2017), such as species belonging to the same genus, *Halophila ovalis* (48.12 % of TFA, SST = 21.5 °C) or *Thallassia hemiprichii* (40.63 % of TFA, SST = 26.1 °C) or *Posidonia sinuosa* (50.21 %, SST = 21.5 °C), contained significantly lower levels of PUFA than shallow *H. stipulacea* (64.6 ± 0.6 % of TFA) (Nichols & John 1985, Beca-Carretero et al. 2018). By contrast, PUFA levels and PUFA/SFA ratios of *H. stipulacea* seemed to be equivalent to

Mediterranean temperate species such as *Posidonia oceanica* or *Cymodocea nodosa* (68.3 % of TFA, SST = 17.5 °C), or even to species living at higher latitudes and much colder environments such as *Zostera noltii* (64.8 % of TFA, SST = 16.8 °C) (Viso et al., 1993; Coelho et al., 2011; Beca-Carretero et al., 2018). These outcomes suggest that the larger levels of PUFA (e.g. ALA and LA) and PUFA/SFA ratios observed in *H. stipulacea*, renders this species to be of higher lipid nutritional value than other seagrass species adapted to similar temperatures and latitudes, including other species of *Halophila*. In terrestrial plants, species such as soybean, tobacco or Arabidopsis, with the capacity to biosynthesize higher levels of PUFA were able to adapt better to colder environments than species which produced lower contents of PUFA (Routaboul et al., 2000; Iba 2002; Alberdi & Corcuera 1991). It has been widely demonstrated that the different levels of unsaturation in the bio-membranes is the most relevant factor controlling the fluidity of membrane lipids of plants (e.g. Cossins 1984; Upchurch 2008) and this was related to their thermal physiological limits for growth and survival (Iba et al., 2002). In seagrasses, the synthesis of PUFA was also linked to their thermal adaptation (decrease of 2.12 % of PUFA (% of TFA) per increase of 1 °C in SST; (Beca-Carretero et al., 2018; (Chapter 8)). Thus, the anomalously high levels of PUFA within *H. stipulacea* may partially explain its capacity to spread to colder habitats outside its native distribution ranges. For example, this species is able to survive winter temperatures in Mediterranean Sea at ~ 13-16 °C (http://www.bio-oracle.org) (Tyberghein et al., 2012; Gambi et al., 2008; Neguyen et al., 2018), while in native areas winter temperatures reach approximately ~ 21 °C.

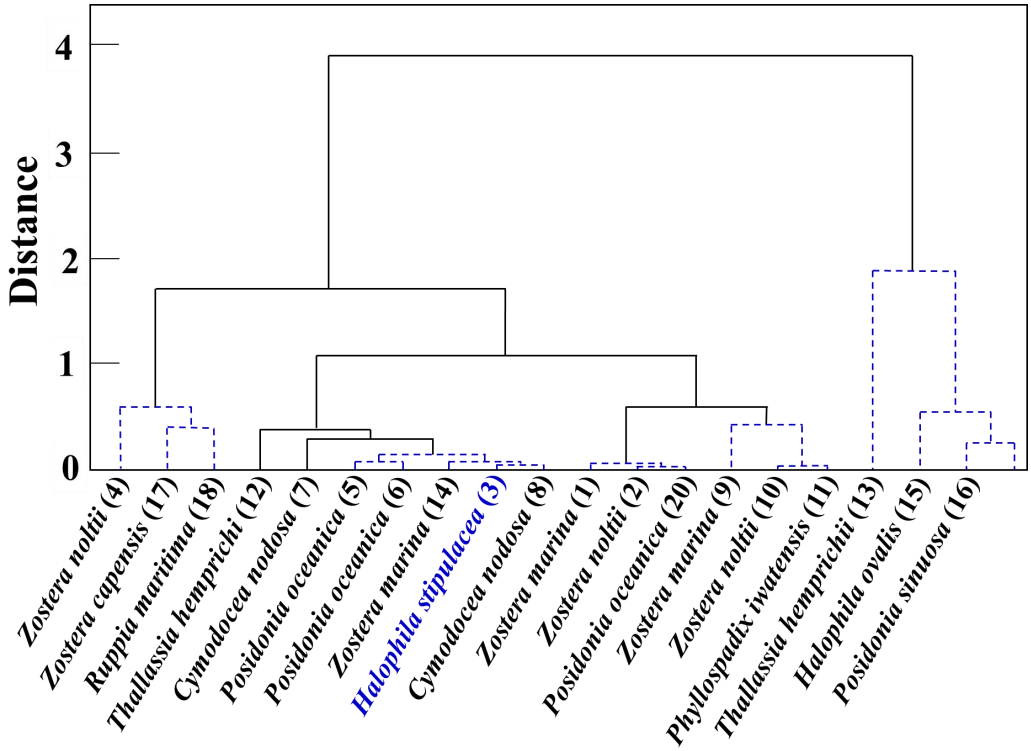
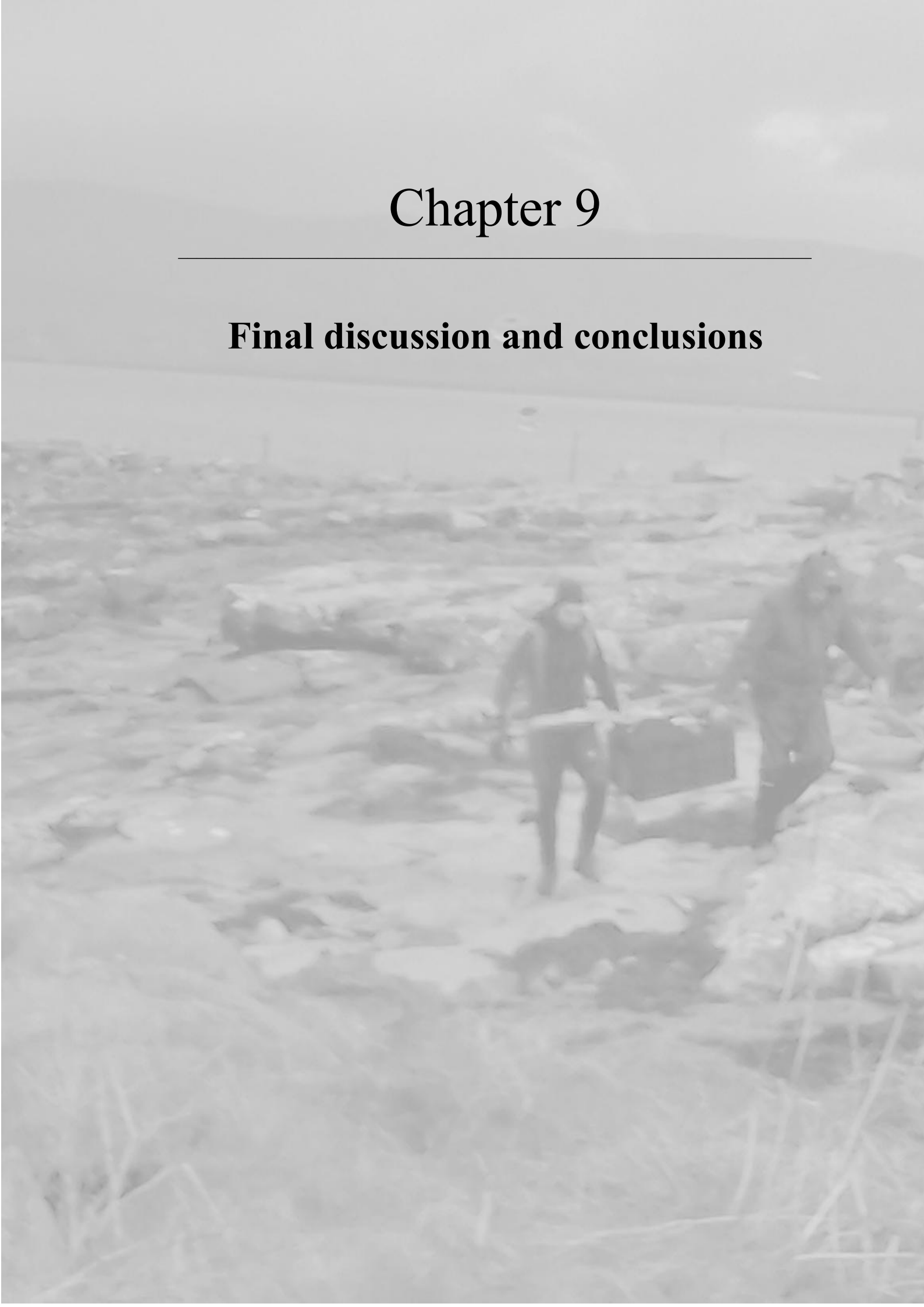


Fig. 8.7 Cluster and SIMPROF analysis of 10 species from 20 seagrass populations worldwide based on content of PUFA (% DW) and PUFA/SFA ratio. Numbers represent the results from the literature review and from the present study (Table 8.2).

In both native and non-native areas, *H. stipulacea* displays a great capacity to survive in (i) environments with low irradiance levels, having been observed as the seagrass species living at greatest depths (e.g. Ruiz and Ballantine 2004; Willette et al., 2014; De Troch et al., 2001), and in (ii) coastal regions with depletion in water transparency and quality due anthropogenic pressures where *H. stipulacea* was reported to be the dominant seagrass species (Kenworthy et al., 1993). Moreover, recent field observations suggest that *H. stipulacea* may be potentially displacing the native *Cymodocea nodosa* in the Mediterranean along the in Tunisian coast (Sghaier et al., 2014). Similarly, studies from the Caribbean have experimentally tested that *H. stipulacea* is physically displacing the local Caribbean seagrass species *Syringodium filiforme*, pushing this species out by monopolizing its space (Willette & Chalifour 2014; Steiner & Willette 2015).

Chapter 9

Final discussion and conclusions



In this thesis we aimed to address the research questions proposed in the introduction which were related to (i) the ecological characteristic of Irish seagrasses regarding temporal growth and population dynamics, (ii) variations in fatty acid content and composition in respond to variations in climate and other environmental conditions, (iii) the potential larger extent of seagrass meadows in Ireland, and finally (iv) the effect of projected climate scenarios in the affect the lipid nutritional value of seagrasses.

Firstly, we conducted seasonal monitoring projects comparing both shallow and deep adapted populations. Using the same populations, we also investigated their biochemical composition, such as fatty acid and pigment contents, and related these to morphological variations. Additionally, we studied morphological and biochemical variation from *Halophila stipulacea* plants from the Red Sea living under different irradiance conditions. These projects allowed us, for the first time, to elucidate the role that FA, and particularly PUFA and SFA, may play in seagrass adaptations. Moreover, we applied our obtained knowledge in FA synthesis, to study potential effects of projected warming in seagrasses in Irish populations and Mediterranean species. Finally, we implemented different approaches to discover new seagrass habitats/meadows in Ireland at a relative low cost, allowing us to map large areas of new meadows in Ireland.

9.1. Temporal morphological and population responses of western Irish *Zostera marina* meadows

Our objectives were to confirm that Irish eelgrasses may show comparable temporal growth patterns and population structures to *Z. marina* populations inhabiting similar climate regimes. In our study, the descriptors at individual and population level displayed clear temporal responses. Most parameters revealed maximum values attained in July, intermediate in April and November, and lowest values observed in January. Such results could have been expected as optimal conditions for photosynthesis in cold-temperate *Z. marina* populations are typically reached in

summer periods, where eelgrasses increase their active leaf structures and to maximise the storage of energy-rich compounds such as carbohydrates (Bay et al., 1996; Alcoverro et al., 2001). On the contrary, during less favourable darker and colder periods, eelgrasses reduce their leaf structures and metabolism and use their energy reserves (e.g. Duarte 1989; Olesen & Sand-Jensen 1993; Lee et al., 2005; Chapter 2). Also, our results highlight that Irish *Z. marina* populations form monospecific stands with growth rates similar to other perennial populations in temperate regions; this is considered a typical strategy of eelgrass in stable environments (i.e. Olesen & Sand-Jensen 1993, 1994; Cabello-Pasini et al., 2003; Clausen et al., 2014). The shoot densities of the Irish meadows studied (50 to 800 shoots per m^2) were similar to other cold-temperate *Z. marina* (e.g. Aioi et al., 1980; Olesen & Sand-Jensen 1994; Bostrom et al., 2006; Watanabe et al., 2005), whereas higher densities (> 3000 shoots per m^2) were observed at similar latitudes (e.g. Wium-Andersen & Borum 1984; Krause-Jensen et al., 2000). The influence of local environmental fluctuations on the reproductive effort and flowering of *Z. marina* has been extensively documented (e.g. Meling-Lopez et al., 2014; Harwell et al., 2002; Lee et al., 2006). In the meadows studied, the presence of early-stage reproductive shoots was observed in March-April (SST of ~ 10 °C); in July (SST of ~ 16 °C) we reported the highest density with mature male and female flowers and seeds and finally, in November (SST of ~ 10 °C), we observed the presence of flowering plants and seeds, pointing out that the reproductive period of Irish eelgrasses spans 9 months (Chapter 2).

The second objective was to assess potential temporal differences in morphological and population responses of plants living at their deepest distribution limit, versus plants inhabiting shallow sites. Populations living in deeper environments appeared to be characterized by a relative presence of larger shoots with higher biomass than shoots from shallower populations. These morphological characteristics were accompanied by 2-3 fold decreases in above-ground biomass, eelgrass cover and density with increasing depth; this trend has been widely observed in deep cold-temperate *Z. marina* populations (e.g. Lalumiere et al., 1994; Olesen et al., 2002; Nielsen et al., 2002). Overall, these results support previous evidence in cold-adapted eelgrass populations which indicated that deep adapted plants favour vertical growth

rather that the horizontal growth, which is a well-known response of seagrasses to depletion in irradiance with increases in depth (i.e. Sand-Jensen & Madsen 1991; Ralph et al., 2007). Such results were expected since light is considered the controlling factor of eelgrass metabolism, distribution and abundance, especially towards the lower depth limit of a meadow (Duarte 1991; Krause-Jensen et al., 2000; Watanable et al., 2005) (Chapter 2). These results were accompanied with a biochemical acclimatization with increases in pigments and PUFA levels with decreases in irradiance (Chapter 3).

The results presented here are the most detailed Irish monitoring baseline to date, and represent a vital contribution for the management and conservation of this precious ecosystem in Ireland. These new data will support future projects assessing potential shifts at both shoot and population levels, which is particularly relevant in the context of climate change. We also suggest completing these monitoring programs investigating other parameters such as photosynthetic responses or analysing phenol contents which provide relevant information regarding the physiological state of marine plants. Moreover, we propose to establish continuous monitoring programs in other geographical regions in Ireland to obtain a more complete overview of Irish seagrass ecology.

9.2. Temporal and depth-induced morphological and biochemical adjustments

We proposed that biochemical adjustments in seagrasses, in particular the synthesis of fatty acids (FAs) and photosynthetic pigments occur in response to varying climate parameters, such as temperature and irradiance. Also, we hypothesise that understanding these variations may allow us to assess the eco-physiological status of eelgrass populations. To the best of our knowledge, for the first time, the results included in Chapter 3 point out clear and significant spatial and temporal patterns in key biochemical compounds such as FA content and composition and photosynthetic pigments of shallow and deep adapted Irish eelgrass populations. Particularly, in winter seagrass leaves accumulated 2-3 times more TFA and photosynthetic

pigments than in summer. Increases in TFA were mainly linked to the larger synthesis of polyunsaturated fatty acids (PUFA) and the lower production of saturated fatty acids (SFA) in colder periods whereas in warmer months the opposite pattern was observed in the production of these compounds. These results can be explained by the low temperatures and irradiances increasing the degree of unsaturation of thylakoid membrane lipids, which were previously considered to be a physiological mechanism of primary producers to maintain optimal fluidity conditions in cold seasons (Murata et al., 1982; Alberti et al., 2002). In more favourable environmental conditions, we observed the accumulation of storage lipids, such as TAG containing mostly SFAs, which was previously described as a physiological mechanism to transform and store the excess energy generated by photosynthesis (Goncharova et al., 2004; Pal et al., 2011; Solovchenko 2012). Increases in energetic compounds such as carbohydrates in summer months, is considered to be an adaptive strategy of eelgrasses to survive coming colder and darker months in winter (Alcoverro et al., 2011). These temporal results confirmed for the first time that seagrasses behave similarly to other terrestrial plants and seaweeds, readjusting their lipid profiles regarding their physiological demands.

Moreover, in Chapters 3 and 8 we studied depth acclimatization of seagrasses comparing biochemical and morphological responses of Irish *Z. marina* populations and *H. stipulacea* from the Red Sea. Overall, for the two species, the results suggested similar patterns, with more extensive leaf structures observed with reductions in irradiance, which were accompanied by a greater content of photosynthetic pigments and increases in TFA levels. For example, leaves from-deep adapted *Z. marina* plants were 24 % larger than shallow populations (Chapter 2), whereas in *H. stipulacea* leaf length increased by 9 %. Increases in leaf surface area is widely known to favour optimal light absorption and the photosynthesis (i.e. Olesen et al., 1993; Cummings & Zimmerman 2003). In both Irish eelgrasses and *H. stipulacea* populations from the Red Sea, we observed significant increases in pigments at higher depths. Such changes have been widely documented in seagrasses as lower irradiances increase the pigment production to ensure optimal light absorption (e.g. Cummings et al., 2003; Olive et al., 2013). Furthermore, we observed that deep-adapted Irish eelgrass and *H. stipulacea* populations produced

higher levels of TFA, particularly n-3 PUFA, than shallow adapted populations which were significantly, and positively, correlated with the pigment production. These results suggest that seagrasses adapted to darker environments differently adjust their thylakoid membranes by increasing their degree of desaturation compared to shallow plants. Similar conclusions were also derived from Chapter 6, where *Z. marina* plants exposed to low irradiance accumulated slightly larger amounts of TFA and PUFA than plants incubated at higher irradiance levels. Previously, experimental studies of microalgae reported that under low light conditions, PUFA accumulation occurred, alongside the development of larger thylakoid membranes (e.g. Khoeyi et al., 2012; Goss & Wilhelm 2010).

These results are particularly relevant because, for the first time, we demonstrated the potential role that FA, and particularly PUFA, may play as a acclimatory mechanism for seagrasses to cope with different irradiance levels, which couple with the synthesis of photosynthetic pigments and biometric responses. Future projects may combine variations in biochemical composition of seagrass leaves with pulse amplitude modulated (PAM) fluorometry measurements and/or oxygen production, to better elucidate the role that FA content and composition may play in the eco-physiological status of seagrasses. Moreover, future investigations may address how these compounds may vary in other structures of the seagrasses, such as, rhizomes or roots, and how FA responses are correlated with variations in other energy-compounds such as carbohydrates. Additionally, future projects may investigate if there are specific thresholds in the production of PUFA and SFA regarding the thermal physiological limits of seagrasses.

9.3. Fatty acid production and composition under different thermal conditions

Prior studies of terrestrial and marine primary producers revealed a considerable dependence on the synthesis of lipids and fatty acids to thermal regimens, and related different levels of unsaturation in the membrane of the thylakoids with their thermal physiological limits (Iba et al., 2002; Cossins 1984;

Sanina 2004; Upchurch 2008). Therefore, we proposed that, in seagrasses, we should observe clear responses of these target compounds in response to thermal stress conditions. Results from Chapter 6 (Irish *Z. marina* populations) and in Chapter 7 (two Mediterranean seagrasses) reported significant depletions in PUFA/SFA ratios under higher temperatures, pointing out that warming may negatively affect the lipid nutritional value of seagrasses. These results were reinforced by data from the latitudinal study comparing samples of *Z. marina* populations collected in summer (Chapter 3). These results highlighted that southern-distributed plants produced the largest proportions of SFA, whereas Greenland populations produced a higher content of PUFA, representing, to date, the seagrass population with the highest n-3 PUFA worldwide. Reduction in PUFA under warming conditions can be explained by a lower requirement for PUFA to maintain optimal membrane fluidity. These results are particularly interesting in the context of understanding the effects that projected scenarios of climate change may play in the synthesis of important nutritional components, and how these variations may affect the health or susceptibility of consumers from higher trophic levels. Recent investigations revealed that predicted changes in temperature and acidification increase the susceptibly of herbivores for consuming seagrasses. (Hernan et al., 2017, 2018). Therefore, future studies on the specific FA composition of the major lipid classes will lead to a better understanding of the underlying metabolic processes causing the responses to experimental warming, and effects on plant-consumer interactions.

Effects of temperature are driving changes in *Z. marina* populations worldwide with southern-adapted populations experiencing die-back and *in-situ* extinctions, whereas for northern population climate conditions are favouring their growth and productivity. Therefore, understanding how current and projected climate regimes can affect central eelgrass populations is a major concern (i.e. Krause-Jensen & Duarte 2014; Olesen et al., 2015; Beca-Carretero et al., 2018*b*). The second hypothesis of this section was confirmed, since results of the experimental warming (20 °C) induced increases growth and productivity in Irish seagrasses compared with current summer temperature (16 °C).

Results from Chapter 6 and 7 suggest that changes in the production of key fatty acids in response to climate regimes could be used as an additional reliable eco-

physiological indicator. Indeed, evaluation of the species-specific responses of seagrasses and their capacity to adjust to climate change may be essential to support current efforts in seagrass monitoring and management in Europe.

9.4. Modelling and mapping new seagrass habitats at local and regional scale in the coast of Ireland

In Chapters 4 and 5 we developed different mapping strategies which allow us to identify undiscovered seagrass meadows. In total we discovered and mapped more than 14 km^2 which represent around 30 % of the mapped distribution to date (45 km^2). Thus, our hypothesis suggesting that the spatial distribution of subtidal Irish seagrass meadows may be larger than is currently reported, was confirmed. Marine coastal ecosystems are characterized by the presence of the water column and the presence to natural forces, making it more challenging to generate accurate maps. The seagrass distribution worldwide is estimated to cover 600,000 km^2 (Costanza et al., 1997; Mcleod et al., 2011; Waycott et al., 2009), whereas new research efforts indicated that their extent may be larger than 1,000,000 km^2 (Jayathilake et al., 2018). Thus, the creation of novel mapping strategies to discover new seagrass meadows has emerged as one of the major challenges for the research community.

Here, we combined different approaches to obtain the possible maximum information of potential *Z. marina* distribution in Ireland. We firstly applied MAXENT models to identify which environmental factors better explained the presence of seagrass and also to create maps of the potential habitat suitability of these marine plants along the Irish coast. These models forecasted that the extent of seagrass habitats is larger than currently mapped, suggesting a broader presence of seagrass on the coast of Ireland. Species distribution models, including MAXENT, have been successfully implemented to identify suitable areas for long-term monitoring programs, the prediction of potential impact of humans or climate change or the restoration of seagrass meadows (i.e. Heide et al., 2009; Bekkby et al., 2008; Valle et al., 2013, 2014; Zucchetta et al., 2016).

At a local scale, bathymetry and hard sediment were the most important factors explaining the presence of the *Z. marina*, while fetch and bathymetry were the most relevant variables driving the presence of seagrasses at a regional scale. Overall, the results of both models confirm previous observations which reported that *Z. marina* is a specialist seagrass species with a well-defined ecological niche, preferentially distributed from 0 to 10-meter depth, with preferences for mixed and soft sediments and intermediate current velocities.

Secondly, based on information from the collaborative effort, we obtained a relevant spatial information regarding the seagrasses in Ireland (Chapter 5), mainly regarding subtidal *Z. marina* populations and occasionally intertidal *Z. noltti* and *Ruppia* spp. meadows. Such approaches have previously been successfully used in Portugal assessing the status of kelp (Assis et al., 2009). The next step in our integrated mapping approach was to contrast this information with satellite derived images to be able to elucidate where there was a higher probability of finding seagrasses. Previous studies with marine plants applied remote sensing methods in their identification, quantification and monitoring (i.e. Dahdouh-Guebas et al., 2002; Lathrop et al., 2006; Gullstrom et al., 2016). Finally, by performing the field surveys as was described in Chapter 4, we evaluated 238 new sites and we found seagrass in 198 locations, which accounted for a total of 14 km^2 which were mapped for the first time. These results suggested a success rate of more than 81 % of the evaluated locations, highlighting the robustness of our novel integrated GIS approach. Recent investigations have identified the importance of mapping seagrasses for developing long-term monitoring programs to assess the potential effects of habitat degradation or climate change. This study has indicated the applicability of using GIS-related techniques such as SDMs and satellite-derived images in conjunction with field survey methods. This approach allowed for the prediction of the geographical distribution of the *Z. marina* and the mapping of larger extensions of submerged uncovered seagrass meadows in western Ireland at a relatively low cost.

Finally, this study represents an important contribution to the international efforts in the conservation of these precious ecosystems and, particularly within the European framework. In this project, despite the high number of locations that we evaluated, there are still large areas where potentially seagrasses may be present these remain

unconfirmed. Therefore, we propose that the current ongoing mapping and monitoring efforts should be continued along the Irish coast. Current projects are focussed on mapping and monitoring intertidal meadows along the Irish coast (EPA 2015). Moreover, we propose that this integrated method could be applied to identify and effectively map additional seagrass populations in other parts of the world, or can even be applied to map other subtidal marine species.

9.5. Final conclusion

In summary, the research presented in this thesis represents the most detailed baseline of Irishseagrass ecology to date, providing vital results at shoot and population level. Our findings confirm that shallow and deep-adapted Irish *Zostera marina* populations displayed comparable seasonal responses that perennial temperate eelgrass populations living at similar latitudes. Moreover, our outcomes, for the first time, reported on impacts of climate variation in the production of fatty acids which where positively correlated with photosynthetic pigment production and morphological responses. Additionally, this project investigates the impact of experimental warming in fatty acid composition, suggesting a depletion in lipid nutritional value of leaves of seagrasses under projected scenarios of climate change. Finally, this project demonstrated that the Irish seagrass distribution is mapped is larger than is reportedto date, suggesting that further seagrass habitats may remain unconfirmed.

Chapter 10

Supplementary material

10.1. Chapter 2. Temporal and spatial changes in the structure, morphometry and productivity of pristine *Zostera marina* meadows in western Ireland

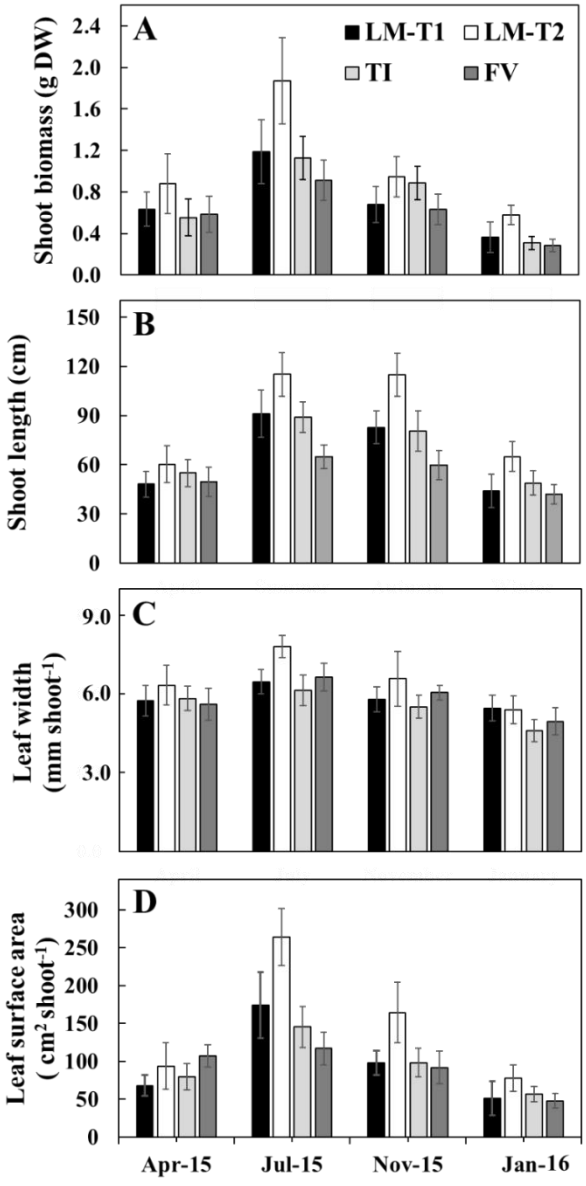


Fig. S2.1 *Zostera marina.* Mean shoot biomass (A), mean shoot length (canopy height) (B), mean leaf width (C) and mean leaf surface area per shoot (D) during the four seasons at the three locations. Data represented as mean ± standard deviation (n=15).

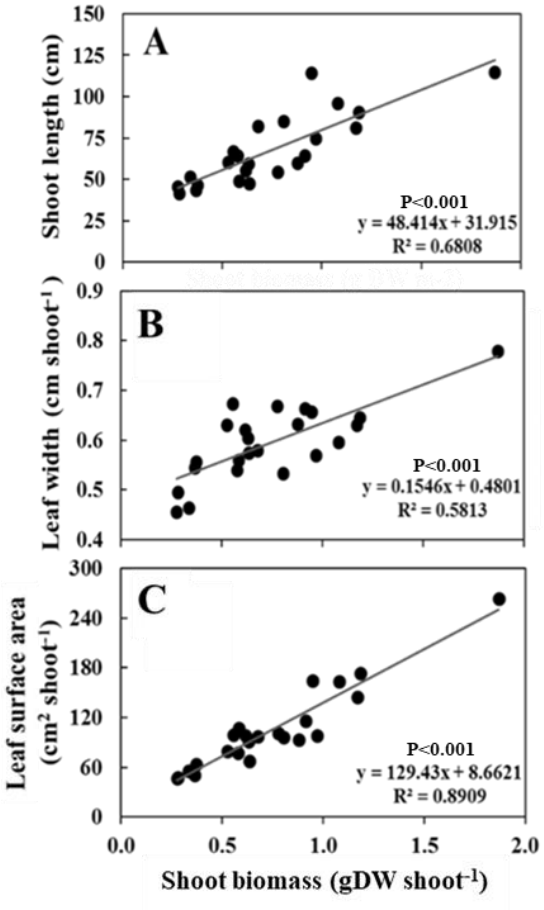


Fig. S2.2 Mean shoot length (Y) versus mean shoot biomass (X) (Panel A); mean shoot length (Y-exe) versus mean leaf width (X) (Panel B); mean shoot length (Y) versus mean leaf surface area (X) (Panel C). Black lines represent the linear regression line (n=24).

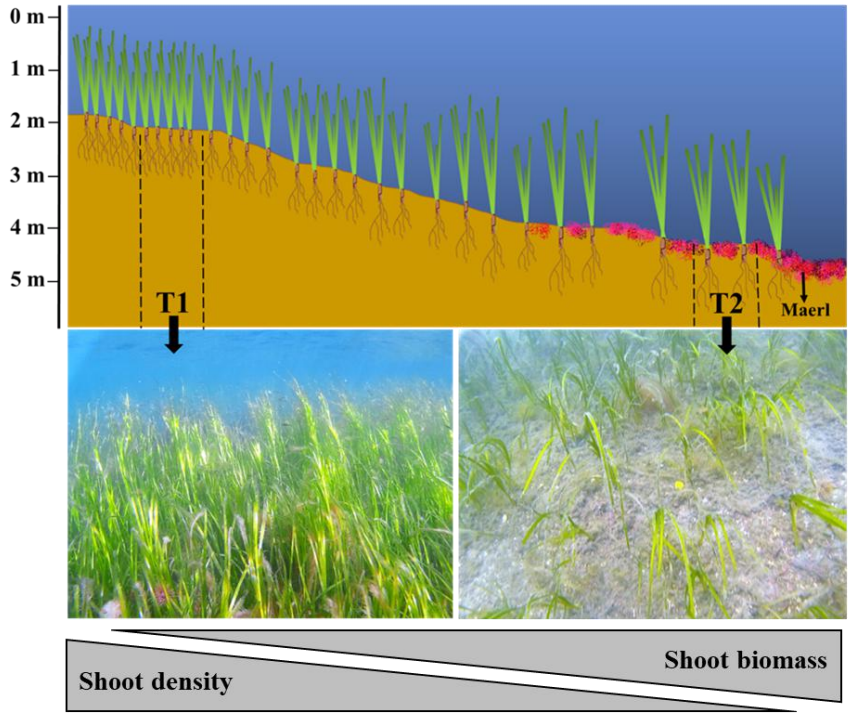


Fig. S2.3 Schematic drawing of demographic and morphological characteristics of the *Zostera marina* population and it co-occurrence with maerl community at two depths at Lettermore (LM). Shallow areas were characterized by high density of eelgrass shoots and no presence of maerl (LM-T1), while, deep waters were characterized by low densities of larger *Zostera marina* shoots and the presence of maerl (LM-T2) which became more abundant at greater depth (*pers. obs*.).

Table S2.1 Grain size distribution (%), mean grain size and grain description (FS= Fine sand; MS= Medium sand; CS= Coarse sand) of *Zostera marina* beds in two permanent transects (T1 and T2) at the three locations (LM, TI and FV (abbreviations)). (n = 5).

Sediment type	**LM-T1**	**LM-T2**	**TI-T1**	**TI-T2**	**FV-T1**	**FV-T2**
Gravel	3.7	31.7	11.3	14.3	1.5	15.1
Coarse sand	34.5	24.7	22	13.4	3	13
Medium sand	28.7	25.1	29.6	20.3	11.6	13
Fine sand	27	13.5	37.1	51.8	82.7	58.3
Mud	6.1	4.9	0.1	0.1	1.1	0.6
Mean grain size (mm)	0.56	1.69	0.66	0.73	0.18	0.75
Grain description	MS	CS	MS	MS	FS	MS

Table S2.2 Data compiled from published literature used to assess seasonal leaf biomass maximum (*B)* and annual leaf formation (*L*) of eelgrass across the entire geographical distribution range.

Source	Location	B	L
McRoy (1970)	Alaska (USA)	x	
McRoy (1974)	Alaska (USA)	x	
Sand-Jensen (1975)	Denmark	x	x
Penhale (1977)	North Carolina (USA)	x	
Jacobs (1979)	France	x	x
Taniguchi and Yamada (1979)	Japan	x	
Aioi (1980)	Japan	x	
Nienhuis and De Bree (1980)	The Netherlands	x	x
Orth and Heck Jr. (1980)	Virginia (USA)	x	
Vaughan (1982) in Roman and Able (1988)	New Jersey (USA)		x
Wetzel and Penhale (1983)	Virginia (USA)	x	
Thorne-Miller et al. (1983)	Rhode Island (USA)	x	
Heck and Thoman (1984)	Viginia (USA)	x	
Robertson and Mann (1984)	Canada	x	
Wium-Andersen and Borum (1984)	Denmark	x	x
Mizushima (1985)	Japan	x	
Kentula and Mcintire (1986)	Oregon (USA)	x	x
Orth and Moore (1986)	Virginia (USA)	x	
Roman and Able (1988)	Massachusetts (USA)	x	x
Thom (1990)	Washington (USA)	x	
Bach (1993)	Denmark	x	
Pedersen and Borum (1993)	Denmark	x	
Lalumière et al. (1994)	Canada	x	
Olesen and Sand-Jensen (1994)	Denmark	x	x
van Lent and Verschuure (1994)	The Netherlands	x	
Cebrián et al. (1997)	Spain	x	
Ibarra-Obando et al. (1997)	Mexico		x
Nelson and Waaland (1997)	Washington (USA)	x	
Sfriso and Francesco Ghetti (1998)	Italy	x	x
Meling-Lopez and Ibarra-Obando (1999)	Mexico	x	
Poumian-Tapia and Ibarra-Obando (1999)	Mexico	x	
Guidetti (2000)	Italy	x	
Hayashida (2000)	Japan	x	

Duarte et al. (2002)	Norway	x	x
Cabello-Pasini et al. (2003)	Mexico	x	
Beal et al. (2004)	Maine (USA)	x	
Boström et al. (2004)	Finland	x	
Lee et al. (2005)	Korea	x	
Watanabe et al. (2005)	Japan	x	x
Hauxwell et al. (2006)	Massachusetts (USA)	x	x
Lee et al. (2006)	Korea	x	x
Hasegawa et al. (2007)	Japan	x	
Kaldy and Lee (2007)	Korea and USA	x	
Naumov (2007)	Russia	x	
Holmer et al. (2009)	Finland and Sweden	x	
Park et al. (2010)	Korea		x
Boström et al. (2014)	Sweden	x	
Carstensen et al. (2015)	Denmark	x	
Olesen et al. (2015)	Greenland	x	x
Röhr et al. (2016)	Finland and Denmark	x	
This study	Ireland	x	x

Table S2.3 Data compiled from published literature used to assess seasonal leaf biomass maximum (g DW m^{-2}) and annual leaf formation (leaves $shoot^{-1}yr^{-1}$) of eelgrass across the entire geographical distribution range and their annual and summer mean seawater surface temperature (°C). nd (not determined).

Source	Location	Sampling place	Latitude	Longitude	Biomass (g DW m^{-2})	Leaves (leaves $shoot^{-1}$ yr^{-1})	SST Annual (°C)	SST Summer (°C)
McRoy (1970)	Alaska (USA)	Craig	55.1	-133.1	52	nd	8.82	14.21
McRoy (1970)	Alaska (USA)	Klawak	55.9	-133.5	68	nd	8.42	13.45
McRoy (1970)	Alaska (USA)	Calder Bay	56.5	-134.1	72	nd	7.68	11.79
McRoy (1970)	Alaska (USA)	Redhead Bay	60.6	-146.9	98	nd	7.98	14.58
McRoy (1970)	Alaska (USA)	Sawmill Bay	60.9	-146.4	82	nd	8.02	14.58
McRoy (1970)	Alaska (USA)	Stockdale Harbour	60.1	-146.9	600	nd	8.17	14.33
McRoy (1970)	Alaska (USA)	Kinzarof Lagoon	55.6	-163.7	1005	nd	5.33	12.49
McRoy (1970)	Alaska (USA)	Izemek Lagoon	55.9	-164.7	821	nd	5.20	12.27
McRoy (1970)	Alaska (USA)	Safety Lagoon	64.5	-164.9	554	nd	2.96	14.38
McRoy (1970)	Alaska (USA)	Port Clareance	65.2	-166.1	80	nd	1.99	12.35
McRoy (1974)	Alaska (USA)	Izembek Lagoon	55.3	-162.8	753	nd	5.21	12.01
Sand-Jensen (1975)	Denmark	Vellerup Vig	55.7	11.8	226	26.1	10.40	20.07
Penhale (1977)	North Carolina (USA)	Phillips Island	34.2	-76.0	162	nd	24.98	29.22
Jacobs (1979)	France	Roscoff	48.7	-4.0	400	18.9	12.97	16.34
Taniguchi and Yamada (1979)	Japan	Iwachi Bay	34.7	138.8	88.8	nd	20.39	27.51
Aioi (1980)	Japan	Odawa Bay	35.2	139.6	90.8	nd	19.90	26.89
Aioi (1980)	Japan	Odawa Bay	35.2	139.6	174	nd	19.90	26.89
Aioi (1980)	Japan	Odawa Bay	35.2	139.6	192.8	nd	19.90	26.89
Aioi (1980)	Japan	Odawa Bay	35.2	139.6	137.9	nd	19.90	26.89
Nienhuis and De Bree (1980)	Netherlands	Lake Grevelingen	51.7	4.0	80	26.1	12.09	19.57
Nienhuis and De Bree (1980)	Netherlands	Lake Grevelingen	51.7	4.0	80	26.1	12.09	19.57
Nienhuis and De Bree (1980)	Netherlands	Lake Grevelingen	51.7	4.0	84	26.1	12.09	19.57
Orth and Heck Jr. (1980)	Virginia (USA)	lower Chesapeake	37.3	-76.4	232	nd	16.63	28.05
Orth and Heck Jr. (1980)	Virginia (USA)	lower Chesapeake	37.3	-76.4	301	nd	16.63	28.05
Orth and Heck Jr. (1980)	Virginia (USA)	lower Chesapeake	37.3	-76.4	238	nd	16.63	28.05
Vaughan (1982) in Roman and Able (1988)	New Jersey (USA)	Little Egg Harbor	39.6	-74.3	nd	23.5	14.29	25.19
Thorne-Miller et al. (1983)	Massachusetts (USA)	Rhode Island	41.4	-71.2	1060	nd	12.67	22.48
Thorne-Miller et al. (1983)	Massachusetts (USA)	Rhode Island	41.4	-71.2	680	nd	12.67	22.48

Thorne-Miller et al. (1983)	Massachusetts (USA)	Rhode Island	41.4	-71.2	355	nd	12.67	22.48
Thorne-Miller et al. (1983)	Massachusetts (USA)	Rhode Island	41.4	-71.2	170	nd	12.67	22.48
Thorne-Miller et al. (1983)	Massachusetts (USA)	Rhode Island	41.4	-71.2	100	nd	12.67	22.48
Thorne-Miller et al. (1983)	Massachusetts (USA)	Rhode Island	41.4	-71.2	100	nd	12.67	22.48
Heck and Thoman (1984)	Virginia (USA)	York River	37.0	-76.4	397	nd	19.10	27.65
Heck and Thoman (1984)	Virginia (USA)	York River	37.0	-76.4	336	nd	19.10	27.65
Robertson and Mann (1984)	Canada	Chezzetcook Inlet	44.9	-63.3	162	nd	8.75	19.22
Wium-Andersen and Borum (1984)	Denmark	0resund	55.8	12.7	281	15	9.88	19.30
Wium-Andersen and Borum (1984)	Denmark	0resund	55.8	12.7	266	18	9.88	19.30
Kentula and Mcintire (1986)	Oregon (USA)	Netarts Bay	45.4	-123.9	256.2	25.26	11.90	16.03
Kentula and Mcintire (1986)	Oregon (USA)	Netarts Bay	45.4	-123.9	226	25	11.90	16.03
Orth and Moore (1986)	Virginia (USA)	Brown's Bay	37.3	-76.4	170	nd	16.63	28.05
Orth and Moore (1986)	Virginia (USA)	Brown's Bay	37.3	-76.4	160	nd	16.63	28.05
Orth and Moore (1986)	Virginia (USA)	Chesapeake Bay	37.3	-76.4	290	nd	16.63	28.05
Orth and Moore (1986)	Virginia (USA)	Chesapeake Bay	37.3	-76.4	400	nd	16.63	28.05
Orth and Moore (1986)	Virginia (USA)	Chesapeake Bay	37.3	-76.4	320	nd	16.63	28.05
Orth and Moore (1986)	Virginia (USA)	Chesapeake Bay	37.3	-76.4	400	nd	16.63	28.05
Roman and Able (1988)	Massachusetts (USA)	Nauset Harbor	41.8	-69.9	305	24.7	11.61	21.17
Roman and Able (1988)	Massachusetts (USA)	Town Cove	41.8	-70.7	199	21.9	12.23	22.19
Thom (1990)	Washington (USA)	Padilla Bay	48.5	-122.5	287	nd	9.85	13.58
Bach (1993)	Denmark	Lake Grevelingen	51.7	4.1	242	nd	12.07	19.56
Pedersen and Borum (1993)	Denmark	0resund	55.8	12.9	441	nd	9.85	19.26
Lalumière et al. (1994)	Canada	Attikuan	54.3	-79.5	342	nd	2.82	10.66
Lalumière et al. (1994)	Canada	Kakassituk	54.1	-79.4	472	nd	2.93	10.66
Lalumière et al. (1994)	Canada	Tees	53.5	-79.1	82	nd	2.82	9.86
Olesen and Sand-Jensen (1994)	Denmark	West Limfjorden	56.9	9.1	222	17	10.55	18.29
van Lent and Verschuure (1994)	Netherlands	Grevelingen	51.7	4.1	403	nd	12.07	19.56
van Lent and Verschuure (1994)	Netherlands	Veerse Meer	51.5	3.8	412	nd	12.10	19.61
van Lent and Verschuure (1994)	Netherlands	Zandkreek	51.5	3.9	303	nd	12.12	19.65
Marbà et al. (1996)	Spain	Cala Jonquet	42.4	3.3	nd	43.8	17.00	23.23
Cebrián et al. (1997)	Spain	Cala Jonquet	42.3	3.3	555.7	nd	17.04	23.31
Cebrián et al. (1997)	Spain	Cala Jonquet	42.3	3.3	202	nd	17.04	23.31
Ibarra-Olbando et al. (1997)	Mexico	San Quintin Bay	30.5	-116.1	nd	44.5	16.90	22.04
Nelson and Waaland (1997)	Washington (USA)	San Juan Island	48.6	-123.1	402	nd	9.89	13.98

Nelson and Waaland (1997)	Washington (USA)	San Juan Island	48.6	-123.1	380	nd	9.89	13.98
Nelson and Waaland (1997)	Washington (USA)	San Juan Island	48.6	-123.1	404	nd	9.89	13.98
Sfriso and Francesco Ghetti (1998)	Italy	The lagoon of Venice	45.4	12.3	360	29.4	17.46	26.72
Meling-Lopez and Ibarra-Obando (1999)	Mexico	Punta Chueca	29.0	-112.2	222	nd	23.37	30.96
Meling-Lopez and Ibarra-Obando (1999)	Mexico	Punta Viboras	29.2	-112.2	194	nd	23.29	30.80
Poumian-Tapia and Ibarra-Obando (1999)	Mexico	North Baja California	30.4	-116.0	77	nd	16.96	21.97
Guidetti (2000)	Italy	Grado Lagoon	45.6	13.4	199.8	nd	17.47	26.29
Hayashida (2000)	Japan	Iwachi Bay, Izu Peninsula	34.7	139.8	68	nd	21.01	27.39
Duarte et al. (2002)	Norway	Hopavagen lagoon	63.6	9.5	149.7	23.5	8.83	14.74
Duarte et al. (2002)	Norway	Hopavagen lagoon	63.6	9.5	142.3	25.2	8.83	14.74
Cabello-Pasini et al. (2003)	Mexico	South Baja California	30.4	-116.0	233	nd	16.96	21.97
Cabello-Pasini et al. (2003)	Mexico	South Baja California	27.7	-114.0	50	nd	18.96	25.30
Cabello-Pasini et al. (2003)	Mexico	South Baja California	26.9	-113.2	50	nd	19.85	27.22
Beal et al. (2004)	Maine (USA)	Cobscook Bay	44.8	-67.2	55	nd	8.41	15.14
Beal et al. (2004)	Maine (USA)	Cobscook Bay	44.8	-67.2	37	nd	8.41	15.14
Beal et al. (2004)	Maine (USA)	Cobscook Bay	44.9	-67.1	64	nd	8.41	15.14
Beal et al. (2004)	Maine (USA)	Cobscook Bay	44.9	-67.1	76	nd	8.41	15.14
Beal et al. (2004)	Maine (USA)	Cobscook Bay	44.8	-67.1	43	nd	8.41	15.14
Boström et al. (2004)	Finland	Åland Islands	60.4	20.1	11.3	nd	7.76	18.96
Boström et al. (2004)	Finland	Åland Islands	60.2	20.1	21	nd	7.94	18.55
Lee et al. (2005)	Korea	Koje Bay	34.8	128.6	695.8	nd	18.02	26.28
Lee et al. (2005)	Korea	Kosung Bay	34.9	128.3	420.4	nd	17.74	26.16
Watanabe et al. (2005)	Japan	Aininkap, Akkeshi Bay	43.1	144.9	900	16.8	8.75	19.38
Watanabe et al. (2005)	Japan	Aininkap, Akkeshi Bay	43.1	144.9	620	nd	8.75	19.38
Hauxwell et al. (2006)	Massachusetts (USA)	Timms	41.9	-70.7	250	30.4	12.14	22.24
Hauxwell et al. (2006)	Massachusetts (USA)	Sage Lot	41.9	-70.7	530	27.2	12.14	22.24
Lee et al. (2006)	Korea	Dadae Bay	34.8	128.6	250	26.1	18.02	26.28
Hasegawa et al. (2007)	Japan	Akkeshi-ko estuary	43.0	144.9	248	nd	8.25	18.90
Hasegawa et al. (2007)	Japan	Akkeshi-ko estuary	43.0	144.9	202	nd	8.25	18.90
Kaldy and Lee (2007)	Korea	Jindong Bay	35.1	129.1	862	nd	18.55	26.96
Kaldy and Lee (2007)	Korea	Jindong Bay	35.1	129.1	993	nd	18.55	26.96
Kaldy and Lee (2007)	Oregon (USA)	Yaquina Bay,	44.6	-124.0	211	nd	11.60	15.69
Kaldy and Lee (2007)	Oregon (USA)	Yaquina Bay,	44.6	-124.0	72	nd	11.60	15.69
Holmer et al. (2009)	Finland	Tvärminne	59.8	23.1	16	nd	8.26	19.49

Holmer et al. (2009)	Finland	Ryssholmen	59.8	23.1	132	nd	8.26	19.49
Holmer et al. (2009)	Finland	Kolaviken	59.8	23.1	97	nd	8.26	19.49
Holmer et al. (2009)	Sweden	Saxnäs	56.7	16.4	109	nd	9.02	19.13
Holmer et al. (2009)	Sweden	Beijershamn	56.7	16.4	94	nd	9.02	19.13
Holmer et al. (2009)	Sweden	Gåsö	57.7	11.8	182	nd	10.25	19.80
Holmer et al. (2009)	Sweden	Lindholmen	57.7	11.8	122	nd	10.25	19.80
Park et al. (2009)	Korea	Koje Bay	34.8	128.6	nd	25.5	18.02	26.28
Naumov et al., 2013	Russia	Kandalaksha Bay	66.5	33.4	198.5	nd	4.14	15.10
Naumov et al., 2013	Russia	Kandalaksha Bay	66.5	33.4	42.453	nd	4.14	15.10
Naumov et al., 2013	Russia	Kandalaksha Bay	66.5	33.4	49.9	nd	4.14	15.10
Boström et al. (2014)	Sweden	Atlantic Norway	58.1	4.7	149	nd	10.31	16.87
Boström et al. (2014)	Sweden	Skagerrak	56.8	11.4	104	nd	10.43	19.89
Boström et al. (2014)	Sweden	Kattegat	55.3	13.1	280	nd	9.91	19.28
Boström et al. (2014)	Sweden	Southern Baltic Sea	54.5	16.8	72	nd	10.17	19.78
Boström et al. (2014)	Sweden	Baltic Proper	56.7	16.5	56	nd	9.05	19.07
Boström et al. (2014)	Sweden	North-eastern Baltic Sea	59.8	22.9	47	nd	8.26	19.47
Carstensen et al. (2015)	Denmark	Flensborg Fjord	55.0	9.7	546	nd	10.28	19.76
Carstensen et al. (2015)	Denmark	Helnaes Bugt	55.2	9.5	312	nd	10.22	19.57
Carstensen et al. (2015)	Denmark	Kertinge Nor	55.5	9.6	240	nd	10.04	19.05
Carstensen et al. (2015)	Denmark	Køge Bugt	55.4	9.7	573	nd	10.11	19.23
Carstensen et al. (2015)	Denmark	Odense Fjord	55.5	9.7	388	nd	10.03	19.03
Carstensen et al. (2015)	Denmark	Roskilde Fjord	55.9	10.3	399	nd	10.14	19.21
Carstensen et al. (2015)	Denmark	South Funen	55.2	9.7	178	nd	10.23	19.58
Carstensen et al. (2015)	Denmark	Archipielago The Sound	54.9	10.0	511	nd	10.39	19.98
Olesen et al. (2015)	Greenland	Kobbefjord	64.2	-51.5	100	6.9	1.58	6.87
Olesen et al. (2015)	Greenland	Qugssuk	64.7	-51.2	250	10.7	1.38	7.14
Olesen et al. (2015)	Greenland	Ameralik	64.1	-50.9	320	8.7	1.70	7.59
Olesen et al. (2015)	Greenland	Kapisillit	64.4	-50.3	300	13.1	1.57	7.04
Röhr et al. (2016)	Finland	Fårö	59.9	21.8	138	nd	8.29	19.24
Röhr et al. (2016)	Finland	Hummelskär	60.3	21.5	70	nd	7.90	19.13
Röhr et al. (2016)	Finland	Jänisholm	60.2	21.0	65	nd	8.03	18.92
Röhr et al. (2016)	Finland	Kolaviken	59.8	22.9	74	nd	8.26	19.47
Röhr et al. (2016)	Finland	Lyddaren	60.1	21.5	86	nd	8.08	19.07
Röhr et al. (2016)	Finland	Långören	59.9	21.4	121	nd	8.28	19.10

Röhr et al. (2016)	Finland	Ryssholmen	59.8	23.2	160	nd	8.28	19.52
Röhr et al. (2016)	Finland	Sackholm	60.1	21.8	110	nd	8.04	19.20
Röhr et al. (2016)	Finland	Tvärminne	59.8	23.2	99	nd	8.28	19.52
Röhr et al. (2016)	Finland	Ängsö	60.1	21.7	91	nd	8.05	19.15
Röhr et al. (2016)	Finland	FIN	60.3	21.7	417	nd	7.84	19.18
Röhr et al. (2016)	Denmark	Agero	56.7	8.6	448	nd	10.52	18.41
Röhr et al. (2016)	Denmark	Agero	56.7	8.6	404	nd	10.52	18.41
Röhr et al. (2016)	Denmark	Dalby	55.5	10.6	76	nd	10.24	19.28
Röhr et al. (2016)	Denmark	Kertinge	55.4	10.6	90	nd	10.34	19.18
Röhr et al. (2016)	Denmark	Lovns	56.6	9.3	141	nd	10.46	18.59
Röhr et al. (2016)	Denmark	Lunkebugt	55.0	10.7	210	nd	10.42	19.80
Röhr et al. (2016)	Denmark	Løgstør	57.0	9.0	149	nd	10.56	18.12
Röhr et al. (2016)	Denmark	Nyborg	55.3	10.8	203	nd	10.33	19.55
Röhr et al. (2016)	Denmark	Thurøbund	55.1	10.7	101	nd	10.40	19.72
Röhr et al. (2016)	Denmark	Visby	57.6	8.5	193	nd	10.56	17.93
Röhr et al. (2016)	Denmark	Funen	55.3	10.4	418	nd	10.66	17.83
This study	Ireland	LM-T1	53.3	9.7	409.1	23.3	11.97	16.88
This study	Ireland	TI-T1	53.3	9.6	568.5	20.4	11.97	16.88
This study	Ireland	TI-T2	53.3	9.6	542.3	19.6	11.97	16.88
This study	Ireland	FV-T1	53.1	9.1	595.6	28.9	11.8	16.78
This study	Ireland	FV-T2	53.1	9.1	680.1	29.4	11.8	16.78

10.2. Chapter 3. Effects of temporal variations, depth and latitudinal gradients in the synthesis of key biochemical descriptors of *Zostera marina* populations

Table S3.1 Total fatty acid content (% DW) and composition (% of TFA) and total pigment contents, chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*) and carotenoids (Car) (mg g^{-1} DW) of Irish *Zostera marina* population. TI = Tír and Fhia; FV = Finavarra. Results are expressed as mean ± SD (n = 5).

Season	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Location	**TI-T1**	**TI-T1**	**TI-T1**	**TI-T1**	**TI-T2**	**TI-T2**	**TI-T2**	**TI-T2**
Total fatty acids (% DW)	3.0 ± 0.3	1.9 ± 0.1	1.4 ± 0.1	2.4 ± 0.0	nd	1.9 ± 0.1	1.9 ± 0.0	2.5 ± 0.2
Saturated FA (% of TFA)	21.3 ± 2.1	27.6 ± 2.2	29.5 ± 1.5	26.6 ± 1.1	nd	28.1 ± 1.1	26.7 ± 0.9	26.7 ± 1.5
Monounsaturated FA (% of TFA)	2.5 ± 0.2	3.1 ± 0.3	4.0 ± 0.6	2.7 ± 0.3	nd	2.9 ± 0.2	3.8 ± 0.5	35.0 ± 0.6
Polyunsaturated FA (% of TFA)	71.6 ± 3.0	63.5 ± 2.9	61.6 ± 2.1	66.8 ± 1.5	nd	62.8 ± 1.6	62.0 ± 1.7	66.5 ± 2.7
Others (% of TFA)	4.6 ± 0.9	5.8 ± 0.4	5.3 ± 0.8	5.9 ± 0.4	nd	6.1 ± 2.3	5.6 ± 0.7	5.0 ± 0.6
Total pigments (mg g^{-1} DW)	8.9 ± 1.5	5.7 ± 0.2	8.1 ± 0.6	8.0 ± 0.6	nd	5.2 ± 0.2	8.1 ± 0.4	8.6 ± 0.8
Chl. a (mg g^{-1} DW)	5.7 ± 0.10	3.5 ± 0.1	5.2 ± 0.4	5.2 ± 0.5	nd	3.6 ± 0.1	5.2 ± 0.4	5.5 ± 0.5
Ch. b (mg g^{-1} DW)	2.2 ± 0.5	1.3 ± 0.1	1.9 ± 0.2	1.9 ± 0.2	nd	1.3 ± 0.1	1.9 ± 0.2	2.1 ± 0.3
Carotenes (mg g^{-1} DW)	1.0 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	nd	0.7 ± 0.1	1.0 ± 0.1	1.0 ± 0.2
Location	**FV-T1**	**FV-T1**	**FV-T1**	**FV-T1**	**FV-T2**	**FV-T2**	**FV-T2**	**FV-T2**
Total fatty acids (% of DW)	2.9 ± 0.3	1.6 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	2.7 ± 0.5	1.5 ± 0.2	1.7 ± 0.1	2.2 ± 0.1
Saturated FA (% TFA)	20.3 ± 1.0	22.3± 2.2	30.0 ± 3.1	25.6 ± 1.1	20.5 ± 1.2	22.8 ± 0.9	29.9 ± 1.1	24.9 ± 1.6
Monounsaturated FA (% of TFA)	2.9 ± 0.1	2.3 ± 0.1	2.9 ± 0.4	2.6 ± 1.0	3.7 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	2.0 ± 0.2
Polyunsaturated FA (% of TFA)	71.5 ± 1.2	71.2 ± 2.9	62.5 ± 3.4	67.5 ± 1.1	71.1 ± 1.4	70.3 ± 2.6	62.6 ± 1.1	68.4 ± 2.0
Others (% of TFA)	5.4 ± 0.5	4.2 ± 0.5	4.3 ± 0.6	4.2 ± 0.3	5.1 ± 0.6	4.2 ± 0.3	4.7 ± 0.2	4.7 ± 0.4
Total pigments (mg g^{-1} DW)	7.1 ± 0.8	5.2 ± 0.4	7.0 ± 0.5	9.5 ± 0.8	8.0 ± 1.35	5.8 ± 0.2	6.8 ± 1.0	10.4 ± 1.3
Chl. a (mg g^{-1} DW)	4.5 ± 0.3	3.2 ± 0.1	4.5 ± 0.8	6.0 ± 0.8	5.1 ± 0.8	3.6 ± 0.1	4.2 ± 0.8	6.5 ± 0.8
Ch. b (mg g^{-1} DW)	1.7 ± 0.1	1.1 ± 0.1	1.6 ± 0.4	2.85 ± 0.3	2.0 ± 0.4	1.3 ± 0.1	1.6 ± 0.4	2.8 ± 0.4
Carotenes (mg g^{-1} DW)	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	0.8 ± 0.1	1.15 ± 0.1

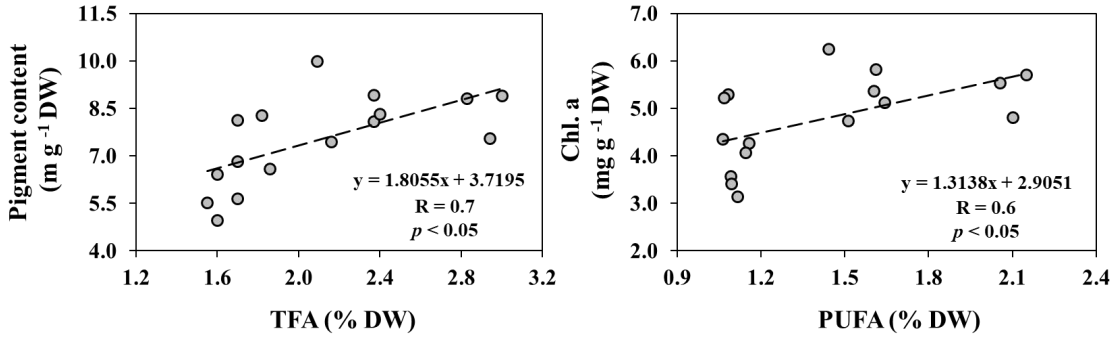


Fig. S3.1 TFA content (% DW) and PUFA content (% DW) and corresponding pigment content (mg g^{-1} DW) (Panel A) and Chl. a content (mg g^{-1} DW) (Panel B) of leaves of Irish *Zostera marina* populations. Black lines represent the linear regression line.

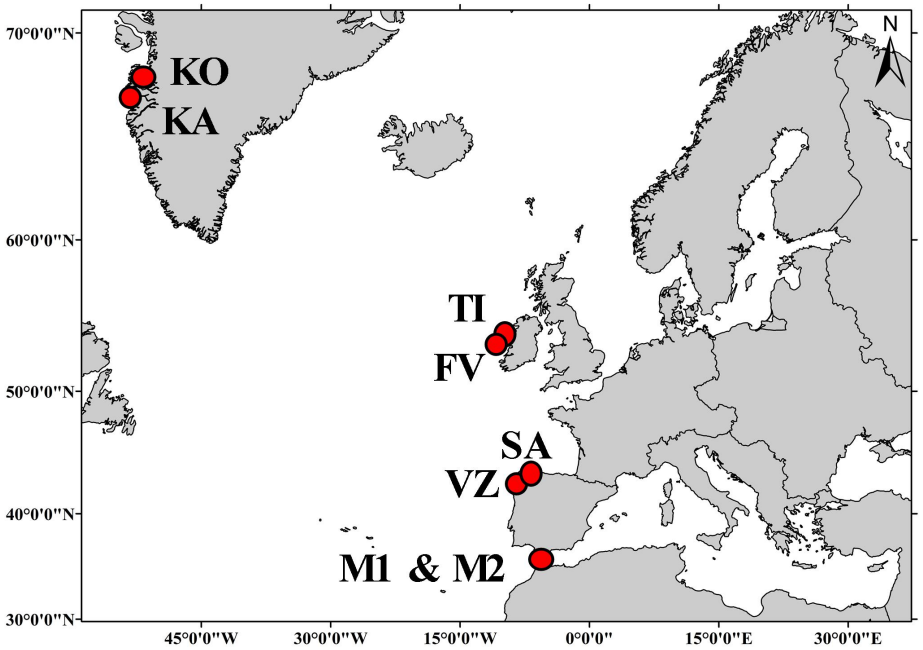


Fig. S3.2 Map of distribution of Z. marina population used for the latitudinal comparison. Southern (Meadow 1 (M1) and Meadow 2 (M2)) and northern Spain (Vouzas (VZ) and (Sada) (SA)), Ireland (Tír an Fhia (TI) and Finavarra (FV)) and Greenland (Kapossillit (KA) and Kobbejord (KB)).

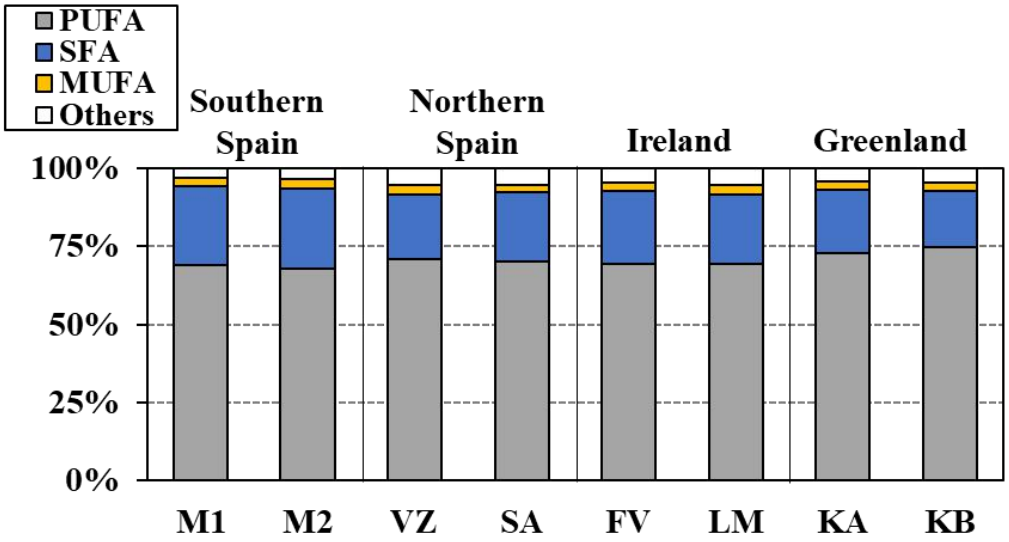


Fig. S3.3 Total fatty acid composition (% TFA) of leaves *Zostera marina* populations across the latitudinal range. Southern (Meadow 1 (M1) and Meadow 2 (M2)) and northern Spain (Vouzas (VZ) and (Sada) (SA)), Ireland (Tír an Fhia (TI) and Finavarra (FV)) and Greenland (Kapossillit (KA) and Kobbejord (KB)).

10.3. Chapter 4. A novel integrated GIS approach indicates that the distribution of *Zostera marina* in Ireland is more extensive than reported

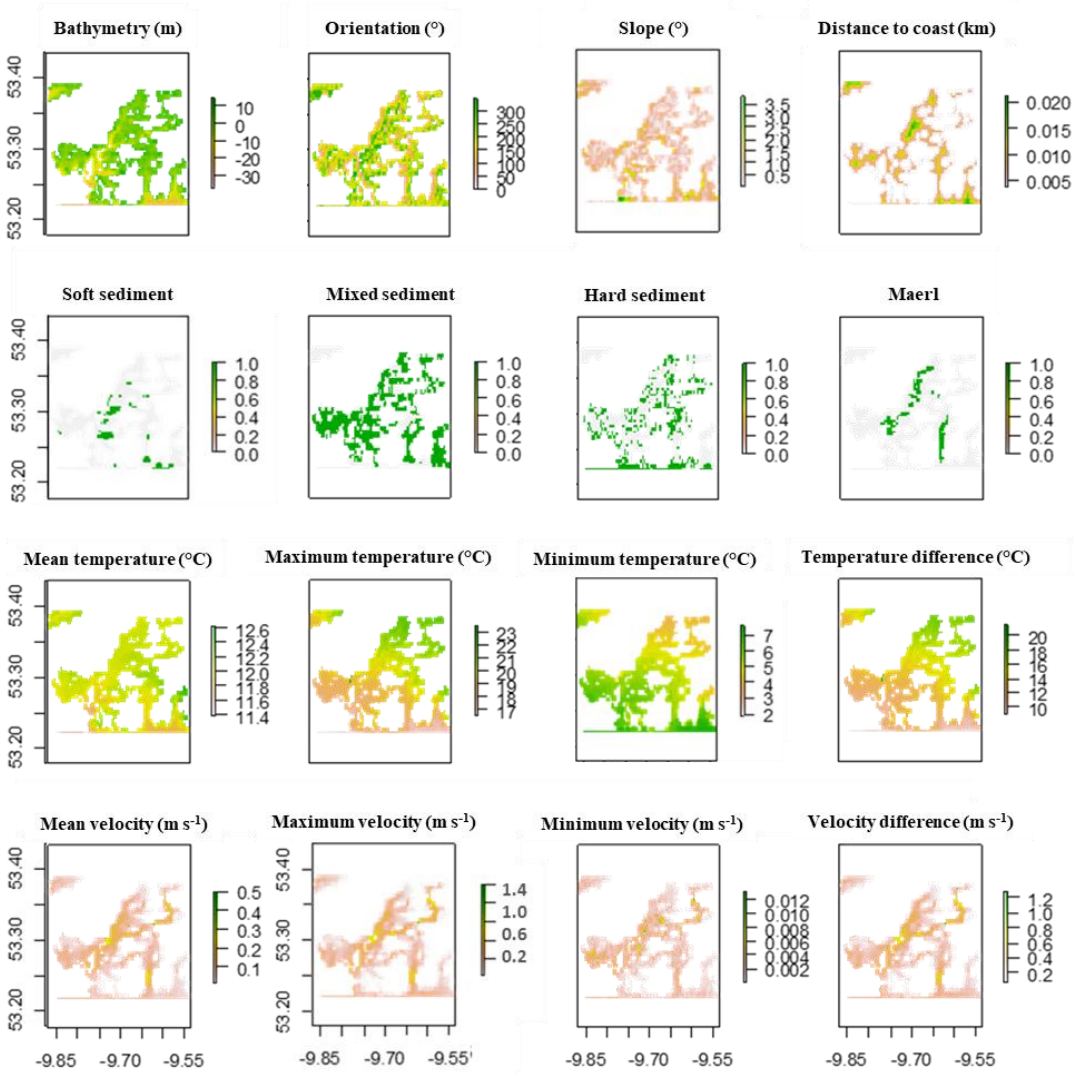


Fig. S4.1 Predictor variables used for performing the *Zostera marina* distribution model (MAXENT)

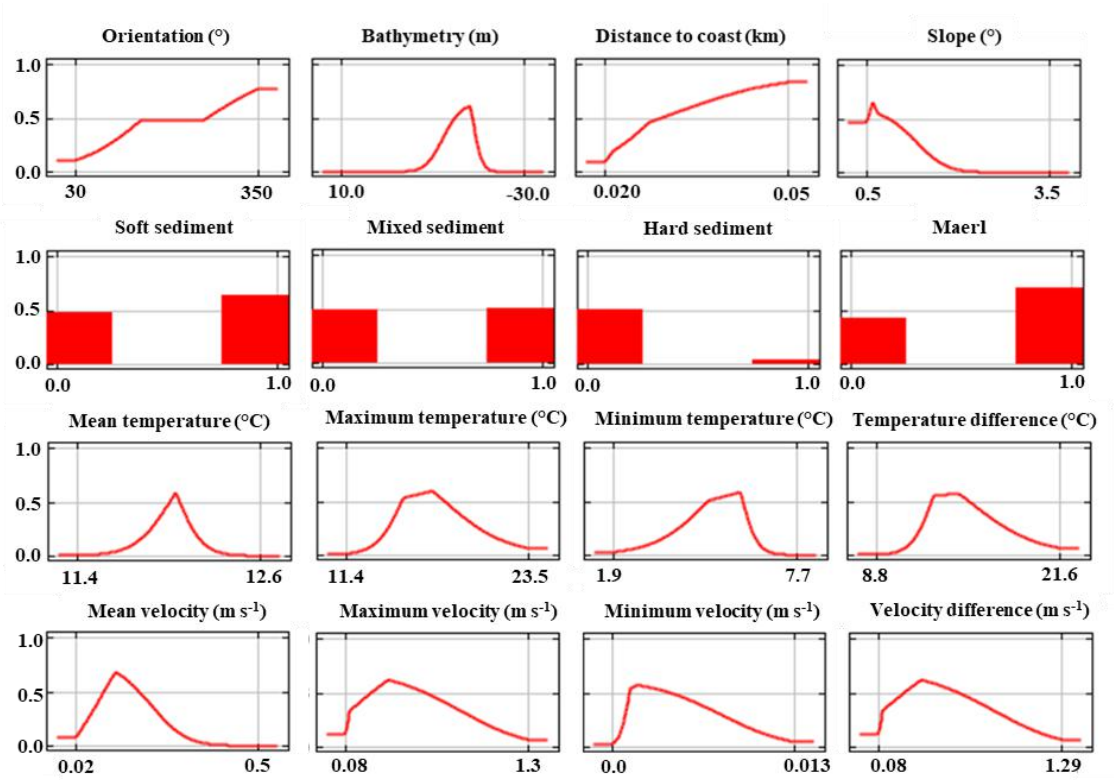


Fig. S4.2 Variables response curves estimated from MAXENT indicating the log response of the *Zostera marina*.

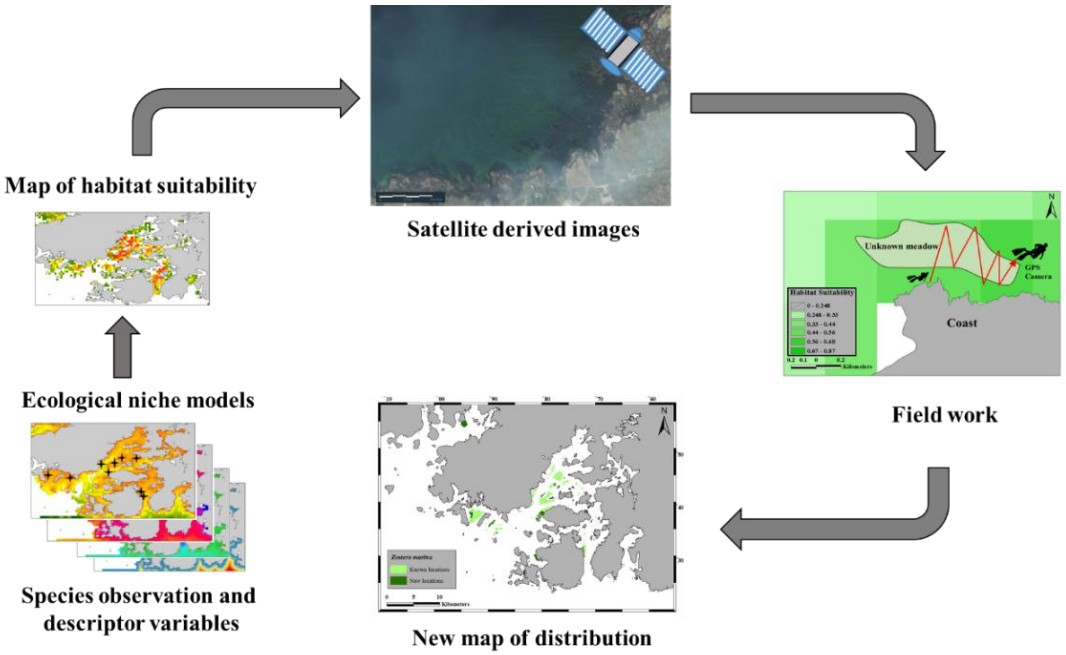


Fig. S4.3 Graphical abstract

10.4. Chapter 7. Effects of an experimental heat wave on fatty acid composition in two Mediterranean seagrass species

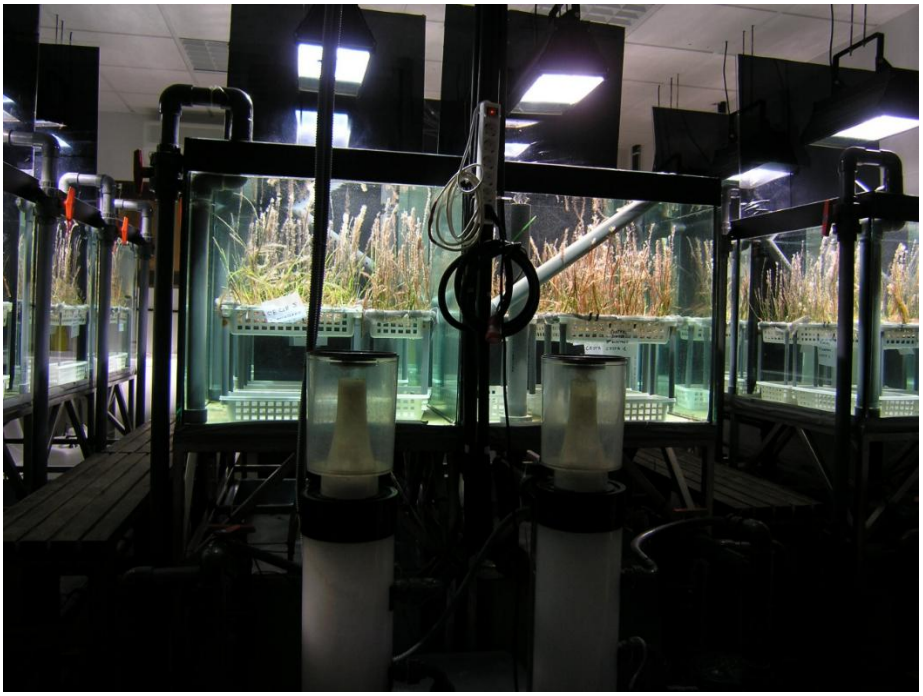


Fig. S7.1 Image of the mesocosm in the IEO of Murcia, Spain. Photograph provided by Juan Manuel Ruiz.

10.5. Chapter 8. Depth-induced adjustment of fatty acid and pigment composition suggests high biochemical plasticity in the tropical seagrass *Halophila stipulacea*

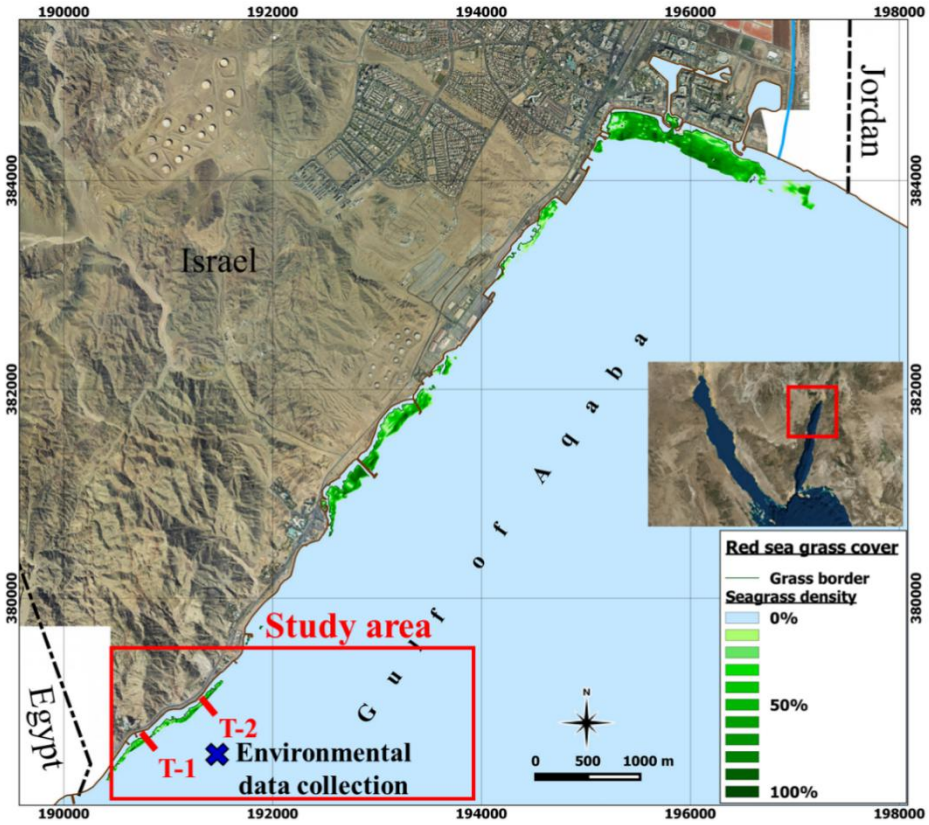


Fig. S8.1 Map of study area in the Gulf of Aqaba (GoA), Red Sea, Israel, with the two transects (T-1, T-2) extending ~ 25 m from shallow (6 and 9 m) to intermediate (15 m) and deep areas (21 m). Blue cross represents the position (29.493 N, 34.909 E) of environmental data collection in adjacent waters.

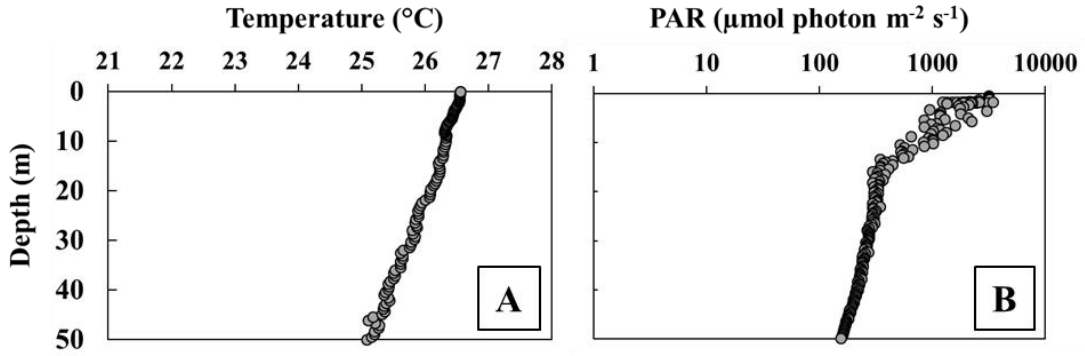
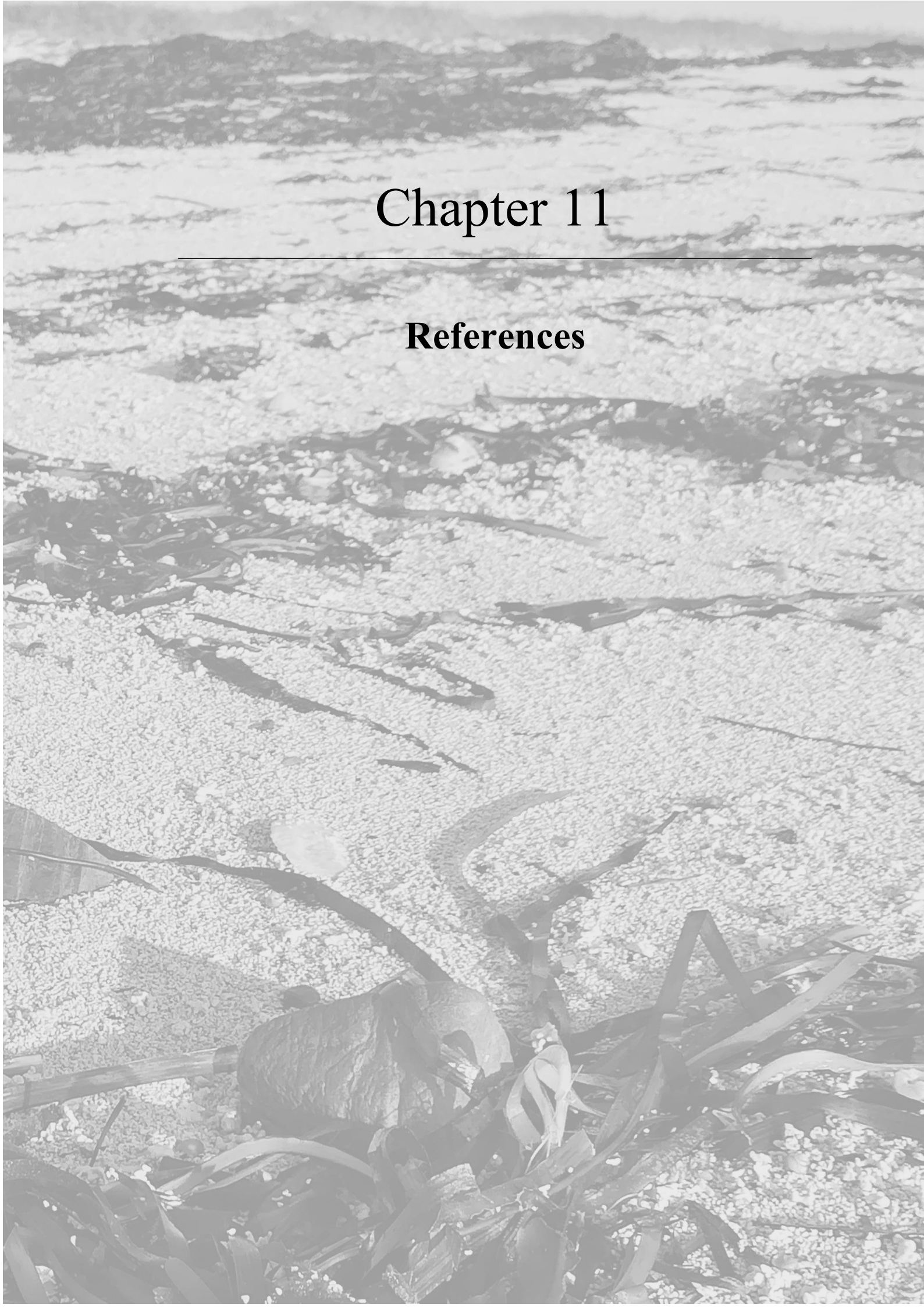


Fig. S8.2 Water temperature and irradiance (PAR) changes across a depth gradient (0 – 50 m) of July 2016 in adjacent waters (29.493 N, 34.909 E) from Israel's National Monitoring of the Gulf of Eilat modified from Winters (2016).



Chapter 11

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Scientific articles

2018 **Beca-Carretero P**, Winters G & Stengel DB. Depth-induced adjustment of fatty acid and pigment composition suggests high biochemical plasticity in the tropical seagrass *Halophila stipulacea*. Submitted: *Marine Ecology Progress Series* (Accepted 09-2018)

2018 **Beca-Carretero P,** Olesen B, Marbá N, Krause-Jensen D. Response to experimental warming in northern eelgrass populations: comparison across a range of temperature adaptations. *Marine Ecology Progress Series* 589: 59–78

2018 **Beca-Carretero P,** Guihéneuf F, Marín-Guirao L, Stengel DB, Ruiz JM. Effects of an experimental heat wave on fatty acid composition in two Mediterranean seagrass species. *Marine Pollution Bulletin* (Published)

2017 Nordlund LM, Jackson EL, Nakaoka M, Samper-Villareal J**, Beca-Carretero P**, Creed JC. Seagrass ecosystem services – what's next? *Marine Pollution Bulletin* (Published)

Under review **Beca-Carretero P**, Rotini A, Mejia A, Migliore L, Vizzini S, Winters G. Structural and biochemical descriptors of *Halophila stipulacea* in its native habitat (northern Red Sea) disclose its plasticity and invasiveness capacity. (Submitted 04 – 2018) *Marine Environmental Research*

Under review **Beca-Carretero P**, Varela S, Stengel DB. A novel integrated GIS approach indicates that the distribution of *Zostera marina* in Ireland is more extensive than reported. (Submitted: 03 – 2018) *Aquatic Conservation: Marine and Freshwater Ecosystem*

Under review **Beca-Carretero P**, Stanschewski C, Julia-Miralles M, Sanchez-Gallego A, Stengel DB. Temporal responses in the structure, morphometry and productivity of pristine *Zostera marina* meadows in western Ireland. (Submitted 05 – 2018) *Aquatic Botany*

Ready for submission **Beca-Carretero P,** Land J. Otero BH, Taylor S, Groom, Álvarez-Salgado XA. Climate drivers of the seasonal and inter-annual variability of net primary production in the NW Iberian margin. For submission to *Progress in Oceanography*

Ready for submission	**Beca-Carretero P**, Guihéneuf F, Krause-Jense D, Stengel DB. Effects of temporal variations and geographical distance in the synthesis of key biochemical descriptors of *Zostera marina* populations. For submission (10 – 2018) to *Marine Environmental Research*
Ready for submission	Beltran R, Marbá N, Jiménez MA, **Beca-Carretero P**, Traveset A. Responses of macrofaunal to *Posidonia oceanica* beach-cast: variations in diversity and community structure. For submission to *Food Webs*
Review in progress	Eklöf J, Boström C, Krause-Jensen D, Alcoverro T, Connolly R, Rossi F, Pages J, Coles R, Sherman C, Macreadie **Beca-Carretero P**, Salo T, McMahon K, Frouws A. Seagrass Ecosystem Resilience – a semi- systematic review
Review in progres	Winters G, Beer S, Willette DA, Chiquillo K, Viana V, **Beca-Carretero P**, Migliore L, Rotini A, Arland M, Blaustein J, Rilov G, Belmaker J, Wise G, Gamliel I, Alexandre A, Procaccini. The invasive tropical seagrass *Halophila stipulacea*: A review of what we know and identifying gaps of knowledge. For submission to *Marine Biology*

Conference and Workshops Presentations

09/2018

Beca-Carretero P, Guihéneuf F, Julia-Miralles M, Stengel DB. Experimental and biochemical assessment of the impacts of global change on Irish seagrass (*Zostera marina*) populations. Irish Algal Research Conference (iARC). *Galway, Ireland* (Poster presentation). Awarded with the best Poster presentation.

06/2018

Beca-Carretero P, Guihéneuf, Stengel DB. Effects of temporal variations and geographical distance in the synthesis of key biochemical descriptors of *Zostera marina* populations. 13th International Seagrass Biology Workshop (ISBW13). *Singapore* (Oral presentation).

05/2017

Beca-Carretero P, and Stengel, DB. Assessment of environmental conditions affecting the performance of eelgrass *Zostera marina* meadows in western Ireland. Environ, *(Ireland)* (Oral presentation).

06/2017

Beca-Carretero P, Rotini A, Mejia A, Migliore L, Vizzini S, Winters G. Seasonal changes in *Halophila* meadows of Eilat. Workshop: Developing an integrated framework for studying *Halophila stipulacea*, the world's first globally invasive marine angiosperm (Seagrass). *Eilat (Israel)* (Oral presentation).

10/2016

Beca-Carretero P, Julia M, Varela S and Stengel DB. A first detailed assessment of current environmental pressures affecting the performance of *Zostera marina* in western Irish populations. 12nd International Seagrass Biology Workshop (ISBW12). *Wales (UK)* (Poster presentation).

10/2016

Beca-Carretero P, Varela S and Stengel DB. The Irish seagrass distribution may larger than reported. INFOMAR conference. Marine Institute. *Orannmore (Ireland)* (Poster presentation).

10/2016

Beca-Carretero P, Julia M, Varela S and Stengel DB. The Irish seagrass distribution may larger than reported. 10th Irish Earth Observation Symposium. *Cork (Ireland)* (Oral presentation).

06/2016

Beca-Carretero P, Varela S and Stengel DB. Modelling the potential distribution of the eelgrass, *Zostera marina* in Irish coast. ISEH 2016, ISEG 2016 & Geoinformatics. *Galway (Ireland*) (Oral presentation).

05/2015

Beca-Carretero P and Stengel, D.B. Irish seagrasses ecology, nutritional composition and habitat mapping in the light of climate change. 4th Mediterranean Seagrass Workshop. *Sardinia (Italy)* (Poster presentation).

List of Co-supervised Under and Post-graduate Theses

09/2018-12/2018 **Erasmus co-supervisor**. Tomás Azcárate (Cádiz University - NUI Galway student). Seasonal comparison of *Zostera marina* and *Z. noltii* growth and physiological responses across their latitudinal distribution range.

2017-2018 4th year project co-supervisor. Emmeline Cosnett (NUI Galway student). Eco-physiological responses to climatic fluctuations of two selected seagrass species along the Irish west coast.

2016-2017 4th year project co-supervisor. Sean McLoughlin (NUI Galway student). Seasonal, spatial and depth related variations in morphological and biochemical characteristics of two Zostera marina meadows in western Ireland.

2015-2016 **4th year project co-supervisor**. Clara Stanschewski (NUI Galway student). Characterization of *Zostera marina* meadows on the Irish west coast and responses to experimental warming scenarios