Investigating the responses of seagrasses to environmental drivers of water quality around the UK and Brazil.

By

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1. General Introduction

This thesis aims to investigate the effect of environmental drivers on seagrasses by studying shoot-scale and meadow-scale responses, focussing on *Zostera marina*, *Zostera noltii* and *Halodule wrightii*. Seagrasses are plants that have evolved from being terrestrial to living in an entirely marine environment which means they have become highly adapted. The conditions that effect seagrass growth can also be described as drivers as they have the ability to modify seagrass meadows in a variety of ways. These drivers can either be natural environmental factors or anthropogenic processes directly or indirectly affecting the marine environment in which seagrasses are found. These responses environmental conditions allow seagrasses to be used as indicators of the health of our coastal waters with poor water quality causing substantial impacts on seagrasses. Better knowledge of seagrass responses to local environmental conditions will help the identification of stressors which can then be managed. It will also help to comprehend the degree of risk to be expected from the threat of climate change including increased storm events, rises in sea level and sea temperature, and ocean acidification. Mitigating existing or potential impacts that lead to a reduction in water quality will improve the overall health and resilience of the seagrass to future threats from climate change.

1.1 Seagrasses and their ecosystem role

Seagrasses are marine angiosperms, the only truly marine flowering plants that have adapted to total submergence in the marine environment. They can be found in shallow coastal habitats around the World, typically growing in sandy or muddy substratum down to a depth determined by light availability. Seagrasses are known as ecosystem engineers as they are able to modify their abiotic environment (van der Heide et al., 2007). They can form dense meadows in subtidal and intertidal zones, with complex root and rhizome systems, which bind and stabilise substrata (Fig. 1.1). Their leaves absorb nutrients from the water column (Moore, 2004) and act as baffles to wave action, slowing water flow and increasing sedimentation from the water column (Gacia and Duarte, 2001) and are thus also responsible for reducing turbidity. Seagrass meadows provide many important ecosystem services. The below-ground structure of seagrass meadows can help to protect shorelines from erosion as well as provide a stable and oxygenated substratum for benthic fauna to dwell in (Jackson et al., 2013; Short and Wyllie-Echeverria, 1996). The seagrass canopy filters nutrients from the seawater and provides food and shelter for many organisms, supporting a high biodiversity within the substratum as well as within the meadow (Gell and Whittington, 2002; Orth et al., 1984; Short et al., 2007). The complex habitat created by seagrasses is home to juvenile and larval stages of larger organisms including commercially important bivalve and fish species (Beck et al., 2001; Bertelli and Unsworth, 2013; Heck et al., 2003; Jackson et al., 2001; Unsworth et al., 2008) and intrinsically valuable species such as seahorses (Curtis and Vincent, 2005; Díaz-Ruiz et al., 2000; Jackson et al., 2013; Vincent et al., 2011). Seagrass is also a direct food source for a range of herbivores including fish and invertebrates, but also for a number threatened species including waterfowl such as Brent geese (*Brant bernicla*), wigeon (*Anas Penelope*) (Fox, 1996; Ganter, 2000; Nacken and Reise, 2000), turtles, dugongs and manatees (Short and Wyllie-Echeverria, 1996).

Seagrasses are arguably one of the biggest sinks of carbon on Earth, storing organic carbon from the atmosphere (Fourqurean et al., 2012; Macreadie et al., 2015; Mcleod et al., 2011) whereby carbon is absorbed from the water column and stored as plant tissue as seagrasses grow. Over time, as seagrasses grow and die, meadows can form dense reef-like structures, laying down dead material in the sediments creating a carbon sink (Fourqurean et al., 2012). For these reasons seagrasses are of high conservation and ecological importance and considered to be a foundation species (Davison and Hughes, 1998; Jackson et al., 2013; Orth et al., 2006). Despite this importance, seagrass habitats are being threatened the world over by variety of natural and anthropogenic induced impacts (Björk et al., 2008; Jackson et al., 2013; Jiang et al., 2013; Milazzo et al., 2004; Neckles et al., 2005; Orth et al., 2006; Short and Wyllie-Echeverria, 1996). In a review of the global status of seagrass, Waycott *et al*., (2009) calculated it to be declining at a rate of 110 km^2yr^{-1} since 1980.

Figure 1.1. (Left) Zostera noltii, a small intertidal species of seagrass with leaves between 6-22 cm long (photo from WoRMS, taken in Swale, Kent, 2004) and the larger species eelgrass (right), Zostera marina, which generally has leaves 20-50 cm long but can grow up 2 m in places such as the Isles of Scilly, where this photo was taken (2018).

1.2 Threats to seagrasses

Seagrasses are protected at global, regional and local scales. Under the United Nations Environmental Programme (UNEP), seagrasses are described as important marine ecosystems in need of protection (Jackson et al., 2013), however they are considered to be afforded the least protection of all marine ecosystems (UNEP, 2020; Unsworth et al., 2019). Reasons for this are multifaceted but include the lack of awareness of the issues faced by seagrasses amongst stakeholders (Unsworth et al., 2019) alongside the lack of effective integrated coastal management needed to identify the multiple pressures threatening these ecosystems (Griffiths et al., 2020).

Physical destruction of seagrass meadows can be caused by many factors such as coastal developments, boat moorings, anchor damage, fishing and storm events which can lead to the removal or fragmentation of seagrass meadows. The environmental conditions favourable to seagrass meadows often overlap with areas that are most suitable for harbours which means they are often subjected to boating pressures. Damage to seagrass rhizomes from anchoring and propellers within meadows can be significant where it is not managed. Rhizomes are easily broken and plants ripped up by anchors or scarred from rope or chain drag (Fig.1.2) (Collins et al., 2010; Francour et al., 1999; Milazzo et al., 2004; Montefalcone et al., 2008). Moorings within seagrass meadows will scour the seabed limiting growth and causing characteristic circular scars which can be seen from aerial photos, within which plants are sparse or more than likely absent (Collins et al., 2010; Egerton, 2011; Kelly et al., 2019;

Stamp and Morris, 2013). Physical damage in seagrass meadows opens up space that is at risk of colonisation by opportunists including fast growing algae or non-native species. Disturbance increases the vulnerability to invasive species, which is an increasing problem, with over 56 non-native species having been introduced within seagrass meadows (Williams, 2007). Fragmentation and damage from physical disturbance significantly impacts the stability of this habitat and its ecological value in its capacity to support marine life and protect coastlines. The conflict between boat users and seagrass conservation has been remedied in some places with the use of permanent visitor moorings to minimise anchoring as well as designating seagrass meadows as no anchor zones. More recently, the development of seagrass friendly moorings that reduce the scouring effects of mooring lines are becoming increasingly popular (Egerton, 2011; Jackson et al., 2013). Raising awareness about seagrass meadows to stakeholders including the damage that can be caused by boats and clearly marking the extent of the beds to aid avoidance will help to reduce these risks, but only if supported and enforced by local government agencies and managers (Jackson et al., 2013; Kelly et al., 2019).

Figure 1.2. Mooring scar caused by chain scour over a seagrass meadow in Porthdinllaen, UK, 2016.

Light is the most important factor to affect the growth, distribution and productivity of any primary producer, and as such, seagrasses are particularly sensitive to chronic and temporary light reduction. Reduced water quality either directly or indirectly compromising light attenuation in the water column is thought to be the most significant threat to seagrass meadows worldwide (Biber et al., 2009; Hemminga, 1998; Jiang et al., 2013). Light can be attenuated by increased turbidity caused from boating activity, terrestrial run-off or resuspension of particulate matter in the water column from fishing and dredging activities (Biber et al., 2009; Jiang et al., 2013). Nutrient loading from agricultural and urban run-off can lead to eutrophication, particularly in sheltered areas, causing algal blooms and increasing epiphytic growth which reduce light availability (Jackson et al., 2013; McClelland and Valiela, 1998). Nutrient over-enrichment has been attributed to one of the major reasons for seagrass die-off worldwide (Burkholder et al., 2007; Ralph et al., 2007; Short and Wyllie-Echeverria, 1996). Not only does nutrient loading decrease light levels but it can also lead to the overgrowth of macroalgae that can outcompete seagrasses if not managed. As a marine angiosperm, seagrass has a relatively high minimum light requirement (MLR) in comparison to other marine primary producers in the marine environment (Kenworthy and Fonseca, 1996). This is due in part to the large proportion of non-photosynthetic tissue that make up the roots and rhizomes, often rooted in anoxic sediments due to the high microbial activity within (Hemminga, 1998). Oxygen is transported to the roots from the leaves via internal aeration canals called lacunae (Beer et al., 2014; Carruthers et al., 2001; Waycott et al., 2007). The root and rhizome system of seagrasses is extensive, they are more closely related to ginger or lilies than true grasses (Jackson et al., 2013) and carbohydrates are stored in the rhizomes as a reserve for periods when respiration may outweigh photosynthesis (Burke et al., 1996). The root system enables seagrasses to absorb nutrients from within the substrate as well as across the leaves from the water column which gives them an advantage in nutrient poor waters (Hemminga, 1998). However, this turns to a disadvantage in deteriorating light conditions with the non-photosynthetic below-ground tissue increasing the respiratory demand on the above ground photosynthetic tissue (Collier et al., 2012; Hemminga, 1998). In these circumstances, the balance between carbon demand (for growth, respiration and reproduction) and carbon gain (via photosynthesis) becomes close to or even below zero (Duarte and Kirkman, 2001; Leoni et al., 2008). The MLR of seagrass can also be seasonal, particularly in temperate zones where the fluctuations in daily light period and temperature are great. Increased summer temperatures will increase the metabolic activity of the plants resulting in increased rates of photosynthesis and respiration and therefore an increase in

MLR (Marsh et al., 1986). When temperatures drop, metabolic activity slows and plant growth is reduced lowering the minimum light requirement, but allowing survival aided by carbohydrate stores within the rhizomes (Olesen and Sand-jensen, 1993).

The effects from climate change will have considerable implications for seagrasses especially where they are growing at edges of geographical ranges. An increase in sea level will modify seagrass distribution, with potential expansion of shallow edges where conditions are suitable, but shrinkage from deeper edges where light attenuation will increase. Rises in sea temperature are predicted to lead to the 'Tropicalization' of temperate seagrass meadows, especially in the southern hemisphere, due to a poleward shift in warmer waters (Hyndes et al., 2016). Rises in temperature will increase the MLR of seagrasses due to increased respiration rates. Also, increased storm events will lead to increased run-off, affecting turbidity as well as causing physical damage. The effects of ocean acidification (OA) are more complex, with indirect effects from the impacts on calcifying organisms such as calcareous algae, corals, molluscs, and crustaceans. These organisms make up some of the epiphytes, grazers and predators found within seagrass meadows around the world. A reduction in epiphytic grazers for example, could have huge consequences for seagrasses. However, seagrass could help to reduce the effects of ocean acidification, as they have the capacity to modify the pH within their canopy (Hendriks et al., 2014). Their ability to uptake dissolved inorganic carbon (DIN) in the form of CO2 or HCO_3^- means they have the potential to modify local carbonate chemistry and buffer the effects of ocean acidification (Koweek et al., 2018; Unsworth et al., 2012). However, existing pressures on seagrasses means that this capacity and their resilience to adapt to climate change has already been undermined (Ehlers et al., 2008).

One of the most well documented losses of seagrasses was the dramatic die-off of *Z. marina* in the 1930s across the whole of Europe and North America, which coincided with intensive use of artificially produced fertilisers and herbicides in agriculture (Hughes et al., 2018). This significant loss has been attributed to an infection called 'wasting disease', a type of slime-mould *Labyrinthula zosterae* which causes blackened lesions in leaves which inhibit photosynthetic capacity (Ralph and Short, 2002). This led to vast swathes of seagrass meadows being decimated with losses of around 90% (Muehlstein, 1989). Wasting disease is still prevalent in eelgrass meadows today and is thought to be ubiquitous in the marine environment (Vergeer and den Hartog, 1994). The lack of such die-back events in recent years has led scientists to believe other stressors, such as poor water quality, causes seagrasses to become less resilient and therefore more susceptible to the disease (Ralph and Short, 2002).

The sensitivity of seagrasses to declines in water quality defines seagrasses as sentinels of coastal degradation (McMahon et al., 2013; Orth et al., 2006) and in many cases seagrasses are integrated into management plans to assess the ecological status of coastal waters (Dennison et al., 1993; Foden and Brazier, 2007; Krause-jensen et al., 2005). However poor water quality is still causing a steady decline in seagrass meadows worldwide and we are at risk of losing the very sentinels of coastal health on which we depend (Dennison et al., 1993; McMahon et al., 2013; Orth et al., 2006; Short and Wyllie-Echeverria, 1996). Effective integrated coastal management or ecosystem-based management is needed to identify and manage the threats to seagrasses which includes consistent and frequent monitoring (Griffiths et al., 2020; Ruiz-Frau et al., 2017).

1.3 Adaptations of seagrasses to environmental drivers related to water quality

The main environmental driver related to water quality problems is a reduction in light availability. Light attenuation from the water column means that seagrasses need morphological and physiological adaptations to living in lower light conditions than their ancestral land-based relatives. Seagrasses have also been classified as shade-adapted marine plants due to their overall ability to survive at low levels of irradiance, for example *Posidonia oceanica* can grow up to depths of up to 45 m in the Mediterranean (Pergent et al., 2010). Yet other seagrasses, such as *Zostera* species found in turbid estuarine habitats, can have a depth limit of less than 2 m (Collier et al., 2007; Dennison et al., 1993; Duarte, 1991). The band of light spectrum that is utilized by seagrass and other plants, known as photosynthetically active radiation (PAR), is comprised of wavelengths between 400-700 nm (measured in µmol photons $m^{-2}s^{-1}$) (Beer et al., 2014; Carruthers et al., 2001). In the marine environment, a substantial amount of light from the surface is either reflected, refracted or absorbed by the water which means levels of PAR drop quite significantly with depth. Seagrass light acclimation varies greatly on spatial and temporal scales. Some species of seagrasses tolerate and adapt to high levels of irradiance, especially in tropical regions with increased water clarity and high levels of sunshine. *Halophila stipulacea* was shown to adapt to high light irradiance with changes in chlorophyll content, maximal photosynthesis rates and maximal quantum yield demonstrating an ability to acclimate to high and low light conditions within weeks (Sharon et al., 2009). In high irradiances, *H. stipulacea* also demonstrated chloroplast clumping, making the plant appear more transparent, and then when the light intensity lowers the chloroplasts disperse again. This is thought to protect the chloroplasts against high irradiance and UV radiation damage (Beer et al., 2014).

Seagrasses exhibit various changes in morphology and physiology to light limitation. Some shading experiments have shown that as leaf length decreases, leaves become narrower and thinner, with fewer leaves per shoot (McMahon et al., 2013). Above ground biomass is reduced in this way in order to reduce the respiratory and energetic costs that come from the production and maintenance of new leaves (Collier et al., 2012; Fourqurean and Zieman, 1991). Chlorophyll content increases under low light, with the chlorophyll *a:*b ratio lowering to increase photosynthetic efficiency (Silva et al., 2013). However, photosynthetic performance measured using chlorophyll fluorescence, decreases within a relatively short time-frame (Bité et al., 2007; Ralph and Gademann, 2005). In general leaf size and number of leaves per shoot decreases rather than increases which reduces the respiratory demand of the shoot, but also results in a decrease in photosynthetic capacity (Campbell and Miller, 2002; Collier et al., 2012; Ralph et al., 2007). A reduction in PAR means growth is affected resulting in a reduction in shoot production, leaf growth, root extension and rhizome extension. These changes indicate that seagrasses are able to acclimate to a changing light environment, but the ability to maintain a positive carbon balance will depend upon stores within the rhizomes which will have been built up in higher light conditions (Dennison and Alberte, 1985; Yaakub et al., 2013). This is exhibited by a reduction in rhizome sugars and reduced carbon uptake indicated by the carbon nitrogen ratio (C:N) in the shoots (McMahon et al., 2013). When reserves have been depleted, the resilience of seagrasses to further light reduction or stresses will be weakened. The responses of seagrasses to short and long-term light reductions have been well studied, and understanding the MLR threshold of survival has been highlighted as an important component which needs to be integrated into management and monitoring plans (Dennison et al., 1993; Erftemeijer and Lewis, 2006; McMahon et al., 2013; Yaakub et al., 2013). Local light history will affect the MLR threshold of seagrasses. For example, *Halophila ovalis* subjected to a chronic low light or turbid environment was found to have diminished resilience to further light reductions in comparison to *H. ovalis* from a clear site (Yaakub et al., 2013). The maximum depth limit of a seagrass meadow will give an indication of the MLR of seagrass and some idea of the mean annual light regimes of the location (Carruthers et al., 2001). An overview of the maximum depth limits of seagrass meadows was compiled by Duarte (1991) and the average minimum surface irradiance needed for seagrasses found to be 11% of surface irradiance (Duarte, 1991). However, estimates of the MLR of seagrasses can vary greatly between species and within species (Longstaff and Dennison, 1999). There is, therefore, the scope and the need to understand seagrass resilience and ability to adapt to environmental stresses such as low light, in different species and within different locations (Procaccini et al., 2012).

Seagrasses absorb nutrients from the water column via their leaves as well as through the sediments via their roots. Although nutrients are necessary for productivity, as with all primary producers, nutrient availability will effect tissue content, particularly the content of nitrogen as well as the ratios of carbon, nitrogen and phosphorus, C:N:P or C:N (Burkholder et al., 2007; Touchette and Burkholder, 2000). However, C:N is also used as an indicator of light availability. Nutrients are depleted under high light conditions whereby photosynthesis rates are increased resulting in higher C:N of plant tissue (Burkholder et al., 2007). The concentration of stable isotopes within leaf tissue can also help determine the source of available nutrients, with $\delta^{15}N$ often indicative of anthropogenic sources (Fourqurean et al., 1997; Udy and Dennison, 1997). Morphological responses of seagrasses to nutrient loading can be inconsistent and are influenced by other environmental conditions such as light and local hydrodynamics, although leaf biomass has been found to decrease with increased leaf nitrogen content (Lee et al., 2004). Seagrass meadows growing in oligotrophic waters will respond differently to nutrient loading to seagrasses where nutrients are not limited. In the former, nutrient inputs can stimulate production leading to longer, wider, denser seagrass, but where nutrients are excessive, also causing algal growth and shading, seagrass meadows will reduce in biomass and density (Short et al., 1995).

1.4 Aims

The aim of this study is to investigate the effects of water quality related environmental drivers on the morphological and physiological adaptations of seagrasses by studying responses under controlled conditions as well as *in situ* using existing environmental gradients. The aims were as follows:

1) To test a range of bioindicators of light stress on the seagrass *Zostera marina* under controlled laboratory conditions by consistently measuring changes over time under different light regimes at a shoot level (Chapter 2).
2) Investigate the status of *Zostera noltii,* a small pioneering seagrass species, within a heavily industrialised waterway looking at meadow scale changes in abundance and extent over time using long-term monitoring data (Chapter 3).
3) Investigate morphological and physiological bioindicator responses of a small pioneering seagrass species, *Halodule wrightii,* growing under a range of environmental stressors and anthropogenic impacts in Brazil (Chapter 4).
4) Compare the plasticity of *Z. marina* meadows growing in a range of environmental conditions, including a potentially impacted site within an industrialised waterway, using shoot and meadow scale bioindicators, and analyse any available long-term monitoring data to determine the stability and status of these seagrass meadows (Chapter 5).

Chapter 2: Light Stress Responses by the eelgrass *Zostera marina* (L)

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Abstract

Zostera marina is the dominant seagrass species in the Northern Hemisphere where it grows in sheltered bays and estuaries. As a consequence of its distribution its conservation is commonly threatened by poor coastal water quality. The high minimum light requirements of seagrasses results in water quality degradation (high turbidity and eutrophication) being a significant risk. Bioindicators of light stress can be used to interpret seagrass responses to light limitation and therefore act as sentinels for conservation management. However, there exists limited experimental inter-comparison of the effectiveness of multiple individual bioindicator responses. Meta-analysis suggests that rhizome sugars, shoot C:N, shoot growth and number of leaves per shoot provide the most consistent response variables to increasing light limitation in seagrass, but this premise remains largely untested at the plant level as a direct comparison of multiple bioindicators. The present study aimed to test the morphological, physiological and photo-physiological bioindicator responses of *Z. marina* to light stress applied within controlled laboratory conditions. These bioindicators were used to assign minimum light thresholds. Growth rate and photophysiological parameters (alpha, E_k and ETR_{max}) were rapidly (1st week) and drastically affected by low light shade treatments (20 µmol photons $m^{-2}s^{-1}$ and lower). After three weeks at low light, significant reductions in maximum leaf length and leaf width were observed. Principal Component Analysis identified leaf length, shoot growth, shoot surface area, ETR_{max}, E_k and alpha as having the strongest responses to reduced light. Shoot growth, ETR_{max}, E_k and alpha were found to provide the best early warning of light limitation after 5-8 days. These results provide evidence for bioindicators of light stress in *Z. marina* and highlights the importance of understanding these responses for the successful management and conservation of this species.

2.1. Introduction

Chronic and temporary light reductions caused by reduced water quality are the biggest threat to seagrasses globally (Biber et al., 2009; Hemminga, 1998; Jiang et al., 2013). The sensitivity to light reduction and high nutrient levels defines seagrasses as sentinels of coastal degradation (McMahon et al., 2013; Orth et al., 2006) and in many cases seagrasses are integrated into management plans to assess the ecological status of coastal waters (Dennison et al., 1993; Foden and Brazier, 2007; Krause-jensen et al., 2005). However, poor water quality has resulted in a steady decline in seagrass meadows for decades worldwide and we are at risk of losing the very sentinels of coastal health we have defined (Dennison et al., 1993; McMahon et al., 2013; Orth et al., 2006; Short and Wyllie-Echeverria, 1996).

Seagrasses have been found to exhibit various changes in morphology and physiology in response to light limitation. These responses can be used as bioindicators of reduced light levels attributed to anthropogenic disturbance or other causes for decline in water quality. Light limitation generally causes a decrease in above ground biomass, enabling plants to reduce the respiratory demand of the shoots, but resulting in a decrease in photosynthetic capacity (Campbell and Miller, 2002; Collier et al., 2012b; Ralph et al., 2007). This is shown in morphological responses exhibited during shading experiments such as decreases in leaf length, leaf width, shoot growth and fewer leaves per shoot reducing overall plant surface area (Biber et al., 2009; Collier et al., 2012b; Ochieng et al., 2010; Olesen and Sand-jensen, 1993; Yaakub et al., 2013). The photosynthetic performance of seagrasses, measured using chlorophyll fluorescence, has been found to be affected by light stress within a relatively short time-frame from within days to just seconds (Bité et al., 2007; Ralph and Gademann, 2005). The reduction in light availability results in an increase in the light capture efficiency of the photosystems, but an overall decrease in electron transport rates and carbon fixation (Bité et al., 2007; Ralph and Gademann, 2005). Light reduction can also result in an increase in chlorophyll content, with the chlorophyll *a:b* ratio lowering to increase photosynthetic efficiency (Collier et al., 2009; Collier et al., 2012b; Sharon et al., 2009; Silva et al., 2013). However some studies have found the opposite effect under very low light conditions (Biber et al., 2009; Collier et al., 2012b). These responses in morphology and physiology indicate that seagrasses are able to acclimate to a changing light environment. However the ability to adapt and maintain a positive carbon balance will depend upon the stores within the rhizomes which will have been built up in higher light conditions, as well as the strength and length of light attenuating events (Dennison and Alberte, 1985; Yaakub et al., 2013). This can be shown by a reduction in rhizome sugars and reduced carbon uptake indicated by the carbon nitrogen ratio (C:N) in the shoots (Alcoverro et al., 1999; McMahon et al., 2013). If light levels drop below the minimum light requirement (MLR) threshold, plants are unable to maintain this carbon balance and plant mortality follows.

The range of responses and adaptations of seagrasses to changing light environments could be a reason for the variety of methods used in monitoring. In Europe, 49 seagrass indicators and a total of 51 metrics have been identified in a review of monitoring strategies (Marbà et al., 2013). A more consistent approach would prove useful for managers in determining the status of seagrass meadows aided by identifying the best metrics to measure. A detailed meta-analysis by McMahon et al., (2013) revealed a number of consistent and robust bioindicators to light stress from an array of tropical and temperate seagrass species across a geographical range. In particular, rhizome sugars, shoot C:N, shoot growth and number of leaves per shoot were found to exhibit early responses, with shoot density and above-ground biomass as meadow-scale, long-term responses. However, it should be taken into consideration that response thresholds to light reduction are species-specific and dependent on morphological plasticity, storage products and growth rates (Collier et al., 2012b; Olesen et al., 2002; Ralph et al., 2007). Although there exists a number of reviews and meta-analyses of bioindicators (Biber et al., 2005; Lee et al., 2007; McMahon et al., 2013; Ralph et al., 2007), there are limited case study examples that simultaneously compare a plethora of morphological, photophysiological and biochemical indicators of seagrass response to light availability.

Zostera marina (eelgrass) is a prolific temperate seagrass found growing from the intertidal to depths of around 10m depending on water clarity (Dennison and Alberte, 1982; Jackson et al., 2013). Its distribution in temperate and subpolar regions makes *Z. marina* particularly vulnerable to light limitation, with significant reductions in light during winter months (Alcoverro et al., 1999; Backman and Barilotti, 1976; Moore et al., 1997; Zimmerman et al., 1995). The MLR of seagrass is relatively high in comparison to other marine primary producers (Kenworthy and Fonseca, 1996), due in part to the large proportion of non-photosynthetic tissue that makes up the roots and rhizomes. By using maximal depth limits, the MLR for *Z. marina* is estimated to be 17.6 ± 5.3% SI (Dennison et al., 1993; Erftemeijer and Lewis, 2006; Lee et al., 2007). Surface irradiance (SI) levels vary spatially and temporally with photo-acclimation to local light regimes thought to be responsible for the large within-species variation (Lee et al., 2007). Other studies suggest that eelgrass requires 5-6 hours of irradiance-saturated photosynthesis per day to maintain a positive carbon balance (Alcoverro et al., 1999; Zimmerman et al., 1996, 1995). Nonetheless, the understanding of the MLR threshold of survival has been highlighted as an important component which needs to be integrated into management and monitoring plans (Collier et al., 2016; Dennison et al., 1993; Erftemeijer and Lewis, 2006; McMahon et al., 2013; Yaakub et al., 2013).

The aim of the present study was to test a range of light stress bioindicators simultaneously by systematically monitoring the morphological and physiological responses of *Z. marina* to varying degrees of light stress treatment under experimental conditions. Plants under low and extremely low light treatments were expected to show a rapid change in photosynthetic performance and shoot growth followed by morphological responses as plants become constrained by low light, leading to mortality. Plants under medium light (close to minimum light requirements) were expected to show less extreme responses and better adaptation to lower light conditions to allow survival. By monitoring the rate of numerous responses to light stress over time, this study aimed to identify the most robust bioindicators of light stress specific to *Z. marina* and provide a quantitative estimate for the MLR threshold for this seagrass species.

2.2. Materials and methods

2.2.1 Experimental design

Thirty independent *Z. marina* cores (10 cm x 10 cm, 15-20 cm deep) were collected on a low spring tide from across a shallow subtidal seagrass meadow at Durgan, Helford River, UK (50º06’27.19”N; 5º06’54.70”W). Care was taken to ensure the plants were extracted with as little damage as possible and that the shoots had substantial rhizome sections and sediment for re-planting. The cores of seagrass were put into separate large plastic bags and then transferred to pots and placed in a cool box for transportation to Swansea University, with ice packs and fresh seawater. Extra samples of rhizomes and shoots were taken as site control samples for carbohydrate analysis (n=4), chlorophyll content (n=4) and C:N ratios (n=5) to compare with plants at the end of the experiment. These samples were stored in a freezer at -20°C. The individual cores were replanted into tubs (n=5 per treatment). Extra sediment collected from the field site was used to ensure natural presence of benthic fauna.

In the laboratory, the plants were left to acclimatize for 10 days before the experiment started in a flow-through system providing fresh filtered seawater. At the start of the experiment, the plants were placed under 4 different light treatments in a flow-through seawater microcosm providing continuous fresh seawater. Pumps and air stones were provided to ensure circulation and the shade treatments were created using shade cloths. The flow-through system was connected to a cooling unit to ensure maintenance of a temperature of 14 ± 1°C, simulating the sea temperature at time of collection. The temperature was monitored in each tank with a Tiny Tag (Aquatic 2, Gemini data loggers, Chester, UK) temperature logger. Frames with LED aquatic lights (AquaBeam 2000HD) were fitted and shades of varying weave put in place above and between the tubs to create independent shade treatments (and high light with no shade). A light meter (ULM-500, with spherical micro quantum Sensor US-SQS/L, Waltz GmbH, Effeltrich, Germany) was used to set up the light treatments by measuring the average PAR (Photosynthetic Active Radiation) in each tank section at the top of the seagrass leaf canopy. The HL treatment was set up to have a level that corresponds to HL treatments in other studies of around 100 ± 10 µmol photons $m^{-2}s^{-1}$ (Biber et al., 2005; Olesen and Sand-jensen, 1993; Shafer and Kaldy, 2013) also comparable to highest growth rates found previously (between 100-150 µmol photons $m^{-2}s^{-1}$, Olesen and Sand-jensen (1993)). The medium light treatment (ML) was set up using 40% shade cloth which gave an average of 27 ± 5 µmol photons $m^{-2}s^{-1}$ at plant height, to create light levels that correspond to levels of light compensated growth (between 19-47 µmol photons $m^{-2}s^{-1}$, Olesen and Sand-jensen, 1993). The low light treatment (LL) was created using a 90% shade cloth, reducing the light to around 7 ± 3 µmol photons $m^{-2}s^{-1}$. The extreme low light treatment (EL) was placed under a 98% shade cloth found to have a PAR of around 1.8 ± 0.5 µmol photons $m^{-2}s^{-1}$. Lights were fitted on a timer to give a photoperiod of 10 hours of light and 14 hours of dark reflecting the natural daylight hours at time of collection. An Odyssey PAR light logger (Dataflow Systems Ltd), calibrated against a Li-Cor quantum light sensor (LI 192), was deployed at mid-depth of plant canopy under each treatment for 1-2 days logging every 10 mins, to provide an average PAR per day (Table 2.1). The experiment was set up to run for 6 weeks after acclimation time.

Table 2.1. Total amount of light recorded by Odyssey PAR logger placed within tanks (calibrated with a LI-COR, Li-250A light meter) and temperature range logged for each treatment tank.

Treatment	Amount of light per day (mol photons $m^{-2}day^{-1}$)	Average PAR per treatment (µmol photons $m^{-2}s^{-1}$ ±SD)	Temperature range (°C)
High	5.61	155.76 ± 11.42 (100%)	13.2 – 14.0
Medium	0.73	20.15 ±0.36 (12.9%)	13.2 – 14.0
Low	0.35	9.78 ± 0.57 (6.3%)	13.4 - 13.9
Extreme low	0.14	3.76 ± 0.41 (2.4%)	13.4 - 13.9

2.2.2. Morphometric measurements

Morphometric measurements for each shoot (n=5 per treatment) were taken weekly, these included; sheath length, leaf length (taken from top of sheath to tip of leaf), leaf width and shoot growth. Leaves were wiped clean of epiphytes throughout the experiment although older ends of leaves were difficult to clean without causing damage so were left, but these tended to be chlorotic and often dead (Drake et al., 2003). Lengths measurements were taken with a measuring tape to the nearest mm, and the maximum leaf length for each shoot was analysed as a measure that can be attributed to canopy height in seagrass meadows (Longstaff and Dennison, 1999). Leaf width was measured using callipers to the nearest 0.05mm at 5 cm above sheath or in the middle of each leaf <5 cm long. Average leaf width was calculated for each shoot. Shoot surface area was calculated from the length and width measurements of all leaves of all shoots to provide potential surface area for photosynthesis. For shoot growth, each plant was marked at the top of its sheath with a needle so the growth of each leaf could be measured using the method outlined by Short and Duarte (2001). If new leaves were found, they were marked at the same location on the sheath. The new growth could then be measured against the mark on the sheath, the outer part of which is usually dead and does not alter. If the outer leaf became detached then the top of the sheath would remain so growth could still be measured from this position. Raw values for maximum leaf length, shoot surface area, shoot growth and average leaf width per shoot were used for statistical analysis.

2.2.3. Photosynthetic measurements

Pulse Amplitude Modulated (PAM) fluorometry using a Diving-PAM (Waltz), was used to measure chlorophyll fluorescence weekly, as a non-invasive technique for assessing photosynthetic activity. Photosynthetic parameters were obtained by performing rapid light curves (RLC) using the internal stepwise function of the PAM fluorometer. All RLCs were carried out between 11am and 2pm. These were conducted on the mid-section of the youngest mature leaf using a leaf clip, enabling the measurement of a proxy for quantum yield (F_v/F_m), using the first data point obtained from the rapid light curve which provides maximum quantum efficiency, where F_v is the variable fluorescence yield and F_m is maximal fluorescence yield (Collier et al., 2009; Ralph and Gademann, 2005). The change of the fluorescence ($\Delta F/F_{m'}$) is measured at each irradiance step of the light curve. Steps ranged from 7-1652 µmol photons $m^{-2}s^{-1}$ lasting 10 s, with initial saturating pulse >>2,000 µmol photons $m^{-2}s^{-1}$ (Beer et al., 2001) for 0.8 s. Electron Transport Rate (ETR) was worked out using the equation; ETR = $\Delta F/F_{m'} * I_i * AF * 0.5$, where AF is the absorption factor, calculated to be 0.78 (± 0.02 S.D., n=5) (Beer et al., 2001; Saroussi and Beer, 2007) and I_i is the incident irradiance (from the light stages of the light curve programmed into the Dive-PAM). The stepwise RLCs were fitted to the non-linear least-squares regression model by Eilers and Peeters (Eilers and Peeters, 1988) to estimate the ETR_{max} (maximum rate of photosynthesis), α (the initial slope of the rapid light curve, a measure of the light harvesting efficiency) and E_k (the minimum saturating irradiance worked out from the intercept of α and the maximum photosynthetic rate). As the E_k is worked out from the value of ETR_{max} /α on the RLC, it will show correlation which needs to be taken into account when interpreting results.

2.2.4. Physiological measurements

2.2.4.1. C:N content of seagrass

At the end of the experiment, leaf material from each shoot was taken to be compared with site control samples taken from the field site at Durgan. Avoiding the older chlorotic leaves, leaf material was scraped free of epiphytes, blotted dry and weighed before being dried at 60°C for 48 hours. The dried seagrass was ground up with a pestle and mortar to a fine homogenous powder before being weighed (Ohaus balance, max 100g d=0.1 mg, Switzerland). Samples were sent to IBERS (Aberystwyth University) for analysis of the % composition of C and N by weight using a continuous flow isotope ratio mass spectrometer (Anca SL 20-20, Europa Scientific, Crewe, UK).

2.2.4.2. Rhizome sugars

At the end of the experiment the rhizomes from each plant were separated, weighed and also stored at -20°C to be compared with samples taken from the field site at Durgan. The rhizomes were dried in an oven at 60°C for 4-5 days. The dried rhizome sections were ground up with a pestle and mortar to a fine homogenous powder to enable analysis by HPLC. Samples were sent to IBERS at Aberystwyth University for analysis. Soluble rhizome sugars were extracted based on the method outlined in Cairns & Pollock (1988).

2.2.4.3. Chlorophyll pigments

Samples of *Zostera marina* from Durgan were collected from the study site and compared with samples from all of the plants at the end of the experiment. Chlorophyll was extracted in 90% acetone and quantified using a spectrophotometer (Schimadzu UV-2550 UV VIS Series) based on the methods outlined by Dennison (1990) and Granger and Izumi (2001).

2.2.5. Statistics

Two-way repeated measures ANOVA was used to analyse time series collected data, with light treatment (between-subject effects) over time (within-subject effects) using SigmaPlot Version 11 (Systat Software, San Jose, CA). Data did not completely fulfil the assumptions of ANOVA therefore in order to minimize the risk of Type I error, significance was only accepted based on p-values of <0.01 (Collier et al., 2012b; McDonald, 2014; Underwood, 1997). ANOVA was still performed due to the robust nature of the test and the relative insensitivity of the F test to departures from normality (Glass et al., 1972; Lix et al., 1996; McDonald, 2014).

Results from the repeated measures ANOVA were interpreted with the Holm-Šídák pairwise comparisons test in SigmaPlot.

C:N ratio, rhizome total water soluble carbohydrate (WSC) content and chlorophyll content were analysed using one-way ANOVA in RStudio (R version 3.2.2) to compare plants after treatment with site control plants that were taken directly from Durgan (untreated). The Bartlett test for homogeneity was used to test for equal variance (Bartlett, 1937). Tukey's test was used to look at multiple comparisons of means.

Principal Component Analysis (PCA) using Primer6 was used to identify patterns of which morphological and photophysiological factors contributed to the biggest responses of plants to shade treatment over time. Principal components with eigenvalues greater than 1.0 were considered, and eigenfactors or variable coefficients ≤ -0.3, or ≥ 0.3 were selected.

2.3. Results

At the end of the experiment none of the HL shoots had died but all plants subjected to shade treatment (ML, LL and EL) showed signs of mortality at the basal meristem (all leaves coming free from within the sheath) by day 43, with one LL shoot dying after just 4 weeks.

2.3.1 Morphological characteristics

2.3.1.1. Maximum leaf length

At the start of the experiment all plants exhibited a maximum leaf length within the range of 455 mm to 727 mm with an average of 564.00 ± 18.53 mm. The average max leaf length decreased in all plants throughout the experiment although reduction was lowest in HL plants. All plants under shade treatments showed a significant reduction in average max leaf length in comparison to those under HL from day 15 for ML (p=0.009) and LL plants (p=0.01) and day 22 for EL plants (p=0.013, Fig. 2.1). ML plants decreased steadily in length from day 15 resulting in a significant reduction in max leaf length to 391.60 ± 51.61 mm by day 43 (p<0.001). LL plants also decreased steadily from day 15 although the differences in max leaf length were not significant. EL plants decreased in max leaf length from day 22 with a significant reduction shown between day 15 and day 37 (p=0.002). HL plants remained with a max leaf length above 610 mm until day 43 when average max leaf length had decreased to 489.80 ± 38.47 mm.

2.3.1.2. Leaf width

The leaf width of all plants at the start of the experiment ranged between 4.6 mm to 6.85 mm. All plants decreased in average width throughout the experiment and this reduction was lowest in HL plants. All plants under shade treatments showed significant reductions in width by day 29 with no significant change in leaf width for HL plants throughout the experiment (Fig. 2.1). ML plants decreased steadily from day 0 and showed a significant reduction in width from 5.35 ± 0.11 mm to 5.02 ± 0.12 mm on day 22 (p=0.002). LL plants decreased significantly in width from 5.93 ± 0.35 mm on day 0 to 5.14 ± 0.30 mm on day 43 (p<0.001) with the first significant reduction by day 29 (5.33 ± 0.33 mm, p<0.001). For EL plants leaf width decreased significantly from 5.92 ± 1.25 mm on day 0 to 5.40 ± 0.26 mm on day 43 (p<0.001) with the first observed significant decrease in width observed on day 29 (5.48 ± 0.22 mm, p<0.001). Results showed there was a significant interaction between light treatment and time on average leaf width (p=<0.001) (Table 2.2).

2.3.1.3. Shoot growth

All plants decreased in average growth rate throughout the experiment, although this reduction was lowest in HL plants. HL plants had significantly higher growth of 28.29 ±1.63 mm day^{-1} on day 9 in comparison to 16.55 ± 2.36 mm day^{-1} for ML plants (p=0.001), 13.10 ± 1.66 mm day^{-1} for LL plants (p=<0.001) and 13.80 ± 3.03 mm day^{-1} for EL plants (p<0.001) (Fig. 2.1). Shoot growth of HL plants remained significantly higher than shade treated plants until day 37 and continued to remain higher than shaded plants until day 43. ML plants showed a significant decline in growth from day 9 to day 37 (p=0.002). The growth of LL and EL plants was significantly lower than HL plants from day 9, but not significantly different to ML plants or to each other suggesting growth was already affected by light reduction for shade treated plants between before day 9. Results showed there was a significant interaction between light treatment and time for shoot growth (p=0.001) (Table 2.2).

2.3.1.4. Shoot surface area

At the start of the experiment shoot surface area ranged from 4563.3 mm^2 to 10580.0 mm^2 and averaged 8218.6 ± 326.6 mm^2. All plants decreased in average shoot surface area throughout the experiment with the lowest reduction in HL plants. All plants subjected to shade treatments showed a significant decline in surface area by day 29 (fig. 2.1). HL plants remained significantly larger in surface area than all shade treated plants from day 29 until day 43 (p=<0.001-0.002). ML plants reduced in surface area by 41.5% (a reduction from 7831.89 ± 245.9 mm^2 to 4584.1 ± 464.5 mm^2, p=<0.001) and LL plants were reduced by 44% by day 29 (from 7883.3 ± 756.6 mm^2 to 4413.8 ± 1345.7 mm^2, p=<0.001). EL plants displayed a reduction of 30.5% on day 29 (p=0.002) and had reduced by 44.3% by day 43 (p=<0.001). For plant surface area there was a significant interaction between light and day (p=<0.001) (Table 2.2).

Table 2.2-2.3. Results of two-way repeated measures ANOVA testing for the within-subjects effects of time and treatment on the morphological parameters maximum leaf length, width shoot surface area and shoot growth rate, and on the photosynthetic parameters Alpha (α), ETR$_{max}$ and Maximal yield ((F$_m$'-F)/F$_m$'). No transformations were applied to the data for statistical analysis. Also shown is the p-value that was considered significant (adjusted if variances were not homogenous)

		Max leaf length (mm)			**Leaf width (mm)**			**Shoot surface area (mm^2)**			**Shoot growth rate (mm day^{-1})**		
	DF	**MS**	**F**	***P***	**MS**	**F**	***P***	**MS**	**F**	***p***	**MS**	**F**	***p***
% Light	3	1.41E+05	4.532	**0.018**	3.735	2.856	0.070	5.11E+07	3.897	**0.029**	1791.182	22.496	**<0.001**
Day	6	5.90E+04	17.305	**<0.001**	0.97	37.633	**<0.001**	5.11E+07	29.086	**<0.001**	367.994	12.472	**<0.001**
% Light x Day	18	3660.635	1.073	0.391	0.091	3.519	**<0.001**	5.15E+06	2.809	**<0.001**	84.04	2.848	**0.001**
Significance level				0.05			0.05			0.05			0.05

		Alpha (α)			**ETR$_{max}$**			**Ek**			***F_v/F_m***		
	DF	**MS**	**F**	***p***	**MS**	**F**	***p***	**MS**	**F**	***p***	**MS**	**F**	***p***
% Light	3	1.108	21.632	**<0.001**	3314.746	21.908	**<0.001**	1.92E+04	8.71	**0.001**	1.108	21.632	**<0.001**
Day	6	0.158	7.425	**<0.001**	2256.437	43.597	**<0.001**	6373.13	16.44	**<0.001**	0.158	7.425	**<0.001**
% Light x Day	18	0.040	1.878	**0.027**	184.494	3.565	**<0.001**	1057.88	2.73	**0.001**	0.040	1.878	**0.027**
Significance level				0.01			0.01			0.01			0.01

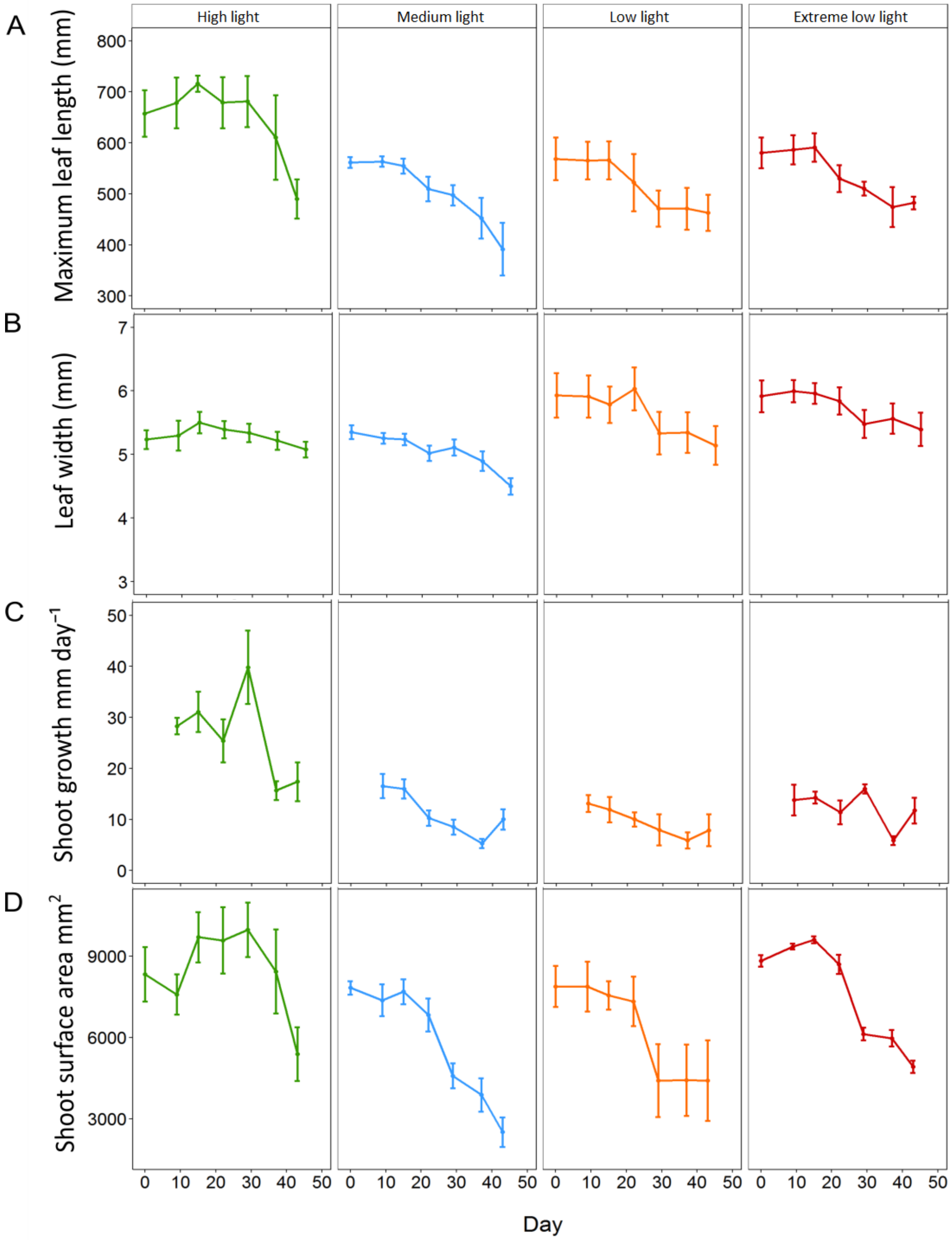

Figure 2.1. Effect of light shading on the morphological characteristics of *Zostera marina* over time. Plants were kept in a flow through aquaria using natural filtered seawater under artificial light. (A) Maximum leaf length (B) leaf width, (C) shoot growth and (D) total shoot surface area. Values are means ±SE, *n*=5 (except low light plants on days 37 and 45 where *n*=4).

2.3.2. Photosynthetic characteristics

2.3.2.1. Alpha

At the start of the experiment the light harvesting efficiency or alpha (α) ranged from 0.53 to 1.27 with an overall average of 0.87 ± 0.05. Alpha responded rapidly (by day 5) to shading treatments relative to the HL plants (fig. 2.2A). There was no significant change in α for HL plants throughout the experiment. ML plants, displayed a significant increase in α from 0.91 ± 0.07 on day 0 to 1.19 ± 0.02 on day 19 (p=0.003). EL plants showed a significant increase in α from day 5 from 0.69 ± 0.08 to 1.25 ± 0.08 (p=<0.001). LL plants showed no significant change in α over time, although levels remained significantly higher than HL plants throughout the experiment. Results showed there was a significant interaction between light treatment and time for alpha (p=0.001) (Table 2.2).

2.3.2.2. ETR$_{max}$

The maximum Electron Transport Rate (ETR$_{max}$) ranged from 26.82 µmol electrons m^{-2}s^{-1} to 97.60 µmol electrons m^{-2} s^{-1} with an average of 57.98 ± 4.75 µmol electrons m^{-2} s^{-1} at the beginning of the experiment. The ETR$_{max}$ was significantly affected in all shading treatments by day 5 (Fig. 2.2B). Not all plants gave a reliable ETR$_{max}$ value as some curves did not saturate and these results were omitted. HL plants remained with significantly higher ETR$_{max}$ than all shade treated plants (p<0.001-0.012) with EL plants showing the biggest drop of 76 % from day 0 to day 5 (79.39 ± 9.11 to 18.96 ± 0.50 µmol photons m^{-2}s^{-1}; p<0.001). ML plants showed a reduction in ETR$_{max}$ of 42 % on day 5 (55.03 ±6.58 to 31.94 ± 2.34 µmol electrons m^{-2} s^{-1}; p<0.001). LL plants showed a reduction of 57 % (43.66 ± 5.89 µmol electrons m^{-2} s^{-1} to 18.85 ± 0.24 µmol electrons m^{-2}s^{-1}; p<0.001) on day 5. On the contrary, HL plants showed a slight increase on day 5 from 60.04 ± 4.18 µmol electrons m^{-2} s^{-1} to 69.10 ± 0.36 µmol electrons m^{-2} s^{-1}.

1.3.2.3. E$_k$

The E$_k$ of plants at the start of the experiment averaged 75.52 ± 11.32 µmol photons m^{-2}s^{-1} with a broad range from 24.64 to 183.57 µmol photons m^{-2}s^{-1}. Not all plants gave a reliable E$_k$ value as some curves did not saturate and these results were omitted. The E$_k$ of all shaded plants was significantly affected on day 5 (Fig. 2.2C). HL had a significantly higher E$_k$ than shaded plants, 72.42 ± 20.38 compared to 33.07 ± 4.80 for ML ($p=0.002$), 16.17 ± 0.19 for LL ($p=<0.001$) and 15.27 ± 0.56 µmol photons $m^{-2}s^{-1}$ for EL plants ($p=<0.001$). All plants showed a decrease in E_k throughout the experiment with EL plants showing the biggest reduction of 80.1% by day 43 ($p=<0.001$).

1.3.2.4. Maximum quantum efficiency (F_v/F_m)

A proxy for yield using the maximum quantum efficiency at the first data point of the light curve (F_v/F_m) was significantly affected by shading by day 12 where HL plants had significantly ($p<0.001$ and $p=0.001$ respectively) lower yield (0.74 ± 0.01) than EL plants (0.79 ± 0.003) and LL plants (0.79 ± 0.01). EL light levels resulted in plants having a significantly higher yield than HL until day 43 (Fig. 2.2D).

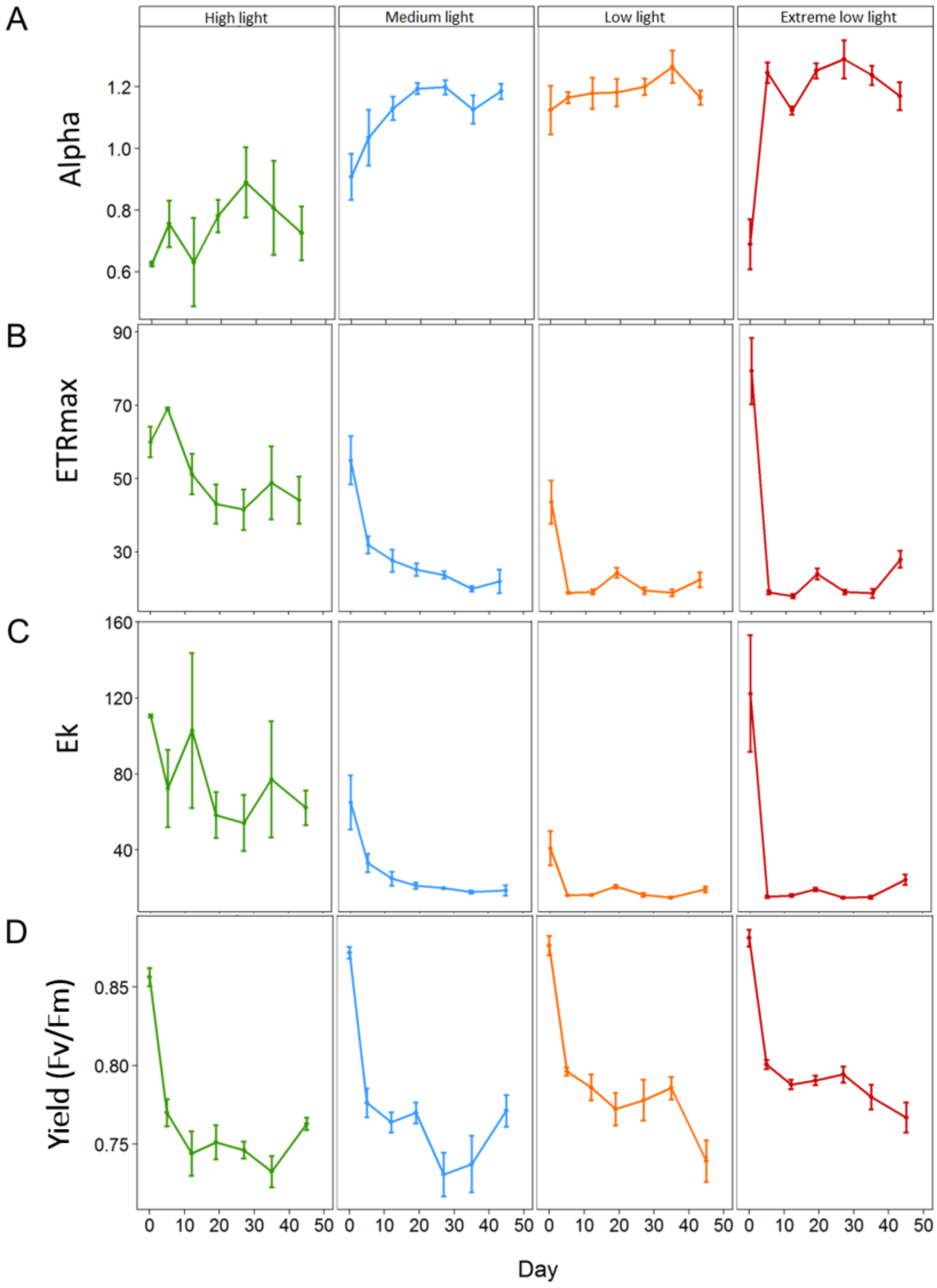

Figure 2.2. Effect of light shading on the photosynthetic properties of *Zostera marina* over time measured by PAM fluorometry. Plants were kept in a flow through aquaria using natural filtered seawater under artificial light. (A) Alpha α, (B) Maximum electron transport rate (ETR_{max}, µmol electrons m^{-2} s^{-1}), (C) Minimum saturation irradiance (E_k, µmol photons $m^{-2}s^{-1}$) and (D) Yield (F_v/F_m). Values are mean ±SE, *n*=4-5 (except HL and EL plants on day 0 where *n*=2 and *n*=3 respectively).

2.3.3. Physiological results

2.3.3.1. C:N

Results of the one-way ANOVA with Tukey's post-hoc comparison showed ML plants had a significantly higher C:N (17.56 ±0.57) than EL plants (15.04 ±0.37), p=<0.006. There were no significant differences in C:N between site control plants (16.48 ± 0.55) and any of the plants after treatment (Table 1.3, Fig. 2.3).

2.3.3.2. Rhizome sugars - WSC (Water Soluble Carbohydrates)

Results of the one-way ANOVA with Tukey's post-hoc comparison showed site control plants had significantly higher total WSC content (52.76 ± 2.36 mg/ml) than all treatments (p=<0.001). There was no significant difference between treatments at the end of the experiment (Table 1.3, Fig. 2.3).

2.3.3.3. Chlorophyll content

Results of the one-way ANOVA with Tukey's post-hoc comparison showed site control samples had significantly higher chlorophyll content (4.13 ± 0.16 µg Chl cm^{-2}) than ML (2.45 ± 0.28 µg Chl cm^{-2}), p=<0.001, and LL plants (3.14 ± 0.09 µg Chl cm^{-2}), p=0.014. ML plants were also significantly lower in chlorophyll content than HL plants, p=0.002, and EL plants, p=0.009 (Table 2.3, Fig. 2.3).

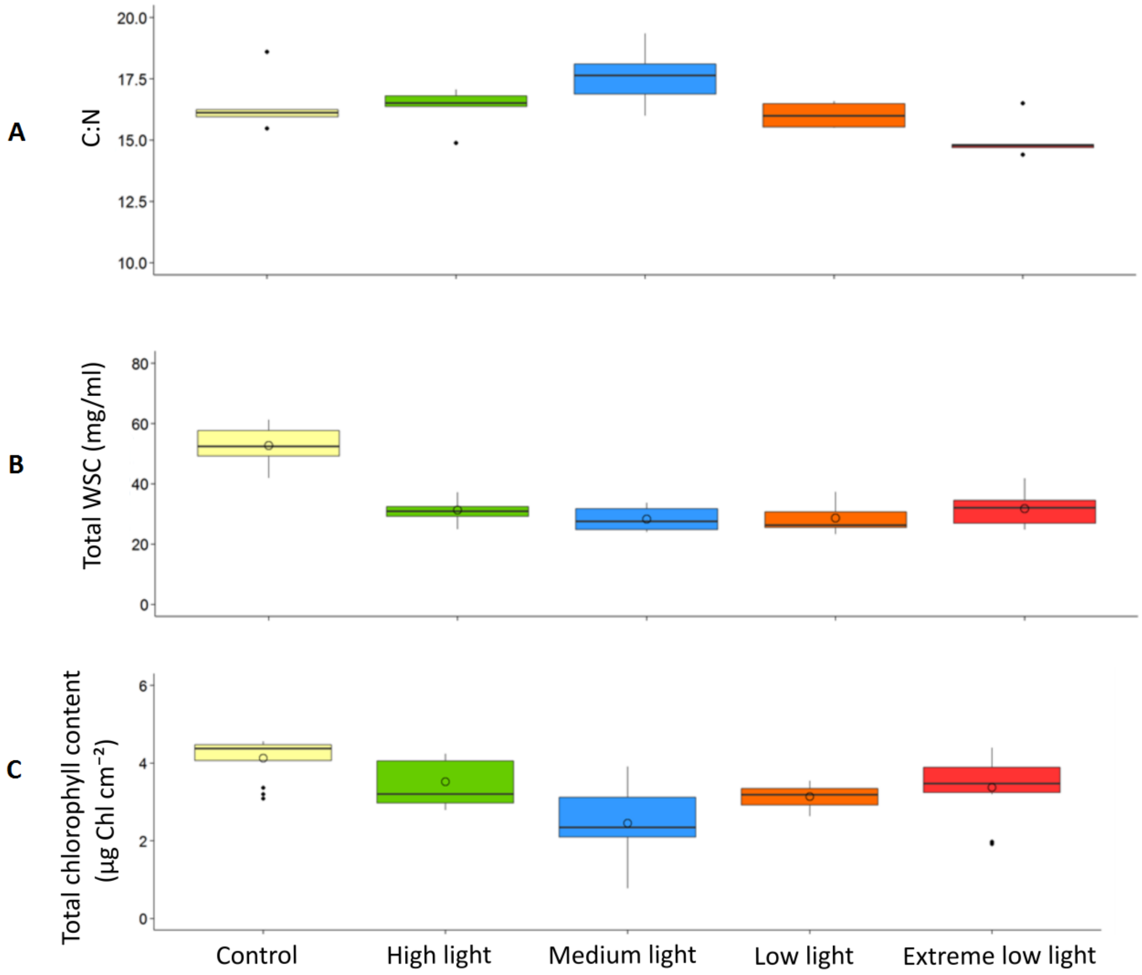

Figure 2.3. Effect of light shading on physiological properties of *Z. marina* with time. Plants were kept in a flow through aquaria using natural filtered seawater under artificial light. Boxplots show results from control site plants compared with plants at the end of the experiment. (A) C:N (B) Total water soluble carbohydrates(WSC), (C) Total chlorophyll content. Central line is the median, o point is the mean, *n*=5.

Table 2.4. Results of one-way ANOVA testing effects of light manipulation on the physiological parameters C:N, water soluble carbohydrate content of rhizomes (WSC), and leave chlorophyll content. All passed Bartlett's test for homogeneity except chlorophyll.

	DF	MS	F	*P*
C:N	4	872.8	34.27	**<0.001**
WSC	4	4.191	4.175	**<0.05**
Chlorophyll	4	5.029	9.179	**<0.001**
Significance level				0.05

2.3.4. Bioindicator analysis

Principal component analysis was used to show which bioindicators contributed to the most variability between treatments and within treatments over time. The first two principal components (PC1 and PC2) had eigenvalues over 1 making up over 70% of the variability. PC1 approximately corresponds with the change from high light to low light, whereas the treatments are more evenly spread across PC2. PC1 had an eigenvalue of 3.85 making up 48.1% of the variability and showed a correlation (above 0.3) between the variables leaf length, shoot surface area, shoot growth, ETR_{max}, alpha and E_k. The first component increases with decreasing leaf length, shoot surface area, shoot growth, ETR_{max}, E_k and an increase in alpha. PC2 has a strong correlation with leaf width and shoot surface area (both above -0.5) and also a correlation with yield (F_v/F_m) (-0.482). PC2 increases with a decrease with these correlating factors. HL plants remain in cluster away from shade treated plants, only moving closer in similarity to shaded plants in week 5 and 6 Fig. 2.4).

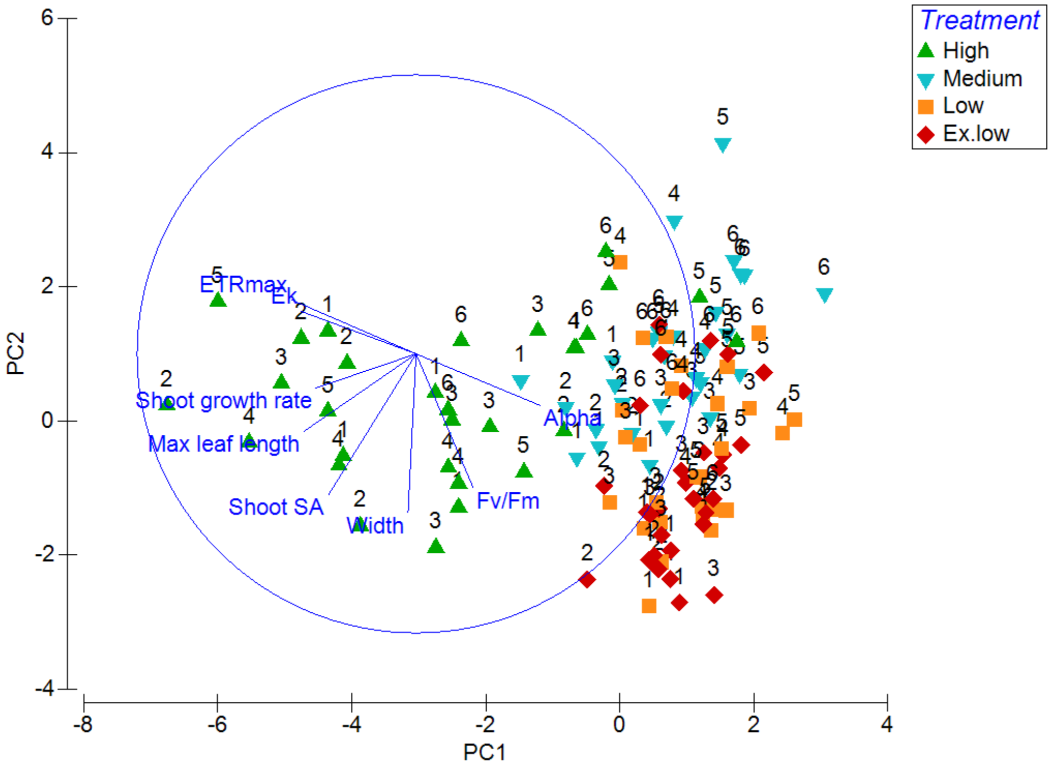

Figure 2.4. Results from Principal Component Analysis of morphological (leaf length, width, shoot surface area and shoot growth) and photophysiological (Alpha, maximum electron transfer rate, minimum saturation irradiance and effective quantum yield) responses with treatment and week (starting from week 1) as factors.

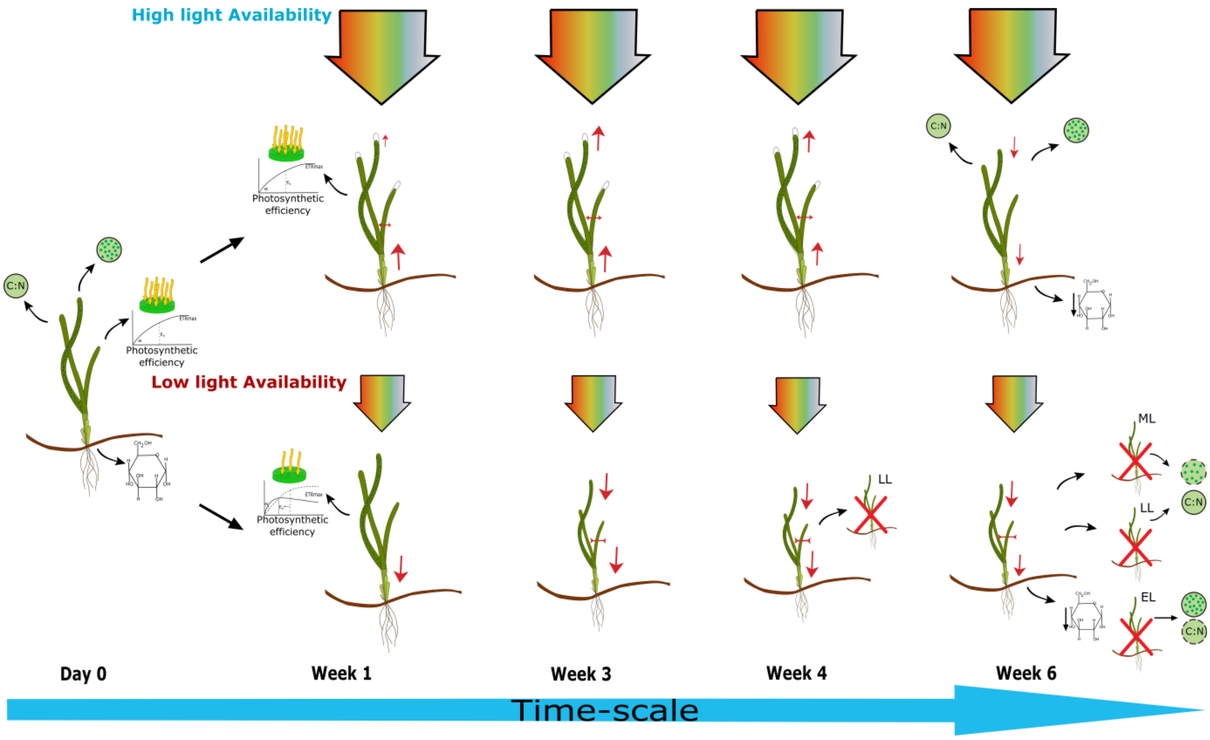

Figure 2.5. Schematic diagram showing effects of light limitation on *Zostera marina* plants over time under experimental conditions. High light treatment (HL) = 5.61 mol photons $m^{-2}day^{-1}$, light limiting treatment < 0.73 mol photons $m^{-2}day^{-1}$ (ML= medium light 0.73, LL= low light 0.35, EL = extreme low light treatments 0.14 mol photons $m^{-2}day^{-1}$). Within the first week photosynthetic efficiency was affected in shaded plants shown by the increase in α, a decrease in ETR_{max} and E_k, along with significantly reduced shoot growth. By week 3 leaf length was reduced in all shaded plants and leaf width reduced in ML plants. By week 4 leaf width and shoot surface area were significantly reduced in all shaded plants with the death of a LL plant. By week six, HL plants also showed decreases in leaf length, shoot growth and shoot surface area. Water soluble carbohydrate content of rhizomes was reduced in all plants at the end of the experiment. A decrease in chlorophyll content was exhibited in LL and ML plants. There was no significant change in C:N of leaf tissue recorded for any of the plants in comparison to samples taken from site location, although EL plants had the lowest ratio. Evidence of shoot mortality was found in all shade treated plants at the end of the experiment.

2.4. Discussion

The present study provides an experimental test of the response of multiple photophysiological and morphological bioindicators to light limitation on *Zostera marina.* The study provides an experimental insight into timescales and levels of response to light limitation specific to *Z. marina* relative to models of light stress developed through meta-analysis (McMahon et al., 2013). Leaf length, shoot growth rate, shoot surface area, alpha ETR_{max} and E_k (which are correlated) showed the strongest responses to light limitation across time suggesting that they act as robust bioindicators of light stress in *Z. marina*. Leaf width and yield were also found to respond consistently to light limitation. Physiological parameters proposed by the meta-analysis (McMahon et al., 2013) to make good bioindicators were not found to be as robust when considered directly against these other variables such as leaf length, alpha and ETR_{max}.

Under light limiting conditions, plants exhibited significant reductions in length, width, surface area and growth rate within three to four weeks, resulting in a reduction of above ground tissue. This reduction potentially poses a respiratory burden to the plant (Collier et al., 2012a; Fourqurean and Zieman, 1991). Growth rate was significantly reduced in shaded plants by day 8, suggesting that the response could have been detected even earlier. At a meadow scale, we propose that the plant response to low light would result in a reduction in overall seagrass density with corresponding impacts upon ecosystem resilience (Maxwell et al., 2016; Unsworth et al., 2015). A reduction in canopy density may actually be considered an adaptive mechanism to maximise available light as this allows more ambient light through to reduce self-shading (Collier et al., 2012a).

The present study shows that a reduction in light to 20 µmol photons $m^{-2}s^{-1}$ (10:14 hour light:dark photoperiod) is enough to cause a significant decrease in leaf length and width resulting in a decrease in plant surface area by 41% after 29 days, with further light stress in the LL and EL treatments causing similar albeit slightly bigger reductions. Light reductions of this scale would result in significant effects at a meadow scale within weeks, indicating that impacts causing light reductions over a similar time scale would have a substantially damaging effect. These results are comparable to *in situ* shading experiments conducted on *Z. marina* (Backman and Barilotti, 1976; Dennison and Alberte, 1985). A decline in shoot surface area results in significant implications on a meadow-scale. A reduced amount of photosynthetic tissue and a decrease in canopy density may affect the ability of the meadow to attenuate the effects of waves and currents. This results in an increase in levels of suspended sediments causing an increase in turbidity, and a reduction in sediment stabilization for the roots and rhizomes (van der Heide et al., 2007). A scenario like this can cause a shift to an alternate state within the meadow system, making it difficult for recovery unless conditions and light levels are restored (Unsworth et al., 2015; van der Heide et al., 2011). Frequent and prolonged periods of reductions in light levels to 20 µmol photons $m^{-2}s^{-1}$ or below should be cause enough to alert managers to take action to improve water quality.

The meta-analysis by McMahon et al., (2013) did not recommend the morphological characteristics of leaf length, leaf width or shoot surface area as robust bioindicators of light stress for seagrasses overall. However, there is evidence for differences between different genera or species in response to light limitation. For instance, *Z. muelleri* was found to have the most rapid and 'plastic' responses in morphology and growth to shading when compared to three other species of differing genus (Collier et al., 2012). The importance of these differences is also highlighted by contrasting interspecific morphological responses, for example, *Posidonia oceanica* has been found to increase leaf width whilst *Zostera nigricaulis, Halophila ovalis* and *Halodule wrightii* exhibit an increase in leaf length as a way of increasing light capture (Bulthuis, 1983; Collier et al., 2007; Dalla Via et al., 1998; Shafer, 1999).

Photophysiological responses to shading were exhibited within the first week and results showed trends consistent with other studies (Beer et al., 2014; Belshe et al., 2008). All plants showed high variability in alpha, ETR_{max}/E_k on day 0, however by day 5, all shaded plants exhibited significant reductions in E_k/ETR_{max}, and significant increases in alpha. HL plants did not show such significant changes in alpha or ETR_{max} as would be expected of plants adapted to higher light conditions. In contrast to the meta-analysis the present study identified alpha as a robust indicator of light stress, along with ETR_{max}/E_k. The use of PAM fluorometry is useful as a non-invasive monitoring tool as it can detect physiological responses of seagrasses to light stress before morphological changes take place (Belshe et al., 2007). However, photosynthesis measurements using PAM display high levels of seasonal, diurnal and shoot-scale variation which need to be fully understood (Durako and Kunzelman, 2002). Also, chlorophyll fluorescence has been found to be good for assessing recovery in *Z. marina*, but not for detecting the onset of mortality (Biber et al., 2009). Even so, if the use of PAM fluorometry is feasible, consistent monitoring would give valuable information about the environmental conditions of a *Z. marina* meadow, especially if used alongside morphological bioindicators.

Physiological responses measured in this study showed no significant changes between shaded and HL plants. There were no significant changes in C:N at the end of the experiment when compared to control site samples. This could have been affected by the time of year the samples were taken. The C:N of *Z. marina* is usually at its highest in summer months and lowest in winter months (Fourqurean et al., 1997) and sampling for the present study took place in late October (autumn in UK). It should also be noted that C:N has been identified as a robust bioindicator for *Z. marina* in a study that used an intensive field sampling strategy providing reliable evidence to this effect (B. L. Jones and Unsworth, 2016).

The water soluble carbohydrate (WSC) content of rhizomes did not vary significantly between treatments at the end of the experiment and therefore the present study did not conclusively find WSC to be a robust bioindicator of light stress. The significant seasonal variation in carbohydrate stores of *Z. marina* (Burke et al., 1996; Dawes and Guiry, 1992; Soissons et al., 2016) suggests sampling time could also have effected this result.

Leaf chlorophyll content did not provide a strong predictor of light limitation. LL and ML plants had the lowest chlorophyll levels, although shaded plants were expected to have higher chlorophyll content than control plants. However, if light stress is too high, plants are unable to respond by producing more chlorophyll. Collier *et al.*, (2012b) also found that chlorophyll levels in very low light treated plants did not increase compared to the HL treated plants. This was thought to be due to the higher level of stress counteracting the energetic benefits of producing more chloroplasts.

Findings from this study indicate that MLR thresholds of *Z. marina* are between the levels of HL and ML treatments: between 156 – 20 µmol photons $m^{-2}s^{-1}$ or 5.61 and 0.73 mol photons $m^{-2}day^{-1}$ under experimental conditions. Maximum growth rates of *Z. marina* have previously been found to be at irradiances between 100-150 µmol photons $\mu m^{-2}s^{-1}$ (Dennison and Alberte, 1985; Olesen and Sand-jensen, 1993), or 5 mol photons $m^{-2}day^{-1}$ (Thom et al., 2008) and the photosynthesis saturating irradiance required for plant growth *in situ* found to be to be 30-40 µmol photons $m^{-2}s^{-1}$ (Alcoverro et al., 1999). As such, HL treatment at 155.8 µmol photons $m^{-2}day^{-1}$ could be considered to be the equivalent of a maximal surface irradiance level and it could therefore be suggested that ML treatment is equivalent to approximately 12.9% of SI. This is similar to the minimum light requirements that have been determined for *Z. marina* in other studies (Koch and Beer, 1996; Olesen and Sand-jensen, 1993; Short et al., 1995). Despite being close to the MLR, the ML treatment in this study is still considerably higher than the light compensation point determined for *Z. marina* in other studies at comparable temperatures; 8.3 µmol photons $m^{-2}s^{-1}$ at 15°C (Abe et al., 2003), 10 µmol photons $m^{-2}s^{-1}$ at 20°C (Dennison and Alberte, 1982) and 18.5 µmol photons $m^{-2}s^{-1}$ at 15°C (Olesen and Sand-jensen, 1993). Consequently, one would expect seagrasses under the ML treatment to be still surviving, although maybe not thriving. However, ML plants showed similar responses to LL and EL plants with the bioindicators not changing in a proportional manner relative to light treatment. This suggests that below a certain light threshold, responses will be consistent. The significant impacts to photosynthetic properties and growth rate within the first week, and significant reductions in leaf length, width and surface area after four weeks of shade treatment indicates 12.9% SI or 20 µmol photons $m^{-2}s^{-1}$ for 10 hours per day is insufficient for *Z. marina* survival.

2.5. Conclusions

This study experimentally tests a wide range of bioindicators of light stress on *Z. marina* plants within controlled laboratory conditions (Fig. 5). The minimum light threshold for *Z. marina* was found to be above 20 µmol photons $m^{-2}s^{-1}$ with photophysiological responses and shoot growth being the first bioindicators to be adversely affected by light stress to this level within the first week. Morphological factors took longer to be affected by light stress; this response was observed between 29 and 39 days. EL plants experienced lowest light levels, but responses were not always as quickly exhibited as in LL or ML plants. This lag in response shows that previous condition, such as larger shoot surface area and rhizomal stores, will slow the effect of light limitation on morphological responses. However, shoot growth, alpha, E_k and ETR_{max} are rapidly effected by light limitation. Environmental monitoring of light levels within *Z. marina* meadows could allow managers to foresee potential risks if light is being attenuated to this level for prolonged periods of time. Using the robust bioindicators identified in this study specifically relevant to *Z. marina* can make it possible to assess whether light limitation has or is occurring. This study shows that light reduction to 20 µmol photons $m^{-2}s^{-1}$ or below for 10 hour daily light period causes significant reductions in above ground tissues and photosynthetic performance leading to shoot mortality within 4 to 6 weeks, under experimental conditions. These results show what the impacts of a light stress event to this level over the time-frame of a few weeks can have on *Z. marina* and its implications at a meadow scale.

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Chapter 3: Finding some seagrass optimism in Wales, the case of *Zostera noltii*

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Abstract

There exists limited understanding of the long-term dynamics of the seagrass *Zostera noltii* and how this is influenced by anthropogenic pressures. Milford Haven is a heavily industrialised estuary and also one of the important sites for *Zostera sp*. in the UK. In this study we examine all available long-term spatial variability and abundance data of *Zostera noltii* within Milford Haven using historic datasets. Results show that *Z. noltii* in all sites have shown meadow expansion when compared to the first obtainable records. Little change in abundance over the past 10-15 years for the two sites confirms certain seagrass populations to be robust and thriving. We hypothesise that these populations are showing a level of resilience to the high nutrient levels, disturbance and high turbidity present within the water column of the Haven.

3.1. Introduction

The functional value of seagrasses by way of stabilizing sediments, providing food and shelter, and carbon sequestration, for example, is well understood (Fourqurean et al., 2012; Guidetti and Bussotti, 2000; E Mcleod et al., 2011; van der Heide et al., 2011). The extent of the decline of seagrasses worldwide is also well documented (Short and Wyllie-Echeverria, 1996; Waycott et al., 2009), with poor water quality thought to be one of the biggest risks (Dennison et al., 1993; Hemminga, 1998). As a consequence, there exists a propensity for studies on long-term dynamics of seagrass to be mostly those that present a negative story, showing a declining meadow caused by a major impact. However, far fewer studies have been published which document the recovery of seagrass meadows (Campbell and McKenzie, 2004; Greening et al., 2014; Walker et al., 2006) or showing long-term stability (Lyons et al., 2012; Yakuub et al., 2014). This could possibly be due to the need for researchers to highlight concerns over sites that appear to be more threatened, so that better management practises can then be advocated.

The dwarf eelgrass, *Zostera noltii*[1], is an intertidal species found growing on muddy or sandy substrates (Den Hartog, 1970), providing stabilization of sediments (Costanza et al., 1997) and an important food source for migrating waterfowl, especially brent geese (*Branta bernicla*) and wigeon (*Anas Penelope*) (Fox, 1996; Nacken and Reise, 2000; Widdows et al., 2008). *Z. noltii* is commonly found in estuaries and sheltered bays, often at risk of conflict with coastal development, and anthropogenic impacts from industrial, agricultural and domestic sources (Bernard et al., 2007; Giesen et al., 1990). For example, in the Berre lagoon, and Bassin d' Arcachon, France, huge losses in *Z. noltii* extent have been recorded where areas have been hugely effected by urban and industrial pollution (Bernard et al., 2007; Plus et al., 2010). Management of coastal waters and waterways is necessary to ensure that habitats such as seagrass beds, are maintained in favourable conservation status whilst also trying to accommodate commercial and recreational uses (CCW, 2005). Successful management of water quality has resulted in recovery of *Z. noltii* in some locations. The Wadden Sea has seen areas of the seagrass double between the early 1970s to the end of the 1980s (Philippart, 1995), and steady expansion of meadows has been observed in Bourgneuf Bay France (Barillé et al., 2010).

In the UK, Milford Haven (west Wales) has been identified as one of the important sites for *Zostera* sp. (Brazier et al., 2007) and is home to subtidal *Z. marina* meadows and several well established *Z. noltii* beds. The Haven is also renowned for its shipping and petrochemical industry. Improvements in water quality in UK waters are also assumed to be contravening historical losses of seagrass (Jackson et al., 2013), but long-term data are spatially limited (Jones and Unsworth, 2016) with some sites recording significant reductions in shoot density (Bull and Kenyon, 2015; Burton et al., 2015). In Milford Haven, monitoring of seagrass meadows has been relatively consistent especially with the contamination risk from oil spills and port operations. Unfortunately, Milford Haven has been subjected to a number of oil spills since 1960 (Petpiroon and Dicks, 1982), the biggest being the Sea Empress in 1996 (Carey

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[1] *Zostera noltii* is now regarded as *Z. noltei* (WoRMS., 2017). For purposes of continuity with previous scientific research, it will remain as *Z. noltii* for this study.

et al., 2015; Hodges and Howe, 2007; Moore, 2006). This has resulted in the area being relatively well monitored in comparison to other locations (Hiscock and Kimmance, 2003). In recent years, concern over the potential decline of *Zostera* and changes in the populations of birds that utilize seagrass in the Haven has led to the demand for assessing long-term changes and understanding more about the health and potential resilience of these seagrass meadows. For this study we aim to review all the long-term monitoring data available regarding *Z. noltii* meadows in Milford Haven including additional data collected for a study by Pratt et al. in 2016, and where possible assess any changes over time. Compiling and summarizing all existing data will contribute to the understanding of the current status of *Z. noltii* in Milford Haven.

3.2. Methods

3.2.1 Study site

Milford Haven waterway is found in the county of Pembrokeshire in west Wales, UK. It is Wales' largest estuary and one of the deepest natural harbours in the World making it a historically significant location for maritime commerce, shipping and more recently, the petrochemical industry (Carey et al., 2015). The large tidal range within the Haven, of over 8 m (Nikitik and Robinson, 2003), results in the presence of large tidal flats providing suitable substrate for *Z. noltii* growth. The Haven is also a part of the Pembrokeshire marine Special Area of Conservation (SAC) containing a number of designated conservation features including seagrass beds (Burton, 2008; Langston et al., 2012). Several sites have been identified as having consistently present populations of *Z. noltii* within Milford Haven (fig. 3.1), all of which have been monitored to varying degrees since 1996.

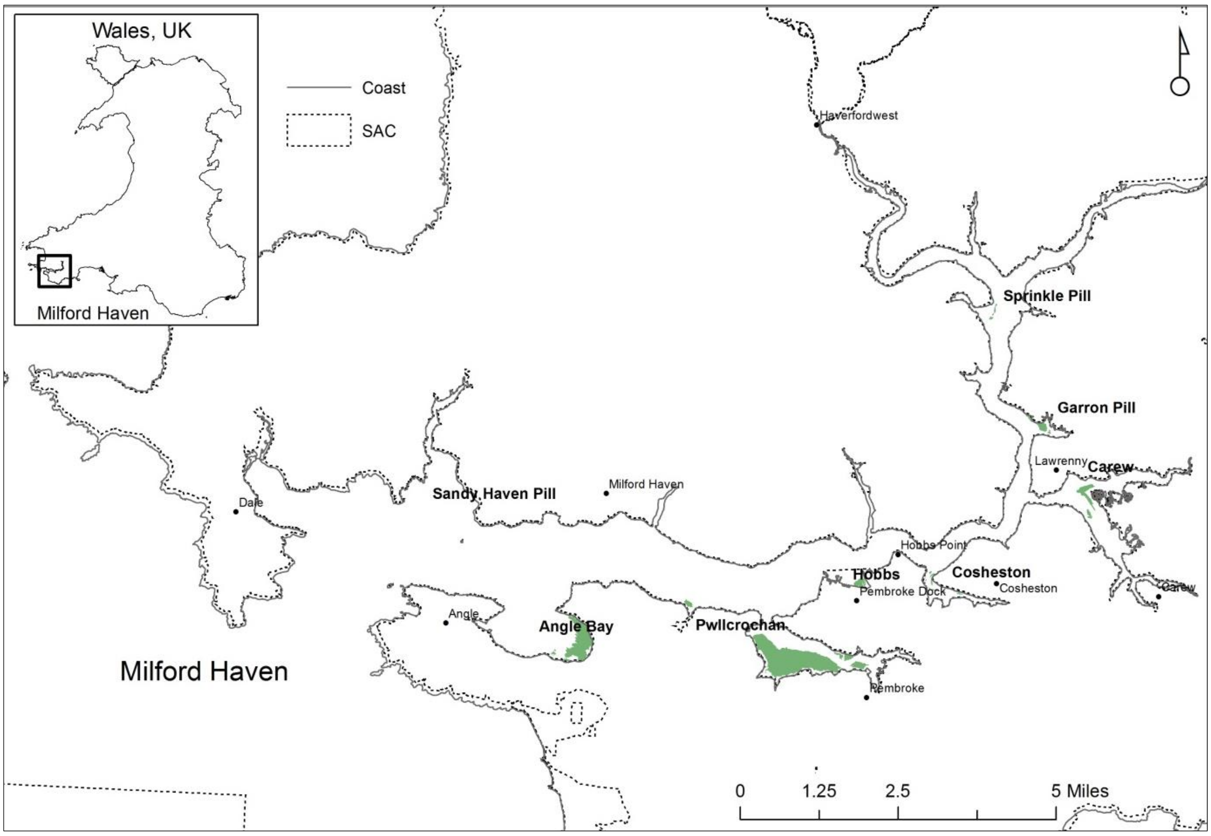

Figure 3.1. *Zostera noltii* mapped locations within Milford Haven, UK, taken from GIS layers provided by NRW.

3.2.2. Monitoring data

A comprehensive review of available data regarding *Z. noltii* in the Milford haven area was conducted in 2016 using sources from monitoring reports undertaken by CCW (Countryside Council for Wales), NRW (Natural Resources Wales, formed in April 2013, largely taking over the functions of the Countryside Council for Wales, Forestry Commission Wales and the Environment Agency in Wales) and consultancy reports where monitoring of *Z. noltii* had been conducted on behalf of industry as a requisite by CCW/NRW. The sites with continued presence of *Z. noltii* in Milford Haven were found to be Angle Bay, Pembroke river, Carew, Cosheston, Garron Pill, Hobbs Point, Pwllcrochan Flats, Sprinkle Pill and Sandy Haven Pill (fig. 1). The Sea Empress disaster in 1996, lead to the more frequent and detailed monitoring of sites where *Z. noltii* beds had previously been recorded, for the 5 years after the spill. However, surveying was limited to Angle Bay, Pembroke River and Sandy Haven Pill, and only the Angle Bay population was monitored for abundance and associated species. The first record of *Z. noltii* extent in Angle Point was estimated as 5.22 ha in 1996 (Table 1) but was more accurately mapped using field survey and GPS from 2007 onwards along with Pembroke River. Other *Z. noltii* meadows within the Haven have been mapped using field survey and aerial imagery from 2008 onwards, although some sites have been missed out on separate survey years (Table. 1). Sandy Haven Pill was only recorded to have two small patches of 1 x 0.5 m, in the report by Hodges & Howe (2007), with no other data available for this study. Monitoring was intensified again from 2008, with more sites being mapped and *Z. noltii* recorded at Garron Pill and Pwllcrochan Flats, locations which were previously found to be absent of the seagrass (Hodges and Howe, 2007). The extent of the *Z. noltii* bed in Pembroke River has been monitored since 2007, with abundance and infaunal surveys conducted from 2009-2015 (Nikitik, 2015, 2014, 2012). Data also includes more recent field surveys conducted by Pratt *et al.* (2016), which involved a detailed assessment of *Z. noltii* distribution throughout south and west Wales, using GPS field survey and UAV (Unmanned Aerial Vehicle) assessments where sites were inaccessible (e.g. Garron Pill). In 2016, most sites in Milford Haven were mapped with the exception of Pembroke River, Sandy Haven Pill and Cosheston.

In order to determine changes in the extent of *Z. noltii* in Milford Haven, all data containing sites with areas of the seagrass mapped using GPS field survey techniques were compiled for comparison. To assess the changes in abundance of *Z. noltii* in Milford Haven, all abundance data (most commonly recorded as percentage coverage) were collated for comparison. These values were taken from raw quadrat data so that changes in abundance could be statistically analysed. The main sites that had long-term abundance records were Angle Bay and Pembroke River, although there were differences in survey effort. Abundance data for Angle Bay was based on a systematic grid system using 0.25 m^2 quadrats throughout the meadow. Therefore, at Angle Bay the sample size varied from *n*=38-72 depending on the extent of the meadow in the year it was sampled. Pembroke River abundance data was collected using *n*=18-20, 1 m^2 quadrats at randomly selected. However, in 2014, a 0.25 m^2 quadrat was used instead. In 2016, abundance data was collected using a 0.25 m^2 quadrat at randomly generated waypoints from within the meadow boundaries (Pratt et al., 2016). All sites were surveyed for percentage cover except Pembroke River and Cosheston.

3.2.3. Statistical Analysis

A simple linear regression was performed on the sum of *Z. noltii* extent in Milford Haven and year. Only data for years from 2008 to 2014, when the majority of sites including the main large meadows had been mapped, were used for the regression, giving the best estimates for total meadow area for the model and standardising effort per year.

For *Z. noltii* abundance at Angle Bay and Pembroke River, data was collected as percentage coverage. Due to the non-normal distribution, non-homogeneity of variance and large dispersion parameters of the data, a quasi-binomial GLM was used for analysis (Crawley, 2005). The GLM model compared all years with percentage cover data available to the earliest year recorded for each site. ANOVA was performed on the GLM using F-test, to see the effect of year on abundance.

For all of the *Z. noltii* sites that were surveyed for abundance in 2016, the same statistical analysis was applied for the percentage cover data (quasi-binomial GLM followed by ANOVA using F-test), but with location as the independent variable instead of year. All statistical analysis was performed in R version 3.2.5 (Crawley, 2005; RStudio Team, 2015). All mean values are presented ± Standard Deviation.

Table 3.1. Summary of *Z. noltii* meadow extent data (ha) over time at different sites within Milford Haven. Also shown is overall change from earliest to most recent record, the mean percentage change per year and mean change in area per year (ha yr-1), - indicates no data available.

	Year												
Site	**1996**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2016**	**Overall Change (ha)**	**Mean % change per year**	**Mean aerial change per year (ha yr^{-1})**
Angle	5.22	16.22	27.53	26.03	29.67	32.53	22.92	32.77	37.80	40.68	35.46	84.91	3.55
Carew	-	-	3.18	3.68	-	1.04	6.23	7.69	8.07	6.92	3.74	14.69	0.47
Cosheston	-	-	0.24	0.21	-	0.37	1.77	0.86	0.69	-	0.45	23.44	0.08
Garron Pill	-	-	4.52	4.83	3.90	4.30	5.59	5.27	5.37	4.65	0.13	0.36	0.02
Hobbs Point	-	-	2.09	2.05	-	0.79	1.61	3.20	1.99	3.49	1.40	8.36	0.17
Pembroke River	-	54.99	93.74	93.73	95.26	97.86	97.35	99.36	97.41	-	42.42	9.64	5.30
Pwllcrochan Flats	-	-	0.17	-	-	0.46	0.13	1.79	2.15	3.85	3.68	270.71	0.53
Sprinkle Pill	-	-	0.16	0.66	-	0.11		0.87	0.71	0.32	0.16	12.73	0.02
Totals			**131.62**			**137.46**		**151.80**	**154.18**		**87.44**		**10.93**

3.3. Results

3.3.1. *Z. noltii* extent in Milford Haven

Overall, all *Z. noltii* meadows in Milford Haven have shown an increase in area (ha) when comparing most recent records with earliest available data (see Table 3.1). All locations show a positive mean percentage change and change in aerial extent per year. The only years where a complete set of extent data are available for all of the main *Z. noltii* beds were 2008, 2011, 2013 and 2014, all showing successive expansion in area (Table 3.1). Results from a simple linear regression model show a significant increase in *Z. noltii* area over time from 2008 to 2014 ($F=17.26$, $p=0.01$, Adj. $R^2=0.76$) (Fig. 3.2).

Angle Bay and Pembroke River hold the largest populations of *Z. noltii* in Milford Haven. In 2016, the extent of the *Z. noltii* in Angle Bay was found to be 40.68 ha, an increase of 35.46 ha from the estimated value in 1996, and a mean annual increase of 3.55 ha yr^{-1}. Pembroke River was found to have an area of 97.41 ha in 2014 (most recent record available), an increase of 42.42 ha from 54.99 ha in 2007, and a mean annual increase of 5.30 ha yr^{-1} (Table 3.1, Fig. 3.3).

Other smaller *Z. noltii* meadows within Milford Haven also show increases in extent overall from 2008 to 2016, albeit with more variation (Table 3.1, Fig. 3.4). The *Z. noltii* meadow at Garron Pill, for example, shows some interannual variation (range 3.90-5.59 ha), but with a mean annual change of 0.02 ha yr^{-1}. Pwllcrochan Flats shows the biggest percentage increase, from 0.17 ha in 2007 to 3.85 ha in 2016 (Table 3.1).

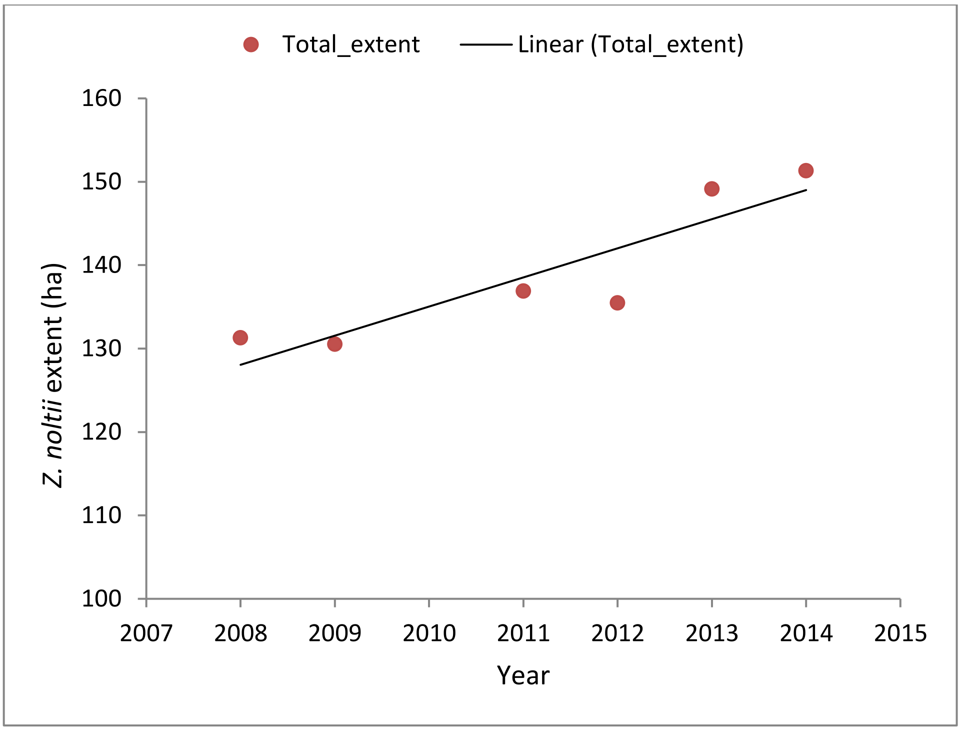

Figure 3.2. Change in extent (ha) for *Z. noltii* in Milford Haven with positive linear regression. Data is total of all available data per year where the majority of meadows (including the two largest sites) had been mapped, excluding Pwllcrochan flats and Sprinkle Pill which were not consistently measured. In 2010 only 3 meadows were mapped and in 2016, Pembroke River was not mapped so these years were omitted from the graph and the regression.

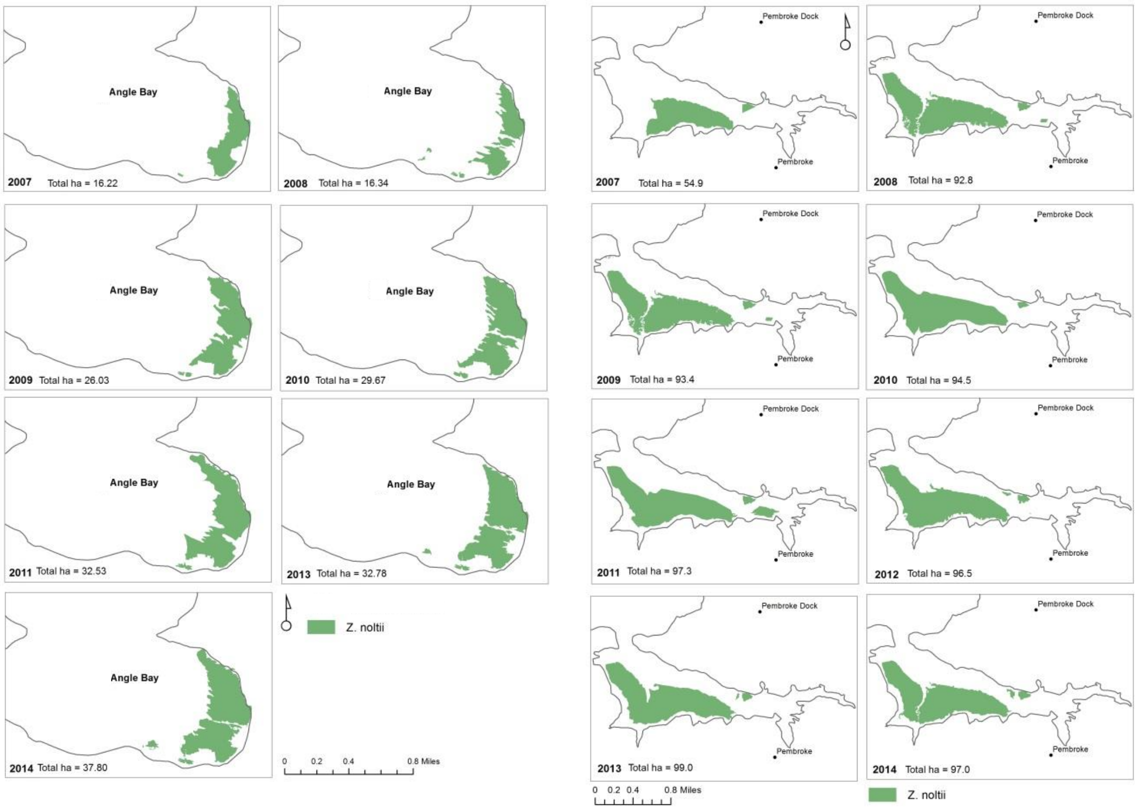

Figure 3.3. Change in *Z. noltii* extent (ha) from 2007 to 2014 in Angle Bay (left) and Pembroke River (right), in Milford Haven. Data for Angle Bay was unavailable for 2012. All data provided by NRW as GIS layers.

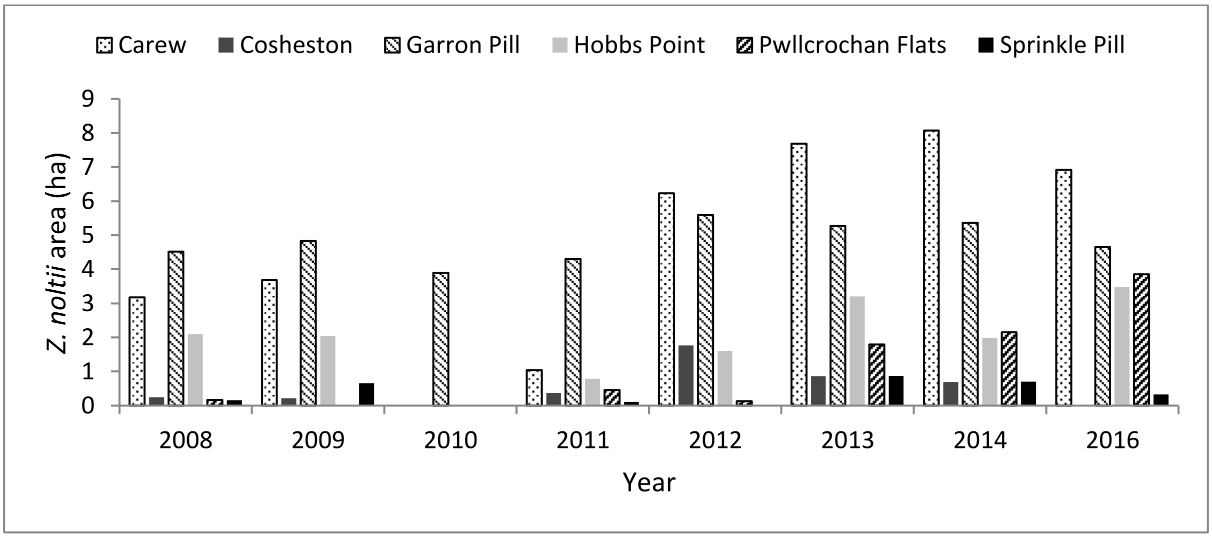

Figure 3.4. Change in meadow extent (ha) from 2008 to 2016, for other major *Z. noltii* sites in Milford Haven. All data provided by NRW as GIS layers except 2016 data from Pratt *et al.* (2016).

3.3.2. *Zostera noltii* abundance

Long-term abundance data, recorded as percentage coverage, was only available for Pembroke River and Angle Bay. Mean percentage coverage ranged from 20.85 ± 31.52 to 48.68 ± 30.37 over time for Angle Bay, and 61.39 ± 12.93 to 82.50 ± 15.80 for Pembroke River. Other meadows in the Haven were measured for abundance in 2016, by way of percentage coverage and shoot density. Percentage coverage ranged from 24.3 ± 18.8 for Sprinkle Pill, to 71.9 ± 23.0 for Carew (Table 3.2).

Table 3.2. Results of abundance data collected at six *Z. noltii* meadows in April and June of 2016, within Milford Haven. Cover was recorded as percentage cover per 0.25 m^2 quadrat, and shoot density taken from 0.2 m^2 cores, all ± S.D., *n*= 30. Data provided by Pratt et al., (2016).

Site	***Z. noltii* cover (% per 0.25 m^2)**	***Z. noltii* Shoot density (per/0.2m^2)**
Angle	30.5±24.5	64.0±41.4
Pwllcrochan	57.815±30.7	116.5±76.6
Hobbs Point	66.1±30.0	116.8±66.6
Carew	71.9±23.0	125.1±61.7
Garron Pill	44.8± 23.5	75.5± 31.0
Sprinkle Pill	24.3±18.8	41.2±43.9
Average	33.3±31.4	62.3±61.2

For Angle Bay, results from the ANOVA of the quasi-binomial GLM show percentage coverage of *Z. noltii* is significantly affected by year (F=5.88, p<0.001 d.f.=402). Pembroke River also shows percentage coverage of *Z. noltii* is significantly affected by year (F=4.42, p<0.001, d.f.=123).

In Angle Bay, the mean percentage cover of *Z. noltii* was significantly higher in years 1998, 1999 and 2000 (p=<0.05) in comparison to the earliest record in 1996. However, in 2008 cover was found to be significantly lower than 1996. This could be due to a change in the method of data collection this year, whereby the area covered in the systematic grid sampling was greatly increased (Fig. 3.5). This led to a higher number of quadrats (n=72 in 2008 c.f. n=43 in 2000) recording 0 % cover than in all other years. In 2016, percentage cover is not significantly different to records from 1996, 30.47 ± 24.49 % c.f. 32.03 ± 28.48 %, although data collection methods were different (a shift from systematic grid sampling to random sampling in 2016 with n=30).

In Pembroke River, the mean percentage cover of *Z. noltii* was significantly lower in 2013 compared to the earliest record in 2009 (*p*=0.04), all other years were not significantly different (Fig. 3.5).

For all the *Z. noltii* meadow sites surveyed for abundance in 2016, results show high variability between meadows with location having a significant effect on percentage cover (F=16.353, *p*<0.001, d.f.=174) (Fig. 3.6). Sprinkle Pill had the lowest abundance followed by Angle Bay with no significant difference from each other. Carew shows significantly higher abundance (*p*=<0.001) than Sprinkle Pill and Angle, followed by Hobbs Point, Pwllcrochan (both *p*=<0.001) and Garron Pill (*p*=0.034) (Table 3.2, Fig. 3.6). All meadows showed consistent patterns for both percentage coverage and shoot density (Table 3.2).

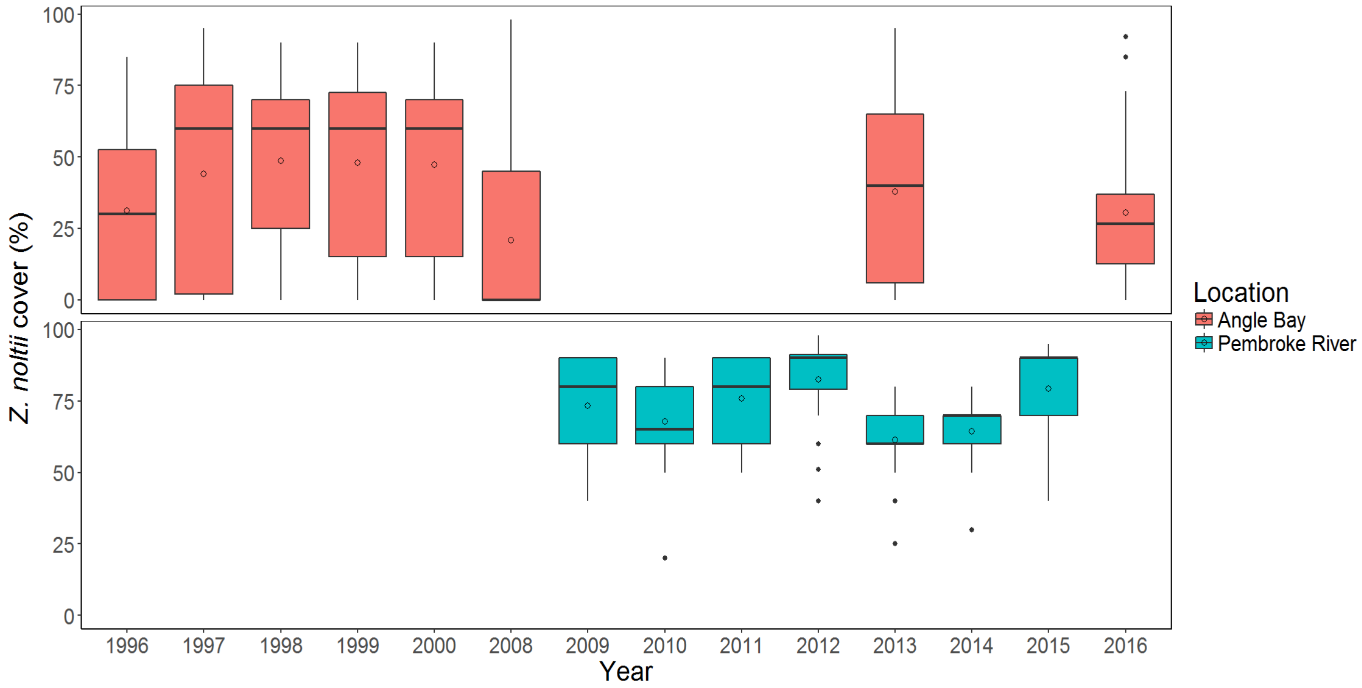

Figure 3.5. Change in *Z. noltii* percentage cover for Angle Bay (top) from 1996 to 2016 (*n*=30-91) and Pembroke River (bottom) from 2009 to 2015 (*n*=18-20). Note that no abundance data was available for either site for 2001-2007. Boxes represent interquartile range and whiskers 1.5 times the interquartile range. Thick black horizontal lines in the box depict the median with mean indicated as open circle within plot. External black points indicate outliers.

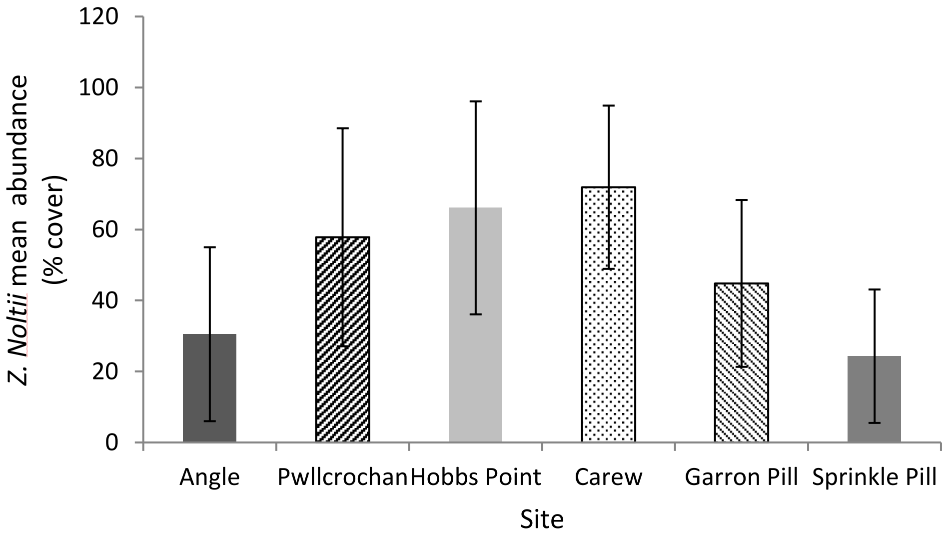

Figure 3.6. Mean *Z. noltii* abundance as percentage coverage (error bars show ± S.D, *n*=30), for the meadows in Milford Haven that were surveyed in April and June 2016, data from Pratt *et al.* (2016).

3.4. Discussion

Seagrass meadows are globally recognised as being at risk with major implications for the ecosystem services they deliver to coastal communities (Orth et al., 2006; Short et al., 2011; Short and Wyllie-Echeverria, 1996; Waycott et al., 2009). Here we present long-term data that provides an example of an extensive intertidal population of seagrass remaining stable and possibly resilient, in a location of intense industrial development and high water pollution concerns (Nikitik and Robinson, 2003; Petpiroon and Dicks, 1982). Despite contamination from a major oil spill in 1996 (Hodges and Howe, 2007), the populations of *Z. noltii* in Pembroke River and Angle Point were not found to be adversely effected in the years that followed the spill (Hodges and Howe, 2007; Moore, 2006), and this study finds that this continues to be the case.

3.4.1. Extent

The present study indicates populations of *Z. noltii* in Milford Haven are expanding in extent, with some small meadows, once thought to be extinct, such as Garron Pill and Pwllcrochan Flats (Foden and Brazier, 2007; Hodges and Howe, 2007), found to be present and thriving over the last decade (Pratt et al., 2016). Prior to 2007, data is lacking for the extent of *Z. noltii* beds in Milford Haven, but data collected after this date strongly indicate an overall trend of meadow expansion. The additional historic data made available for this study supports and reinforces findings made by Pratt *et al.* (2016), whereby *Z. noltii* beds were found to be increasing in extent where historic records of *Z. noltii* were compared with the author's findings. For smaller populations of *Z. noltii* (for example, Sprinkle Pill, fig. 1 & 4), the trend does not appear to be as consistent, with higher variability in area from year to year. This is not unexpected, with small sparser meadows being less stable and thought of as more transient than larger more established populations (Hodges and Howe, 2007). As one of the smaller fast growing and short-lived seagrass species, *Z. noltii* has high production rates in comparison to other seagrasses, enabling it to quickly colonize areas when conditions are favourable and to sustain itself in the presence of considerable disturbance (Borum et al., 2004; Marbà et al., 2013) and remain resilient to changing environmental conditions (Unsworth et al., 2015). This dynamic nature appears more evident for some of the smaller meadows in Milford Haven, such as Cosheston, Hobbs Point and Sprinkle Pill (Fig. 3). For the larger meadows at Angle Bay and Pembroke River, although the extent increased rapidly from

2007, the populations appear to have stabilized in more recent years. Larger meadows are more likely to remain more resilient, as above a minimum size the chances of seagrass patch mortality decreases (Bernard et al., 2007; Duarte and Sand-Jensen, 1990). The expansion of *Z. noltii* meadows is mainly reliant on clonal growth and rhizome extension, as with all seagrass species (C. M. Duarte, 1991). New shoot recruitment is primarily dependent upon propagules from adjacent, well-established meadows (Peralta et al., 2002), with less than 5% of plants thought to originate from seeds (Borum et al., 2004). This is also most likely the case for the *Z. noltii* meadows in Milford haven, with very few seeds found in sediment cores taken from the surveys conducted in 2016 (Pratt et al., 2016).

3.4.2. Abundance

Abundance data was the next most consistent measurement that could be assessed from the historic data to give a better understanding of the status of the *Z. noltii* meadows within Milford Haven. Pembroke River displays a relatively stable pattern of abundance over time. In comparison, Angle Bay shows greater variability in *Z. noltii* coverage between years, which is likely to be explained by the difference in sampling methods (fig. 4) making it difficult for a fair comparison. Percentage cover data for Angle bay was gathered over a longer time period (1996-2016), which will introduce more variability in abundance in comparison to data for Pembroke River. The large increase in extent for Angle Bay in 2008 coincides with a decrease in percentage cover. In this year, what was previously classed as two sub-populations within Angle Bay merged into one bed, resulting in an overall decrease in mean percentage cover caused by the high number of quadrats containing 0% seagrass. The lack of long-term abundance data for other *Z. noltii* sites within Milford Haven means we are limited with our understanding of changes in these meadows in comparison to present status. However, data collected in 2016 shows average percentage cover and shoot density for the majority of sites within the Haven (Table 2). Both parameters exhibit similar patterns across the different meadows showing that both percentage cover and shoot density appear to be effective in determining *Z. noltii* abundance. Although the Angle Bay population is much larger than many of the other sites, it has lower percentage cover and shoot density than many of the other meadows. This is most likely explained by the sampling method, and the amalgamation of sub-populations as previously explained. There is also evidence of bait digging in Angle Bay which was described as localised and fairly minor in impact from 1996-2000 (Hodges and

Howe, 2007), but found to be more extensive in 2008 and 2013 (Duggan-Edwards and Brazier, 2015). This physical impact could be negatively affecting seagrass cover, a concern that is shared in other *Z. noltii* meadows such as in Bourgneuf Bay, France, where increased recreational clam harvesting has been observed (Barillé et al., 2010) and Ria Formosa, Portugal, where clam farming is causing seagrass removal (Guimarães et al., 2012). The high levels of nutrients in Milford Haven Waterway could also be affecting seagrass abundance in certain areas and nutrient loading has been found to negatively effect seagrasses (Burkholder et al., 2007) and cause decreases in *Z. noltii* density elsewhere (Cabaco et al., 2007; Cabaço et al., 2008a). The Waterway is considered to be at a moderate status and hypernutrified compared to Water Framework Directive (WFD) nutrient standards (NRW, 2016) which needs to be taken into consideration.

3.4.3. Status of *Zostera noltii* elsewhere

Although this present study shows that the status of *Z. noltii* in Milford Haven is good, it is difficult to find recent evidence of this trend occurring in other locations. Long-term studies on *Z. noltii* are limited, and show variable trends. In Arcachon Bay, France, long-term spatial studies found severe declines in *Z. noltii* and *Z. marina* between 1988 and 2008, with accelerated declines for *Z. noltii* since 2005 (Plus et al., 2010). In contrast, the spatial distribution of *Z. noltii* meadows in Bourgneuf Bay, France, have been found to have steadily increased within the same time frame, between 1991 and 2005 (Barillé et al., 2010). In the Wadden Sea, similar findings have been observed from aerial surveys which have recorded a three to fourfold increase in *Z. noltii* area from 1994 to 2006 (Reise and Kohlus, 2008). On the contrary, subtidal populations of the common eelgrass *Zostera marina* in Milford Haven are not showing the same pattern as the *Z. noltii*. In Littlewick Bay (near Milford Haven, fig. 1), monitoring surveys have shown small increases in extent in the *Z. marina* meadow, but significant decreases in shoot density and leaf length (Nagle, 2012). The deeper water environments *Z. marina* is subjected to makes it more susceptible to poor water quality, particularly excess nutrients and industrial impacts such as hot water outlets potentially elevating respiratory demands.

3.5. Conclusion

In conclusion we provide evidence of the potential for intertidal seagrass in a well flushed but highly nutrient enriched industrial waterway which has in the past been subjected to a serious oil spill, to resiliently remain in a favourable state over long time periods. We present evidence that *Z. noltii* in Milford Haven is thriving. Extent of the *Z. noltii* in Milford Haven has been expanding over the past decade. Abundance data for the two largest meadows reinforces this although long-term abundance data is unavailable for the other sites within the Haven. Long-term monitoring data provides vital evidence for the status of important habitats like seagrass meadows that exist under the shadow of heavy industry. By including other metrics, such as abundance and possibly leaf length data in monitoring strategies, evidence of the status of the seagrass meadows would be more conclusive. The positive message for *Z. noltii* demonstrates that management of Milford Haven's waterway appears to be proving successful for this dynamic and robust species. The trend is mirrored in other *Z. noltii* meadows in Europe where improvements in water quality were also thought to be responsible for the increase in meadows. However, the moderate status of the water quality Milford Haven Waterway may not be adequate enough to see the same stable trend in its subtidal relative, *Z. marina.*

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Chapter 4 - The response of the seagrass *Halodule wrightii* Ascherson to environmental stressors

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Abstract

Seagrasses are subjected to intense levels of anthropogenic disturbance as a result of the shallow nearshore waters they inhabit. Some seagrasses are known to have dynamic growth patterns, enabling them to colonize unstable shallow environments and adapt to a range of disturbances. This can result in high levels of variability in morphological and physiological attributes. The seagrass *Halodule wrightii* is known to be a fast-growing pioneering species with a large geographic range. The present study examines *Halodule wrightii* in a region under intense anthropogenic stress in order to determine what are the main environmental drivers affecting the morphology, physiology and status of these habitats. Parameters of plant morphology, physiology and status were measured either at the meadow scale (e.g. biochemistry) or at a higher frequency shoot scale (e.g. shoot width). We assigned an impact assessment index to a series of seagrass sites over a gradient of anthropogenic disturbance and found this to be explanatory of a number of the seagrass parameters measured including epiphyte cover, stable isotope $\delta^{15}N$ and ETR_{max} however, it did not clearly explain shoot density, a commonly used bioindicator of environmental stress. At the shoot scale, Principal Component Analysis identified epiphyte and leaf width to have the strongest association. At the meadow scale this was shoot density, dry weight and E_k , albeit with the most impacted sites showing highest shoot density. Stable isotope ($\delta^{15}N$) and leaf length were most significant in explaining the variation between sites and impact category, providing a direct link between anthropogenic sources of nutrients to seagrass meadow density.

4.1. Introduction

The coastal and estuarine waters where seagrasses grow are some of the most heavily impacted regions of the marine environment (Cabaço et al., 2008a; Short and Wyllie-Echeverria, 1996). The anthropogenic pressures from urban and industrial developments occurring in proximity to these areas has resulted in Worldwide declines of seagrass meadows (Short and Wyllie-Echeverria, 1996; Waycott et al., 2009). The biggest threat to seagrasses from coastal development is attributed to poor water quality resulting in the direct or indirect reduction of light availability (Biber et al., 2009; Burkholder et al., 2007; Duarte, 2002; Hemminga, 1998; Jiang et al., 2013). However, in some locations the direct physical damage and removal of seagrass from port/harbour developments and boat anchoring could arguably pose the most critical risk (Grech et al., 2012, 2011).

Seagrasses will exhibit various changes in morphology and physiology in order to adapt to changes in light environment and numerous shading studies have shown decreases in leaf length, leaf width, leaves per shoot and shoot growth (Bertelli and Unsworth, 2018; Biber et al., 2009; Collier et al., 2012b; Olesen and Sand-jensen, 1993; Yaakub et al., 2013). The reduction in above-ground biomass reduces respiratory demand of the plant but decreases its photosynthetic capacity and carbon uptake (Campbell and Miller, 2002; Collier et al., 2012b; Ralph et al., 2007). Light reduction is rapidly reflected in the photosynthetic performance of seagrasses resulting in a decrease in electron transport rates and carbon capture which can be measured *in situ* using chlorophyll fluorescence (Bité et al., 2007; Ralph and Gademann, 2005). Events which frequently reduce light to levels below the minimum light requirement (MLR) can lead to plant mortality and meadow die-off. Responses have been found to be species-specific in some cases (Bité et al., 2007; Collier et al., 2016; Silva et al., 2013) with factors such as seagrass plant size (Roca et al., 2016), life history, habitat and meadow form found to be significant in response time and sensitivity to environmental stressors (Kilminster et al., 2015). The morphological and physiological changes exhibited by seagrasses in response to changes in light availability make seagrasses good ecological indicators of water quality (Grice et al., 1996; McMahon et al., 2013). However, our knowledge of these seagrass responses to water quality is limited to certain species and localities (McMahon et al., 2013). Nutrient availability is exhibited by the long-term uptake within seagrass tissue, and the ratio of leaf nutrients (C:N:P) has been successfully shown to be an indicator of nutrient enrichment in some seagrass species (Burkholder et al., 2007; Carruthers et al., 2005; Fourqurean et al., 1997; Orth et al., 2006). Nutrient inputs can lead to increases in productivity where waters are oligotrophic, but continued nutrient loading has also been found to reduce shoot density (Carruthers et al., 2005; Fourqurean et al., 2003, 1995; Tomasko et al., 1996). The evidence of reduction in shoot density and biomass from nutrient loading highlights the importance of recognising sources of nutrient inputs in seagrass meadows to allow for the management of these habitats for health and resilience. The use of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are being increasingly used as environmental tracers within marine ecosystems (Jennings et al., 1997; Lepoint et al., 2004), and have been used to provide insight into the sources of nutrients in seagrass meadows (Carruthers et al., 2005; Fourqurean et al., 1997; Jones et al., 2018; Lepoint et al., 2004).

Halodule wrightii (Ascherson) is a common shallow and intertidal seagrass in the Tropical Atlantic Bioregion with its southern limit occurring in Brazil. Populations near the southern limit have rarely been recorded reproducing sexually, making them somewhat borderline and spatially discrete (Creed, 1997). *H. wrightii* beds have been found to show large amounts of inter-population variation in morphology as well as shoot density and biomass (Creed, 1997). This suggests environmental factors are affecting the morphology and the development or expansion of these seagrasses. Factors such as high sediment instability, low temperatures, strong wave action at low tides or exposure to air have been described as some of the potential factors that limit the existence of *H. wrightii* (Barros et al., 2013), although such drivers remain poorly explored (Creed, 1997; Creed and Amado Filho, 1999; Pitanga et al., 2012). Direct observations of environmental effects on Brazilian seagrasses are limited (Barros et al., 2013), with data such as leaf nutrient content and photophysiological attributes lacking in the literature. Decline and die-off of *H. wrightii* meadows have also been attributed to anchor damage and overgrowth of epiphytic algae (Creed and Amado Filho, 1999; Papini et al., 2011; Sordo et al., 2011) likely caused by excess nutrients (Balata et al., 2010; Burkholder et al., 2007). Although *H. wrightii* has been found to tolerate eutrophic waters more so than other competing species (Fourqurean et al., 1995; Lapointe et al., 1994; Powell et al., 1989), nutrient enrichment will greatly increase epiphytic biomass which effects seagrass productivity (Wear et al., 1999).

The aims of the present study were to examine the anthropogenic and natural environmental drivers that modify *H. wrightii* at a shoot and a meadow scale in order to describe and define what are the major factors affecting the development of these seagrasses. By better understanding these drivers, it is possible to compare the shoot and meadow scale responses of this tropical species to other seagrasses and ascertain whether these meadows could be under threat from these impacts.

4.2. Method

4.2.1 Study sites

Anecdotal evidence indicates seagrass meadows in Brazil are extensive, yet knowledge of seagrasses in the southwest Atlantic still remains poor (Barros et al., 2013). Recent evidence suggests that although these tropical coastal meadows are of key importance to fisheries (Nordlund et al., 2016) they are under threat, requiring concerted conservation action (Copertino et al., 2016). The coast of Brazil is subjected to intense environmental threats due to the high levels of urbanisation and frequent periods of prolonged intense rainfall (Copertino et al., 2016; Marques et al., 2004). Many areas along the coast are affected by high levels of nutrients from untreated sewage and industrial discharges as well as waters laden with suspended solids diverted from surrounding basins suffering from soil erosion (Marques et al., 2004). This can cause areas of eutrophication where flushing from fresh seawater is reduced and residence time is high such as in coastal lagoons and inevitably have a direct impact any seagrass meadows present. The ecological and environmental characteristics of ten seagrass meadows along the coast of Rio de Janeiro state and São Paulo were quantified within the month of April, in 2017. The study sites were, in Rio de Janeiro: Praia de Manguinhos and Praia dos Ossos (Armação dos Búzios); Saco do Céu and Praia do Abraãozinho (Ilha Grande); Praia Grande and Praia do Catita (Ilha de Itacuruçá); Ilha do Japonês and Praia dos Anjos (Cabo Frio); and in São Paulo: Siriúba and Praia do Sino (Ilhabela) (Fig. 4.1).

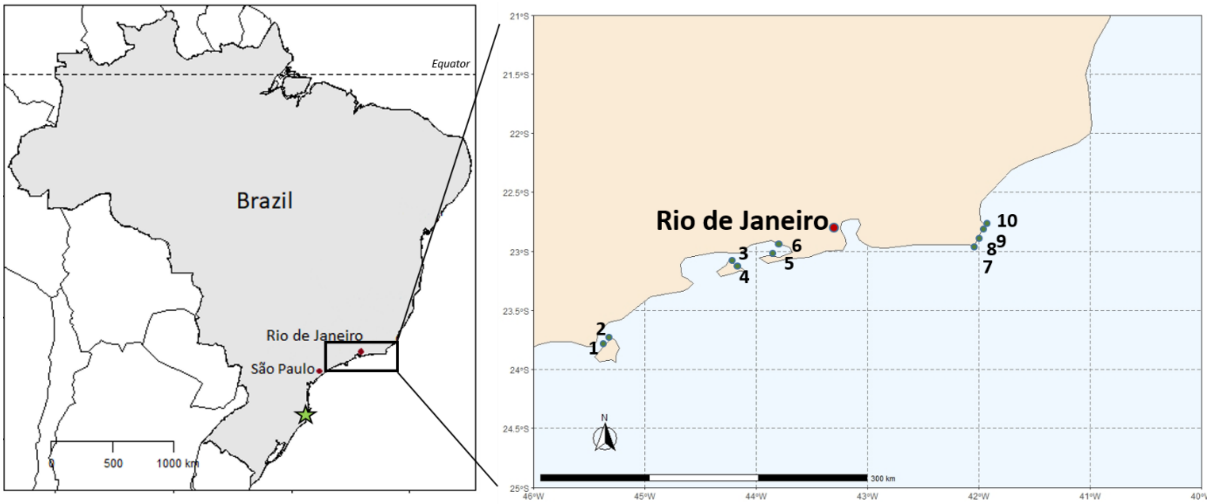

Figure 4.1. Left side shows distribution of seagrass sites along the coast in relation to Rio de Janeiro on the map of Brazil on the right. The sites range from from São Paulo; Siriúba (1) and Praia do Sino (2) to Rio de Janeiro; Saco do Céu (3), Praia do Abraãozinho (4), Praia do Catita (5), Praia Grande (6), Praia dos Anjos (7), Ilha do Japonês (8), Praia de Manguinhos (9) and Praia dos Ossos (10). The green star represents the southernmost extent of *H. wrightii* on the South American coast (Copertino et al., 2016).

4.2.2 Environmental Impacts

Each of the seagrass meadow sites were categorized in terms of environmental impacts. This used a scoring system developed by the authors based on methods and evidence identified from previous studies to provide an index (Creed and Oliveira, 2007; Jones and Unsworth, 2016; Oigman-Pszczol and Creed, 2011; Pitanga et al., 2012) including information collected based on site observations. The anthropogenic impacts that were included in the calculation of this index were as follows; vicinity to highly urbanized area, population, industry, agriculture, boat activity, tourism, freshwater input/sewage outfall, turbidity and enclosed water body/proximity to open sea. Grazing pressure was also included as an environmental stressor. This is because turtle grazing has been found to significantly modify seagrass meadows (Lal et al., 2010), with *H.wrightii* found to be the most important food source for juvenile green turtles found in southern Brazil (Guebert-Bartholo et al., 2011). Each impact was scored from 0 (no impact) to 3 (high impact) and added up to give an overall value. An impact assessment index was calculated by dividing the total score for each site by the maximum possible score giving a value between 0-1 (Table A.1, Fig. 4.2). Impact index scores were then divided up into levels - low-medium (<0.4, SS01-SS03), medium-high (0.4-0.5, SS04-SS07) and high (>0.51, SS08-SS10) categories for statistical analysis.

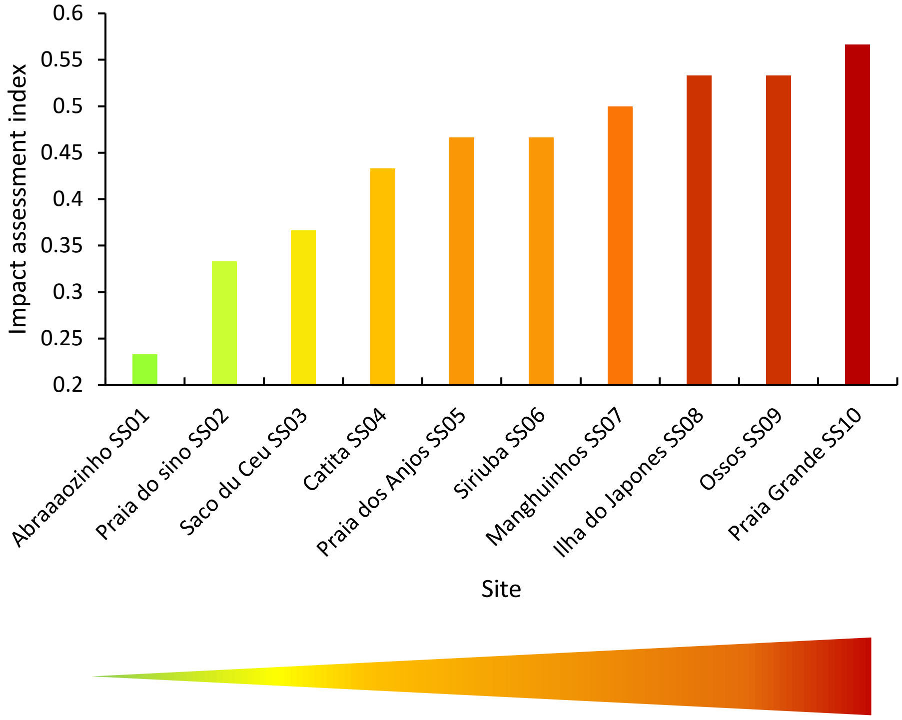

Figure 4.2. Environmental impact index calculated from perceived impacts scored for each seagrass site creating a gradient of increasing impact. Sites are ordered from lowest to highest perceived impact index and labelled SS01-SS10. The perceived impacts included vicinity to industry, tourism, vicinity to highly urbanized area, population, agriculture, boat activity, freshwater input/sewage outfall, turbidity, enclosed water body/proximity to open sea and evidence of grazing.

4.2.3 Environmental data

At each site Hobo light loggers (Onset Hobo UA-002-64) were used to measure light availability and temperature in the middle of the seagrass bed and at the deepest edge to provide light levels at maximum depth threshold. Another light logger was placed in a location on land to record irradiance at or near the sea surface so that the percentage of surface irradiance (%SI) reaching the deep edge could be calculated to represent minimum light requirements. At each site a Secchi disk was used horizontally to estimate turbidity and salinity was recorded using a calibrated refractometer.

4.2.4 Meadow characteristics

The position of mid-meadow and meadow edges were identified by snorkelling along transects throughout each site. Percentage coverage was measured using a 50 cm x 50 cm quadrat. Twelve quadrats were randomly placed along a single transect line (50 -10 m depending on size of meadow) running parallel to the shore through the middle of the seagrass meadow. This was repeated along the outer edge of the meadow. Cores of seagrass were collected using a PVC corer (78.5 cm^2 to a depth of 15 cm) within six of the twelve quadrats (every other quadrat) to obtain other seagrass parameters (Howard et al., 2014; Mills and Berkenbusch, 2009). Within each core sample, the number of shoots were counted, and the length and width of each leaf was measured using a ruler and calipers. An epiphyte cover score was recorded for each leaf based on the index developed for wasting disease (Burdick et al., 1993) and adapted for use for long-term monitoring of epiphyte cover on seagrass (Bull and Kenyon, 2015; Cook, 2011; Cook and Paver, 2007) (Table A.2). Where shoot density was very high, a maximum number of 16 shoots were measured per core and the total number of shoots was recorded. The core samples were washed, dried and then weighed to provide a total dry weight measurement (Table A.2).

4.2.5 Photo-physiological parameters

Pulse Amplitude Modulated (PAM) fluorometry using a Diving-PAM (Waltz), was used to measure chlorophyll fluorescence for assessing photosynthetic activity. Photosynthetic parameters were obtained by performing rapid light curves using the internal stepwise function of the PAM fluorometer once attached to the mid part of *H. wrightii* leaves using a leaf clip. Rapid light curves (RLCs) were started immediately after attachment of the clip to enable the measurement of the proxy for quantum yield ($\phi psII = (Fv)/Fm$), where F is fluorescence yield and Fm is maximal fluorescence yield of the light adapted leaf at each irradiance step (Collier et al., 2009b; Ralph and Gademann, 2005). Steps ranged from 0-2300 µmol photons $m^{-2}s^{-1}$ and were carried out close to midday, between 11am and 1pm. The stepwise RLCs were fitted to the non-linear least-squares regression model by Eilers and Peeters (Eilers & Peeters 1988) using the WinControl software (Waltz) to give photosynthetic parameters for the ETR_{max} (maximum rate of photosynthesis, calculated from ETR at E_k), α (light harvesting efficiency) and E_k (minimum saturating irradiance).

4.2.6 Leaf nutrient analysis

Samples of seagrass were taken randomly from within each of the sites. Leaves were separated, scraped free of epiphytes, and dried. Of the dried plant material collected and transported back to the UK for analysis, unfortunately there was only enough material to provide one sample per meadow once ground up due to the small leaf size of the plants. The dried seagrass was ground up with a pestle and mortar to a fine homogenous powder. Samples were sent to OEA laboratories Limited for analysis of the % composition of Carbon, Nitrogen and Phosphorus by weight using a continuous flow isotope ratio mass spectrometer (Sercon 20-20 IRMS coupled to Thermo EA1110 elemental analyser). The ratios of stable isotope ^{13}C to ^{12}C ($\delta^{13}C$) indicates the deviation of the isotopic composition relative to the Vienna PeeDee Belemnite (VPDB) standard. The ratios of stable isotope ^{15}N to ^{14}N ($\delta^{15}N$) indicates the deviation of the isotopic composition relative to the international standard of air. The elemental ratio of C:N was calculated on a mole:mole basis using atomic weights (C=12.011, N=14.007) (McKenzie et al., 2011). These factors were determined to give values which indicate nutrient availability, anthropogenic sources of nutrients (Jennings et al., 1997; Lepoint et al., 2004) and light availability (Cabaço et al., 2008b; Collier et al., 2009; Grice et al., 1996; McMahon et al., 2013).

4.2.7 Statistical analysis

All the data was divided into measurements collected on a shoot scale and measurements taken at a meadow scale from quadrat data to perceive the environmental effects at these two levels. Parameters measured at shoot scale include average leaf length, leaf width and epiphyte cover. Meadow scale variables included shoot density, dry weight and the photosynthetic parameters Alpha, ETR_{max} and E_k.

All measurement data was analysed using two-way ANOVA tests with impact category and site as a random factor (Brown et al., 1974; McDonald, 2014). In cases where data residuals did not follow a normal distribution, ANOVA was used but with significant p-values set to 0.01 to minimize risk of Type I error (Collier et al., 2012b; McDonald, 2014; Underwood, 1997). Tukey HSD post-hoc multi comparison test was used for comparing ANOVA data.

Principal Component Analysis (PCA) (Clarke and Warwick, 2001) was performed on all quadrat level measurements at both shoot and meadow scale to illustrate the correlations between various shoot response parameters and effects of the perceived impacts upon them. Principal components with eigenvalues greater than 1 and eigenfactors or variable coefficients ≤ -0.3, or ≥ 0.3 were selected.

General Additive Models (GAMs) (Zuur et al., 2009) were carried out in order to identify any non-linear patterns with other possible explanatory factors including abiotic measurements, leaf nutrient content and stable isotopes to determine if these are affecting the abundance of seagrass at sites. Variance inflation factor analysis (VIF) was used to identify collinearity between explanatory variables and those with the highest collinearity were removed from the model. These included %C, %N, E_k, Secchi distance and leaf width. A base GAM model was then created using shoot density as the response variable as it most commonly used factor for monitoring changes in meadows (Burton et al., 2015; Krause-jensen et al., 2005; Mckenzie et al., 2016; Short et al., 2006; Taylor et al., 2003). Alpha, dry weight, leaf length, C:N, δ^{13}C, δ^{15}N, and impact index as explanatory factors and site as a random variable. All covariates were analysed as smooth variables (f) apart from impact index which was treated as a factor variable (F) and nutrient data owing to small sample sizes.

$$(1)\ E[\text{shoot density}] = f(\text{Alpha}) + f(\text{dry weight}) + f(\text{leaf length}) + f(\text{C:N}) + f(\delta 13\text{C}) + f(\delta 15\text{N}) + f(\text{impact index}) + F(\text{site})$$

The best explanatory models were chosen using manual stepwise selection based on the adjusted R^2 and deviance explained values (Table A.7).

All statistics were carried out using RStudio (R version 3.5.1) using R packages *stats*, *devtools*, *gamm4, lme4, mgvc, ggplot2, car* and *carData* (R Core Team, 2018) except for PCA analysis using Primer-e V.6 (Clarke and Gorley, 2006).

4.3. Results

4.3.1 Shoot scale responses

Shoot data showed large variation between sites with average leaf length across sites found to be 25.5 mm (± 14.16), average leaf width 0.43 mm (± 0.22) and average epiphyte score 1.41 (± 0.92).

The results of the two-way ANOVA shows significant interactions between leaf width (F=91.42, *p*=<0.001), leaf length (F=14.74, *p*=<0.001) and epiphytes (F=3.52, *p*=0.03) with impact category and site as a random factor (Table A.4). For leaf width and length, the post-hoc pairwise comparison shows significant variation between plants in med-high vs high, and med-high vs low-medium impact categories (Fig. 4.3, Table A.5), although length was found to increase with increasing impact and widest leaves found in med-high impacted meadows. The mean widths of *H. wrightii* ranged from 0.24mm (±0.07) at SS03, to 0.76mm (±0.13) at SS07. Average leaf length also varied greatly between meadows (Fig. 4.3), from 17.33mm (±9.26) at SS09, to 40.84mm (±24.01) at SS04. Epiphyte cover also varied with the lowest mean score found at SS01 (0.63 ± 0.83) which was significantly lower than all other sites, and the highest score at SS08 (1.86 ± 1.43). Epiphyte cover was found to increase with increasing impact however it was not found to vary significantly between impact categories.

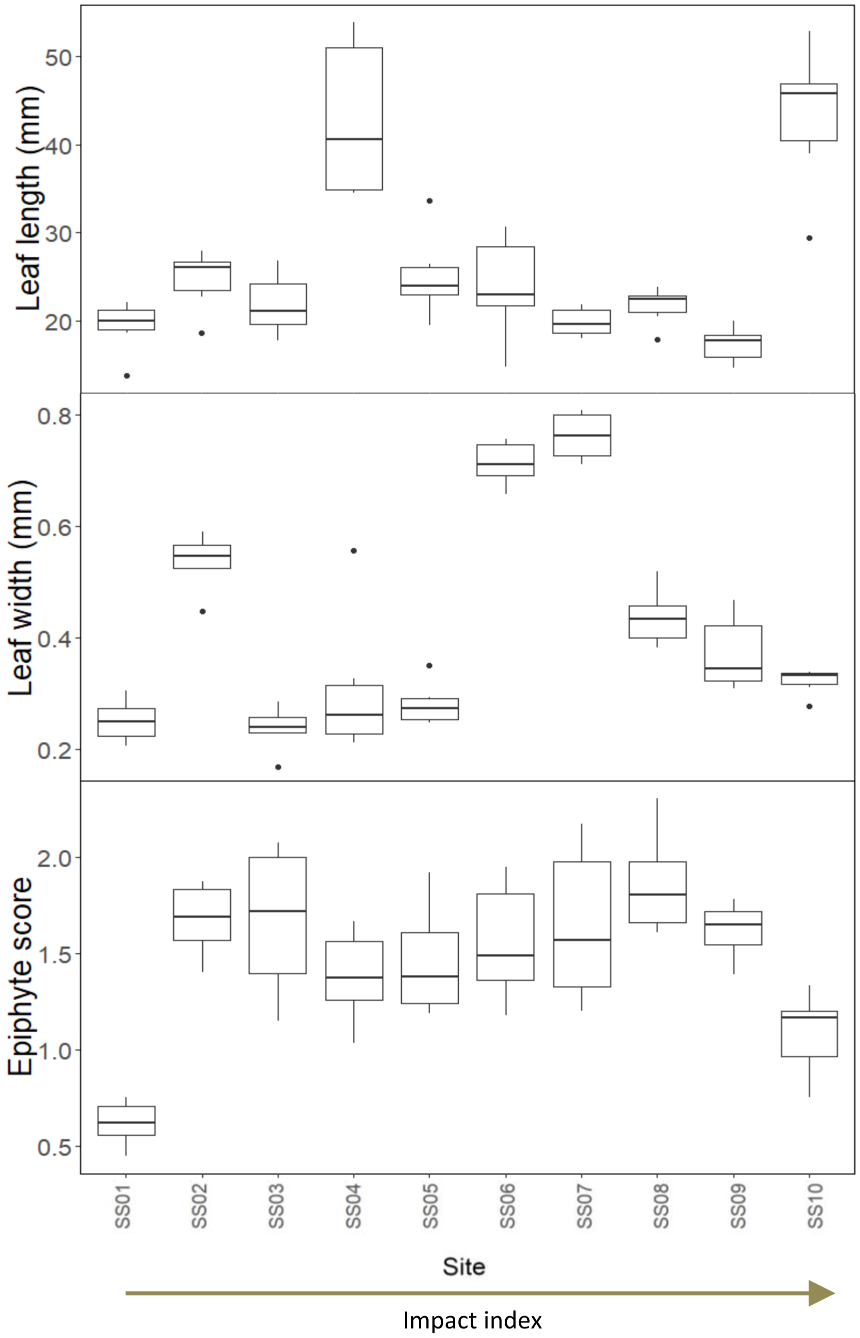

Figure 4.3. Boxplots to show comparison of shoot-scale responses of average leaf length, leaf width and epiphyte cover between seagrass sites (*n*= 55-96 per site). Median is indicated by the solid line, outliers indicated by dots. Site order from lowest impact index score (SS01) on left to highest (SS10) on the right.

4.3.2 Meadow scale responses

Meadow scale variables also showed high variation across sites, with average shoot density ranging from 764.33 (±575.36) to 16645.44.7 (±3668.85) shoots/m^2 and dry weight from 3.74 (±1.27) to 104.46 (±20.4) g/m^2.

The statistical tests showed significant interactions between dry weight (F=50.08, *p*=<0.001) and shoot density (F=66.51, *p*= <0.001) with impact category and site as a random factor (Table A.4). The post-hoc pairwise comparison shows that shoot density varies significantly between each of the impact categories (*p adj*=<0.001) with shoot density actually increasing with impact. For total dry weight, low-medium vs high and low-medium vs med-high showed significant differences (both *p adj*=<0.001, Table A.5), again increasing with impact, not as expected.

Dry weight was highest in SS04 and SS09 with an average of 104.46 (±20.4) and 91.78 (± 19.78) g/m^2 respectively and significantly higher than all other meadows. SS01 had the lowest dry weight at 3.74 (±1.27) g/m^2. SS09 had the highest shoot density, with an average of 16645.44 (± 3668.85) shoots/m^2 (Fig. 3.4). The meadows with the lowest shoot density were SS10 (764.33 ± 575.37 shoots/m^2) and SS03 (1273.89 ± 369.21 shoots/m^2).

4.3.3 Photo-physiological responses

Photosynthetic parameters also showed high levels of variation between sites, with light harvesting efficiency (Alpha) ranging from 0.26 (±0.04) to 0.39 (±0.06), the maximum rate of photosynthesis (ETR_{max}) from 203.01 (±18.65) to 365 (±47.1) and minimum saturating irradiance (E_k) ranging from 588.83 (±91.09) to 1083.62 (±131.42).

Not all photo-physiological parameters showed significant interactions with impact category only ETR_{max} (F=8.62, *p*=<0.001), however they all showed significant variation between sites (Table A.4). The post-hoc pairwise comparison shows only significant variation in ETR_{max} between low-medium vs high (*p adj* = <0.001) and low-medium vs med-high (*p adj*=0.007) impact categories (Table A.5) with ETR_{max} decreasing with increasing impact as would be expected.

The Alpha value recorded for seagrasses at SS04 was significantly higher than several of the other sites (0.39 ± 0.06), followed by SS10 (0.35 ± 0.05) suggesting light limitation at these sites. The sites with the lowest Alpha value were SS08 (0.26 ± 0.04) and SS05 (0.25 ± 0.03) (Fig. 4.4). For ETR_{max}, SS07 and SS03 seagrass showed the highest rates, 365.99 (± 47.43) and 340.3 (± 29.28) respectively. These sites were significantly higher than all other sites. SS10 and SS05 had the lowest values overall (203.01 ± 18.65 and 203.86 ± 33.08 respectively). For the E_k values, the sites that significantly varied from one another included SS08, SS07 and SS03 which had the highest levels (1128.77 ± 122.78, 1183.61 ± 131.42 and 1067.25 ± 131.42 respectively), and SS10 and SS04 which had the lowest levels (588.83 ± 91.09 and 644.71 ± 91.29 respectively, Fig. 4.4).

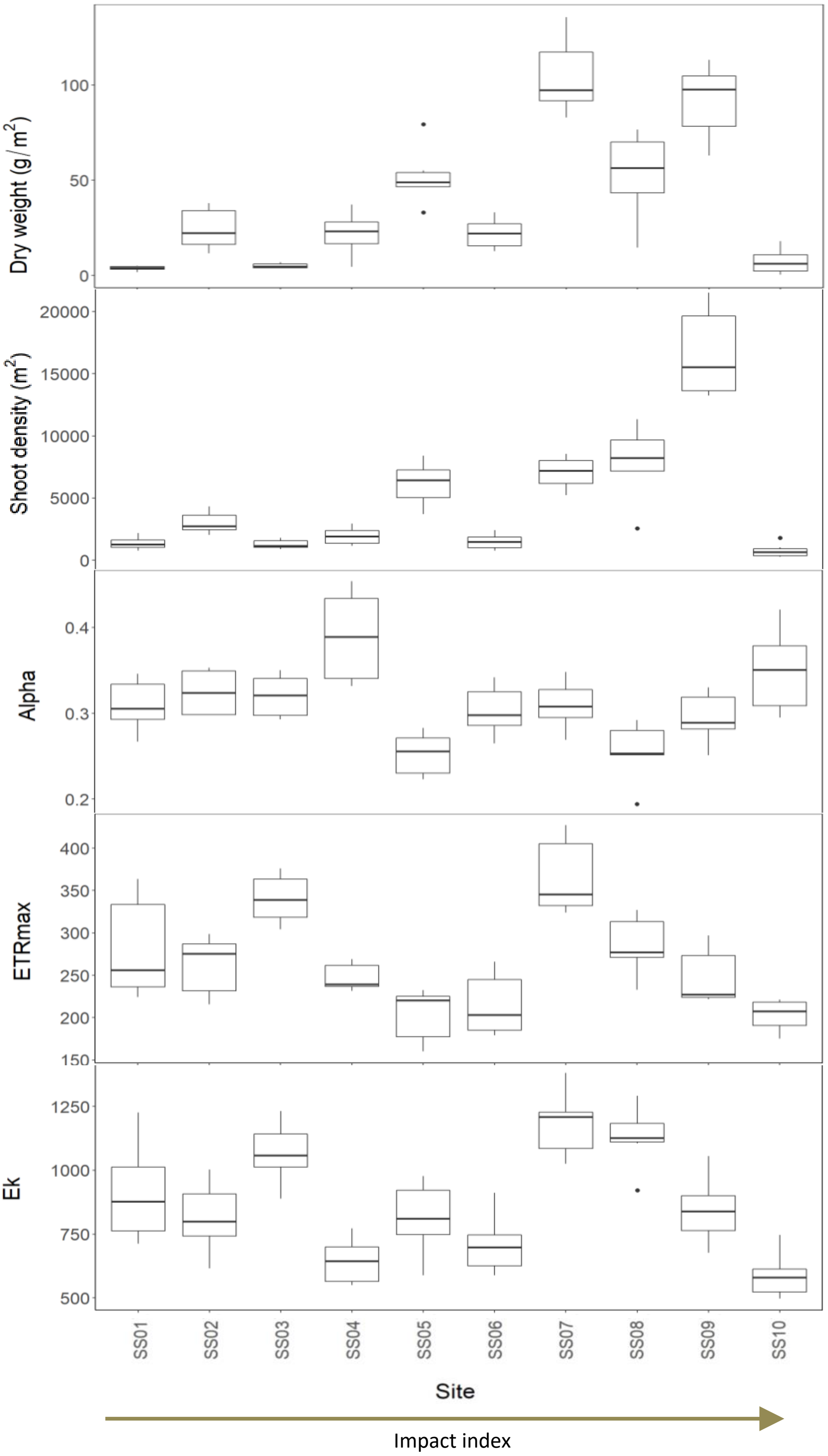

Figure 4.4. Boxplots to show comparison of the meadow and photo-physiological parameters, dry weight, shoot density, Alpha, ETR$_{max}$ and E$_k$ between seagrass sites (n=6). Shoot density and dry weight are shown as per m^2. Median is indicated by the solid line, outliers indicated by dots. Site order from lowest impact index score on left to highest on the right.

4.3.4 Principal Component Analysis

For shoot scale responses (PCA A, Fig. 4.5), PC1 accounted for 48.3% of the variance with an eigenvalue of 1.45. Leaf width and epiphyte cover showed the strongest responses with eigenfactors of -0.668 and -0.613 respectively, showing a negative relationship compared to leaf length (0.422). PC2 accounted for 30.7% of the variance with and eigenvalue of 0.922. Leaf length showed the strongest level of response with an eigenfactor of 0.876, followed by epiphyte cover with 0.464 (Table A.5).

For meadow scale responses (PCA B, Fig. 4.5), PC1 accounted for 48.8% of the variance with an eigenvalue of 2.44. All the seagrass variables included showed strong responses (eigenfactors over 0.3, less than -0.3), although the highest variables were E_k (-0.534), dry weight (-0.504) and shoot density (-0.434) all showing negative association. PC2 explained 28.2% of the variance (eigenvalue 1.41) with all variables showing strong responses except Alpha at 0.246, just below the level of selection (Fig. 4.5, Table A.5).

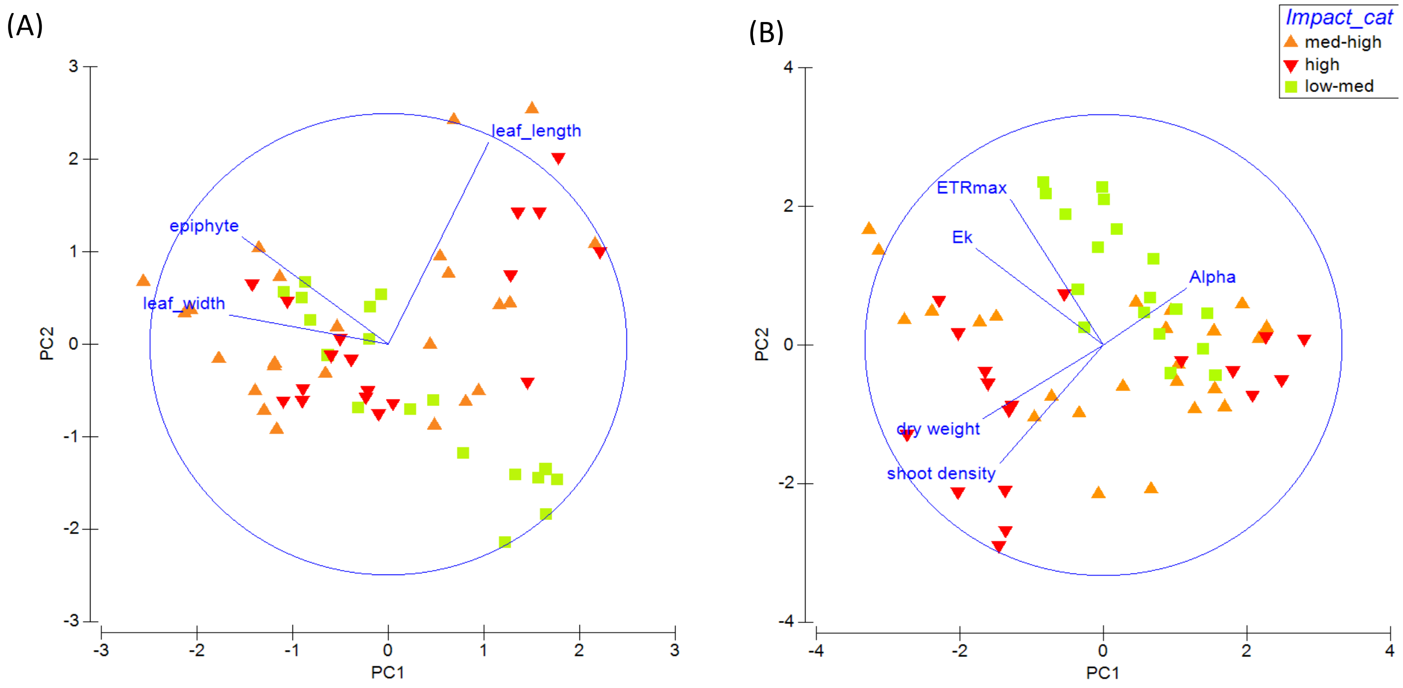

Figure 4.5. Principal Component Analysis of (A) shoot data (leaf width, leaf length and epiphyte cover) variation with impact score as a factor and (B) Principal Component Analysis of meadow-scale data (shoot density, dry weight and photosynthetic characteristics Alpha, ETR_{max} and E_k). Legend separates sites by relative levels of impacts determined by scores and divided into low-medium (<0.4), medium-high (0.4-0.5) and high (>0.5).

4.3.5 Leaf nutrient analysis

Results from the tissue nutrient analysis of *H. wrightii* shoots show that the highest percentage of nitrogen was found in SS10 (3.39%) and SS03 (3.38%), with the average of all sites found to be 3.04 ±0.23 %. The highest percentage of phosphorus was found in the seagrass from SS03 (0.41%) and SS08 (0.40%), with the site average of 0.36 ± 0.04 %. Of the sites, SS03 displayed the lowest C:N ratio (10.44). The highest C:N ratios were found at SS07 and SS05 (12.35 and 12.37 respectively) with the study average found to be 11.63 ± 0.58 (Table 4.2), suggesting these sites are subjected to higher light levels than others.

The values for $\delta^{15}N$ were highest in seagrass tissue from SS10 (7.56‰) and SS04 (7.13‰), followed by SS09 (5.74‰) and SS07 (4.99‰) all relatively higher than the study average (4.15 ± 2.3‰). Sites SS03, SS08 and SS01 had the lowest $\delta^{15}N$ ratios (1.0, 1.68 and 1.72 ‰ respectively) indicating substantially lower anthropogenic nutrient input at these sites than average (Table 4.2).

The ratio of $\delta^{13}C$ in *H. wrightii* shoots averaged -10.11 ± 1.13‰ with little variation between all sites. The lowest $\delta^{13}C$ was found in SS06 (-11.16‰) and SS05 (-11.09) and the highest $\delta^{13}C$ found in the seagrass at SS08 (-7.59) (Table 4.2).

Table 4.2. Results from the elemental analysis of *H. wrightii* leaf tissue taken from the study sites. The stable isotope values for $\delta^{15}N$ (‰), $\delta^{13}C$ (‰).

Site	**$\delta^{15}N$**	**$\delta^{13}C$**	**%N**	**%P**	**C:N**
SS01 (low-med)	1.72	-10.95	3.04	0.38	12.85
SS02 (low-med)	4.14	-10.95	3.07	0.38	13.81
SS03 (low-med)	1.00	-10.22	3.38	0.41	12.17
SS04 (med-high)	7.13	-9.04	2.70	0.27	13.65
SS05 (med-high)	4.70	-11.09	2.84	0.38	14.43
SS06 (med-high)	2.87	-11.16	3.10	0.36	13.48
SS07 (med-high)	4.99	-10.43	2.72	0.34	14.40
SS08 (high)	1.68	-7.59	3.12	0.40	13.20
SS09 (high)	5.74	-9.40	3.07	0.37	13.32
SS10 (high)	7.56	-10.30	3.34	0.31	13.67
Study averages	*4.15*	*-10.11*	*3.04*	*0.36*	*13.50*
S.D.	2.30	1.13	0.23	0.04	0.68
Range	1.0-7.56	-11.16--7.59	2.7-3.38	0.27-0.41	12.17-14.43

4.3.6 Generalized Additive Models to describe shoot density

After removal of collinear variables and those that were non-significant or would not converge within the GAM models owing to unbalanced sample size, the final model consisted of $\delta^{15}N$, leaf length and impact index:

$$(2)\quad E[shoot\ density_i]=f(impact)+f(\delta^{15}N)+f(leaf\ length)+F(site)$$

Basic models were compared for best fit. All variables that were deemed insignificant, having little effect were removed. Site as a random factor and Impact level were also removed to test effect on model fit with results of the final comparisons in Table A.7.

The first GAM model provides evidence that the impact level (low), alpha, leaf length and shoot $\delta^{15}N$ are highly significant in explaining shoot density between impact categories, with highest deviance explained (96.5%, R-sq adj. 0.978) and lowest GVC of all models tested (Table

A.4.7). The tissue $\delta^{15}N$ increases with impact level as expected, however shoot density and leaf length also increase with increasing impact levels. Model 5 which takes out impact level shows leaf length is highly significant as well as site at explaining shoot density, however $\delta^{15}N$ could not be included at this level owing to small sample size.

4.4. Discussion

The plasticity of seagrass meadows to environmental stressors enables them to be bioindicators of ecological change in coastal waters. The present study demonstrates that although such responses occur and do indicate the majority of seagrasses in a region of Brazil to be under anthropogenic stress, their responses are multifaceted and often difficult to interpret with respect to assessing the environmental status of seagrass meadows. One of the major attributes considered for assessing seagrass health and resistance is abundance (Unsworth et al., 2015), with increasing abundance potentially leading to increased resilience (Mckenzie et al., 2016). The present study indicates that this assumption may not always be correct as reduced shoot density and biomass did not always reflect increasing anthropogenic impact. Other indicators were found to be more robust to identifying environmental risks. Epiphyte score, and leaf nutrient content ($\delta^{15}N$, %N, %P and C:N) data supported what were assessed to be the low-medium impacted sites (SS01 and SS03), and leaf nutrients were found to be highest in plants from the site predicted to be of high impact (SS10), indicating nutrient enrichment. Other meadow scale responses also supported the case that SS10 is likely to be the most impacted site displaying the lowest shoot density, ETR_{max} and E_k of all sites, and second highest Alpha. However, other meadow-scale responses specifically the low shoot density and biomass at SS01, do not support the low-medium impact assessment index attributed. The input of naturally occurring nutrients from adjacent mangroves or potential for higher levels of exposure to wave action and recent storm events could have resulted in a decrease in shoot density at this site.

The shoots with the longest leaves were found at SS04 (med-high) and SS10 (high), although leaf widths at these sites were relatively narrow. The seagrass within medium-high impact sites (SS06 and SS07) were found to have the widest leaves, but also amongst the shortest leaves measured. It is expected that nutrient enrichment within the water-column results in increased turbidity and light attenuation, caused by an increase in epiphytic algae growth and eutrophication. Light limitation often results in a reduction of above-ground biomass exhibited by shorter, narrower leaves, with fewer leaves per shoot and becoming less dense at a meadow scale (Bertelli and Unsworth, 2018; Biber et al., 2005; Collier et al., 2012b; Ochieng et al., 2010; Olesen and Sand-jensen, 1993; Yaakub et al., 2013). However, reduction in leaf length and elongation which is exhibited in many seagrass species as a response to light limitation does not always hold true for *H. wrightii*. This study has found the sites with the longest leaves were also found to have highest levels of shoot δ^{15}N, Alpha, turbidity and lowest shoot density and E_k indicating poor water quality and low light conditions. Creed (1999) also found that shoot density to be highest in meadows with shorter leaves. Previous studies have found that *H. wrightii* can show little change in leaf elongation from shading experiments (Czerny and Dunton, 1995), and *in situ, H. wrightii* growing in shade can have longer leaf lengths than neighbouring plants growing under higher light conditions (Shafer, 1999). Leaf length could also have been affected by the presence of turtles observed by the authors, the effect of which has been observed to cause *H. wrightii* in this area to grow wider leaves (Creed, pers. Comm., 2017). There was found to be a relationship between wider leaves of *H. wrightii* and evidence of grazing (turtle presence and cropped leaves, see A.4.1). However this response also appears to be species specific and the converse of findings from other studies that found grazing to have the opposite effect (Fourqurean et al., 2010; Lacey et al., 2014; Lal et al., 2010).

There is a wide body of evidence which shows that light limitation and shading results in a reduction in shoot production and shoot density overall for *H. wrightii* (Biber et al., 2009; Shafer, 1999) and other seagrass species (Lee and Dunton, 1997; Longstaff et al., 1999; Olesen et al., 2002), highlighting this response as a major indication of light stress at a meadow-scale. According to the meadow-scale attributes measured (density, biomass, and photosynthetic stress), SS10 (high) is likely to be the most light-stressed, followed by SS04 (med-high). These sites are located within the semi-enclosed, shallow Sepetiba Bay, with lower flushing, into which flow several rivers draining the catchment of the highly urbanised and industrial city of Rio de Janeiro. This is also supported by the high turbidity and temperatures recorded *in situ*. SS06 also showed similar meadow-scale indicators of stress to SS04 and SS10 which could also be explained by the high level of turbidity found at the site and the low % SI reaching the seagrass (Table A.4.3). This site is within protected and enclosed São Sebastião Channel, also with lower flushing. Shoot density and dry weight show similar patterns for the sites as expected, which are also reflected in the values for alpha which are highest in those meadows which appear to be the most impacted.

The *H. wrightii* at SS10 was found to have the second highest level of nitrogen and the highest $\delta^{15}N$ indicating nutrient enrichment affecting the site is likely to include anthropogenic sources. Seagrass from SS03 was also found to have a high proportion of nitrogen, however its low $\delta^{15}N$ isotopic ratio indicates that there are lower anthropogenic inputs at this site. This site is also within an enclosed embayment, part of the Ilha Grande State Park, where nutrient inputs likely come from leaf litter from the adjacent preserved Atlantic rainforest and mangrove systems rather than from the sparse human habitations. The carbon to nitrogen ratio found within seagrass leaves has been found to be a robust indicator of light stress which responds over a longer time-scale than photosynthetic characteristics (McMahon et al., 2013). The results from this study suggest that SS07 and SS05 (med-high) are subjected to a higher light environment overall, and the site subjected to the lowest light levels over time is likely to be SS03 (low-medium). However, it should be considered that the overall C:N ratios between sites showed little variation suggesting all the sites are subjected to relatively low light conditions and high nutrient status. With global averages for other seagrass species found to be considerably higher at around 20 (Atkinson and Smith, 1983; Duarte, 1990; Grice et al., 1996; B. L. Jones and Unsworth, 2016), the low site averages found in this study support such a premise although more samples should be taken in the future to support this. Increasing irradiance has also been found to lower isotopic discrimination of carbon uptake during photosynthesis in seagrasses and therefore lower $\delta^{13}C$ depletion within the plants. This suggests that the seagrass meadow at SS08 (high) is subjected to the highest light levels of all sites measured in this study which is supported with it being the shallowest meadow and highest % surface irradiance levels recorded (Tables 2, A.3). To the author's knowledge this is the first study that has analysed the C:N and stable isotopes of *H. wrightii* using modern methods of mass spectrophotometry, and despite low sample number, does provide important evidence for this seagrass species. A previous study by Powell *et al*. (1989) quantified the nitrogen and phosphorus content of *H. wrightii* plants before and after nutrient enrichment with results showing concentrations lower than those found at all ten sites measured for this study. Monitoring reports describe seagrasses with tissue nutrient ratios

(C:N) below 15 as very poor (Mckenzie et al., 2016; McKenzie et al., 2011) which suggests that all sites in this study are nutrient enriched. The opportunistic and pioneering nature of *H. wrightii* allows it to grow in relatively unstable conditions compared to other seagrass species (Creed and Amado Filho, 1999; Lapointe et al., 1994; Tomasko and Lapointe, 1991; Wear et al., 1999). These unstable conditions can result in sedimentation and burial, a factor not measured in the present study, but has been found to be one of the most important causes of localised loss (Cabaço et al., 2008b; Ceccherelli et al., 2018). *H. wrightii* has a higher nutrient demand than other seagrasses (Powell et al., 1989; Wear et al., 1999) and so can better tolerate eutrophication (Lapointe et al., 1994) and has been known to displace *Thalassia testudinum* under conditions of prolonged nutrient enrichment (Fourqurean et al., 1995). Yet, enrichment has been found to significantly decrease blade turnover and rhizome growth in other species (Wear et al., 1999), and coupled with sedimentation from anthropogenic caused run-off or natural storm events can cause considerable decline (Ceccherelli et al., 2018). Nevertheless it is expected that the variation in morphology, density and physiology of *H. wrightii* meadows is caused by differences in localised environmental conditions which have been found to be greater at its southern distributional limit (Creed, 1997; Sordo et al., 2011).

4.5. Conclusion

This study assessed seagrass indicators at shoot and meadow-scales across a range of seagrass meadows, and the environmental parameters which are modifying them. The results demonstrate substantial morphological and physiological variation to occur.

The results of this study have shown that although perceived impacts and undesirable environmental conditions are the cause of variation between these relatively isolated populations, it is not straight forward to identify which sites are most impacted in the case of *Halodule wrightii*. The impact assessment index developed in this study identified SS10 as the most impacted site which is reflected in the meadow scale responses; lowest shoot density, ETR_{max} and E_k of all sites, and second highest alpha. The shoot nutrient analysis also confirms that SS10 is probably one of the most eutrophic sites owing to the highest %N content of the leaves. However, the impact index perceives SS01 as the least impacted site but the low shoot density, biomass and leaf widths at this site go some way to contradict this. Other processes that have not been recorded could be responsible for the condition of the seagrass here, its location on the northern side of Ilha Grande is more exposed and potentially subjected to more storm events which could cause burial. It has also previously been rated as the most heavily impacted site within the Baía da Ilha Grande (Creed and Oliveira, 2007). The impact index has been shown to work for assessing the most impacted sites but has not been so useful at determining the least impacted, most likely due to naturally occurring drivers that were not measured.

Of the variables measures, the stable isotope of Nitrogen and leaf length were most significant in explaining the differences in shoot density between sites. Taking more samples for shoot C:N and stable isotope analysis is highly recommended to substantiate this trend. All the seagrass sites used in this study are relatively impacted, with some more than others although *H. wrightii* may also naturally better tolerate more borderline environmental conditions that other seagrasses do not. This highlights the importance of identifying the most species appropriate factors to measure when attempting to assess the health status of seagrass meadows, which could result in the development of monitoring protocols that incorporate species specific responses to site specific threats.

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4.7. Appendix

Table A.4.1. Table of impact assessment scores attributed to each seagrass site for each perceived impact from 0-3. The impact assessment index is calculated by divided the total score per site by the maximum possible score.

Site	Population	Industry	Tourism	Anchoring/ boat activity/	Catchment	Agriculture	Freshwater input/	Turbidity	Open-sea /Lagoon	Evidence of grazing	Total score	Impact assessment index
Abraaaozinho	1	1	2	1	1	0	0	0	1	0	7	0.23333
Praia do sino	1	0	2	1	1	0	2	1	1	1	9	0.33333
Saco du Ceu	1	1	1	2	1	0	1	2	3	0	12	0.4
Catita	1	3	0	1	3	1	0	2	2	0	13	0.43333
Praia dos Anjos	2	1	2	3	2	0	0	1	1	2	14	0.46667
Siriuba	1	0	2	2	1	0	2	3	1	2	14	0.46667
Manghuinhos	2	0	2	2	2	0	1	2	1	3	15	0.5
Ilha do Japones	3	1	2	0	3	0	0	2	3	2	16	0.53333
Ossos	2	0	3	2	2	0	2	1	1	3	16	0.53333
Praia Grande	1	3	1	2	3	1	1	3	2	0	17	0.56667

Table A.4.2. Description of epiphyte cover scores used for assessing coverage based on methods developed by Burdick et al. (1993) and described by Cooke & Paver (2007) for use for determining epiphyte scores in long-term seagrass monitoring programmes.

Score	Description	Percentage cover
0	Uninfected leaf	0
1	Minimal cover apparent	0-2
2	Up to a quarter of leaf covered	3-25
3	Up to half the leaf covered	26-50
4	Over half of all leaf covered	51-75
5	Almost all of leaf covered	76-100

Table A.4.3. Abiotic factors measured at each seagrass site in April 2017. Average light intensity for each site is taken from light recorded within the meadow (middle), % SI is taken as percentage of surface irradiance reaching meadow edge, measured using Hobo light loggers (in lux).

Site	Average light intensity mid-meadow (lux)	SD light intensity	Max light intensity during day (lux)	% SI at deep edge	Max temp (°C)	Min temp (°C)	Secchi distance (m)	Salinity (‰)
Abraãozinho	3835	6129	34445	15.3	27.17	21.09	5.5	38
Praia do sino	3524	5562	35823	13.6	26.98	25.71	3.5	34
Saco du Céu	6692	14608	220446	23.7	29.65	23.87	2.5	36
Catita	5280	13379	198401	11.9	33.43	26.68	2.5	32
Praia dos Anjos	3651	5046	24800	13.2	24.84	22.24	5	37
Siriuba	1409	2231	12400	6.7	26.59	25.61	0.75	35
Manghuinhos	3815	6550	126756	13.8	26.88	23.77	2.5	39
Ilha do Japonês	6555	10472	170846	36.2	32.09	21.19	3	39
Ossos	5522	8202	52356	11.7	26	23.97	3.75	39
Praia Grande	2204	7541	187379	14.0	33.43	26.10	0.5	35

Table A.4.4. Results from Analysis of variance with shoot, meadow and photo-physiological responses to impact index and site included as random factor.

		Df	F-value	Pr(>F)
Width	Impact score	2	91.42	<0.001
	as.factor (Site)	7	209.28	<0.001
Ave.leaf.length	Impact score	2	14.74	<0.001
	as.factor (Site)	7	48.56	<0.001
Epiphyte	Impact score	2	3.52	0.03
	as.factor (Site)	7	13.42	<0.001
Shoot density	Impact score	2	66.51	<0.001
	as.factor (Site)	7	43.62	<0.001
Dry weight	Impact score	2	50.08	<0.001
	as.factor (Site)	7	36.99	<0.001
Alpha	Impact score	2	1.32	0.278
	as.factor (Site)	7	9.83	<0.001

ETRmax	Impact score	2	8.62	<0.001
	as.factor (Site)	7	14.14	<0.001
Ek	Impact score	2	2.77	0.072
	as.factor (Site)	7	17.27	<0.001

Table A.4.5. Results from the post-hoc Tukey HSD pairwise comparison test for each variable measured.

Width	**diff**	**lwr**	**upr**	**p adj**
low-med-high	-0.01120476	-0.03842546	0.016016	0.598286
med-high-high	0.11367441	0.08931648	0.138032	0
med-high-low-med	0.12487917	0.09965718	0.150101	0
Ave leaf length				
low-med-high	-1.347614	-3.918443	1.223215	0.435291
med-high-high	3.770645	1.470187	6.071104	0.000377
med-high-low-med	5.118259	2.736196	7.500322	1.7E-06
Epiphyte				
low-med-high	-0.21339175	-0.40533217	-0.02145	0.024946
med-high-high	-0.13007459	-0.30162185	0.041473	0.176817
med-high-low-med	0.08331716	-0.09456508	0.261199	0.514472
Shoot density				
low-med-high	-51.38889	-62.348165	-40.4296	0
med-high-high	-33.5	-43.751464	-23.2485	0
med-high-low-med	17.88889	7.637425	28.14035	0.000303
Dry weight				
low-med-high	-0.311016667	-0.39909479	-0.22294	0
med-high-high	-0.004573611	-0.08696315	0.077816	0.990139
med-high-low-med	0.306443056	0.22405352	0.388833	0
Alpha				
low-med-high	0.018111111	-0.01028242	0.046505	0.280933
med-high-high	0.013763889	-0.01279583	0.040324	0.428903
med-high-low-med	-0.004347222	-0.03090694	0.022213	0.917598
ETRmax				
low-med-high	49.65061	19.47277	79.82845	0.000655
med-high-high	12.95864	-15.27015	41.18743	0.513189
med-high-low-med	-36.69197	-64.92076	-8.46319	0.007855
Ek				
low-med-high	76.99044	-29.48394	183.4648	0.198266
med-high-high	-16.52768	-116.12534	83.06998	0.915404
med-high-low-med	-93.51813	-193.11579	6.079538	0.069808

Table A.4.6. Results of Principal Component Analysis of shoot scale data (PCA1) and meadow-scale data (PCA2).

PCA1	**PC1**	**PC2**
Summary Values		
Eigenvalues	**1.45**	**0.922**
Percent variation	48.3	30.7
Cumulative percent variation	48.3	79.0
Seagrass variables		
Leaf length	**0.422**	**0.876**
Leaf width	**-0.668**	0.127
Epiphyte	**-0.613**	**0.464**

PCA2		
Summary Values	PC1	PC2
Eigenvalues	**2.44**	**1.41**
Percent variation	48.8	28.2
Cumulative percent variation	48.8	77.0
Seagrass variables		
Shoot density	**-0.434**	**-0.513**
Dry weight	**-0.504**	**-0.319**
Alpha	**0.348**	0.246
ETR_{max}	**-0.390**	**0.632**
E_k	**-0.534**	**0.418**

Table A.4.7. Results from the generalised additive models used to describe shoot density. Models 1-4 showed the best fit by way of deviance explained and R^2 value.

Model 1 = gam(shoot_density ~ as.factor(Impact_cat) + Alpha + leaf length + N15)				
	Estimate	Std. Error	t-value	p-value
(Intercept)	12.065	1.142	10.563	0.0005***
Impact med-high	-0.226	0.366	-0.619	0.570
Impact high	-0.338	0.427	-0.790	0.474
Alpha	-11.925	3.934	-3.031	0.039*
Leaf length	-0.093	0.015	-6.234	0.003**
δN^{15}	0.659	0.114	5.765	0.004**
R-sq. (adj) = 0.978, deviance explained = 96.5%, GVC=**1054.1**				
Model 2 = gam(shoot_density ~ as.factor(Impact_cat) + leaf length + N15)				
(Intercept)	8.914	0.626	14.238	3.08e-05***
Impact med-high	0.166	0.547	0.304	0.773
Impact high	0.285	0.618	0.462	0.664

Leaf length	-0.104	0.022	-4.654	0.006**
δN^{15}	0.445	0.137	3.255	0.023*
R-sq. (adj) = 0.918, deviance explained = 89.1%, GVC=2104.6				
Model 3 = gam(shoot_density ~ as.factor(Impact_cat) + s(Alpha) + s(leaf length)+				
(Intercept)	7.001	0.211	33.169	<2e-16***
Impact med-high	0.243	0.235	5.296	3.02e-06***
Impact high	1.953	0.255	7.660	7.85e-10***
Significance of smooth terms		Ref.df	F-value	p-value
s(Leaf length)	edf	8.957	7.756	1.56e-07***
s(Alpha)	1.00	1.00	0.581	0.450
s(Site)	8.64	1.00	0.000	0.539
R-sq. (adj) = 0.794, deviance explained = 79.5%, GVC=**1410.3**				
Model 4 = gam(shoot_density ~ as.factor(Impact_cat) + s(leaf length)+F(Site)				
(Intercept)	7.056	0.198	35.737	<2e-16***
Impact med-high	1.180	0.219	5.385	2.11e-06***
Impact high	1.857	0.221	8.393	5.33e-11***
Significance of smooth	edf	Ref.df	F-value	p-value
s(Leaf length)	8.64	8.957	7.756	8.01e-08***
s(Site)	6.256e-06	1.00	0.000	0.822
R-sq. (adj) = 0.794, deviance explained = 79.2%, GVC=1374.6				
Model 5 = gam(shoot_density ~ s(Alpha) + s(leaf length)+F(Site)				
(Intercept)	7.046	0.220	31.98	<2e-16***
Significance of smooth	edf	Ref.df	F-value	p-value
S(Alpha)	1.000	1.000	0.327	0.57
s(Leaf length)	1.000	1.000	32.687	3.12e-07***
s(Site)	0.976	1.000	46.213	2.06e-09***
R-sq. (adj) = 0.634, deviance explained = 61.9%, GVC=1874.8				

Chapter 5 – Unravelling the spatial and temporal plasticity of eelgrass meadows

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Abstract

The phenotypic plasticity of seagrasses enables them to adapt to changes in environmental conditions and withstand or recover from levels of disturbance. This plasticity was demonstrated in the large variation found between a range of bioindicators measured within *Zostera marina* meadows around Wales and the Isles of Scilly. Short-term spatial data were analysed alongside long-term monitoring data to determine which bioindicators best described the status of eelgrass meadows subjected to a range of environmental and anthropogenic drivers. Shoot density, leaf length, leaf nutrients (C:N ratio, %N, %P) including stable isotope of $\delta^{13}C$ and $\delta^{15}N$ presented good insight into the longer-term status of the meadows studied and good indication of the causes of long-term decline. The Isles of Scilly had a seagrass meadow with little evidence of impacts when compared to other sites. By contrast, Littlewick had the highest levels of impacts of all sites, with bioindicators showing clear warning signs of nutrient loading reflected in the long-term decline in shoot density, and prevalence of wasting disease. This study highlights the need for continuous consistent monitoring and the benefits of using extra tools in the form of shoot nutrient analysis to determine causes of decline.

5.1. Introduction

Seagrass is protected under International, European and UK legislation and monitoring of meadows has been integrated into management and Water Framework Directives (WFD) as an indicator of good ecological status of coastal waters (de los Santos et al., 2019; Foden and Brazier, 2007; Krause-jensen et al., 2005; Marbà et al., 2013). This has led to an increase in monitoring of seagrass meadows around Europe in recent decades (de los Santos et al., 2019). However, the diverse range of seagrass indicators used (Marbà et al., 2013) and the difference in frequency of monitoring surveys make it difficult to make assumptions on the true status of these habitats. Baselines for monitoring have implications for how the interpretation of the status of seagrass meadows is or has altered over time. Monitoring enables the management and protection of seagrass meadows from direct existing or potential impacts, such as reductions in water quality. This ultimately improves the overall health and resilience of the seagrass to increasing threats from climate change. As an important carbon store in the marine environment, it is even more pertinent that seagrass meadows are protected and where viable restored so that they can continue to absorb CO_2 from the atmosphere (Röhr et al., 2018).

Zostera marina meadows around the British Isles are degraded in status, with estimations of 25-49% decline in the last 35 years (Hiscock et al., 2005; Jackson et al., 2013), although recent evidence has this loss at 92% loss over longer time scales (Green et at., 2021). To be able to set criteria for monitoring and mitigation strategies within management plans, it is important to understand environmental drivers of seagrass meadows. Environmental conditions such as light, temperature and depth will affect many physiological, morphological and structural parameters of seagrass meadows (Martínez-Crego et al., 2008). The plasticity of seagrasses enables them to adapt to changes in environmental conditions and in turn to withstand certain levels of disturbances (Short and Wyllie-Echeverria, 1996). These changes can be used as bioindicators of reduced light levels, nutrient input and other impacts that can be attributed to anthropogenic disturbance or other causes for decline in water quality. Detailed studies of seagrass responses to light reduction have revealed a number of consistent and robust bioindicators such as reductions in shoot density, biomass, growth and production, and shorter narrower leaves (McMahon et al., 2013). Above ground biomass is reduced in this way in order to reduce the respiratory and energetic costs that come from the production and maintenance of new leaves (Collier et al., 2012; Fourqurean and Zieman, 1991). Chlorophyll content of leaves can increase under low light, with the chlorophyll a:b ratio lowering to increase photosynthetic efficiency (Silva et al., 2013). However, if light stress is prolonged, the production of more chloroplasts may prove too costly and resulting in the rapid decline in photosynthetic performance within a relatively short time-frame (Bité et al., 2007; Ralph and Gademann, 2005). Based on such evidence it can be assumed that the morphology and physiology of *Z. marina* can provide an insight into the overall light environments *in situ* and hence the status of coastal waters.

Leaf biochemistry of seagrass can also be used to signify changes in ecological health of coastal waters from eutrophication (Fourqurean et al., 1997; Jones and Unsworth, 2016). Such studies in the UK found most seagrass to be in a poor condition, with nutrient values in excess of global averages (Jones and Unsworth, 2016). Additionally, shoot C:N ratio and the stable isotope of carbon, $\delta^{13}C$ have both been identified as a robust and early indicator of light stress (McMahon et al., 2013), with C:N shown to have a positive relationship with seagrass cover (McKenzie et al., 2011). Also, the stable isotope of nitrogen $\delta^{15}N$ in seagrass can be used to identify anthropogenic sources of nutrient inputs from agricultural or urban effluents (Jones et al., 2018; Lepoint et al., 2004), providing indications of the source of eutrophication threat to the ecosystem (Lee et al., 2004; Short et al., 1995).

In order to understand the status of seagrass, monitoring of abiotic factors such as temperature, turbidity and light are also important (Burton et al., 2015; Jackson et al., 2013; McDonald et al., 2016) as natural environmental processes also effect seagrass growth. Temperature affects the morphology of *Z. marina* with wider leaved plants being found in areas where the annual temperature fluctuation is small such as the Isles of Scilly (Den Hartog, 1970). Also, *Z. marina* growing in higher wave exposure will have significant morphological differences to plants growing where relative wave exposure is lower (Krause-Jensen et al., 2003). Changes in depth limits of seagrass growth is one of the bioindicators used to inform the WFD of changes to water quality as deeper maximum depth limits suggest clearer waters (Dennison, 1987; Dennison and Alberte, 1985; Krause-jensen et al., 2005). Density will also be lower at increased depths as a response to lower light in order to reduce self-shading and reduce respiratory demand (Collier et al., 2007). This supports the need for monitoring a number of robust bioindicators alongside abiotic parameters within seagrass meadows when assessing status. When bioindicators at the meadow or plant-scale change, hypothesising the potential drivers is compromised by gaps in explanatory environmental and seagrass data. Specifically, it is important to determine if changes are natural processes such as yearly fluctuations in sunlight hours and sea surface temperature, or are being caused by anthropogenic sources such as light limitation caused by nutrient loading (Rasheed and Unsworth, 2011). The need to measure factors that can evidence environmental conditions alongside seagrass monitoring data are needed for such changes to be properly assessed (Krause-jensen et al., 2005).

The aims of this study are to investigate the plasticity of *Z. marina* by comparing a range of morphological and physiological indicators alongside environmental conditions across a range of seagrass meadows and hypothesise that these responses can be used to explain changes occurring in these meadows over time using available long-term monitoring survey data. A sixth seagrass site in the Isles of Scilly was included where anthropogenic pressures and impacts from degraded water quality are known to be minimal and seagrass condition has been found to be close to pristine.

5.2. Methods

5.2.1 Seagrass condition in Wales

Six *Z. marina* meadows around the coast of Wales and the Isles of Scilly (UK) were assessed for morphological and physiological factors. The sites were as follows: Littlewick bay 51.706°N, -5.067°E (Milford Haven), North Haven 51.738°N, -5.280°E (Skomer), Pen-y-chain 52.899°N, -4.322°E, Criccieth 52.917°N, -4.227°E and Porthdinllaen 52.943°N, -4.565°E (Llyn Peninsula) and Little Arthur 49.948°N, -6.265°E within the Isles of Scilly (Fig. 5.1, locations in decimal degrees). All sites were surveyed in August and September 2016 using snorkellers, apart from data from Skomer collected by Skomer MCZ (Marine Conservation Zone) team (Natural Resources Wales - NRW) and the Isles of Scilly, collected within yearly monitoring programmes by Natural England, both using SCUBA.

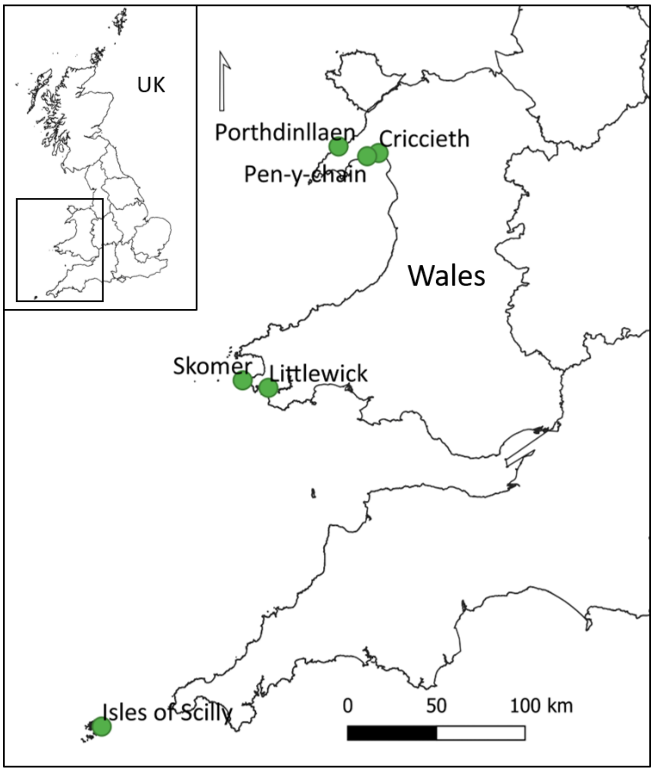

Figure 5.1. Seagrass sites surveyed in August to September 2016 around Wales and on the Isles of Scilly, UK.

At each site a PAR logger (Odyssey, Dataflow systems Ltd) and a temperature logger (Tinytag aquatic 2) were deployed and left *in situ* for a month to record light availability and temperature in the middle of the seagrass meadows. The light logger was placed vertically attached on the mooring block at 50 cm above the seabed so it would be recording at the top of the canopy, and to avoid shading. A Secchi disk was used to measure turbidity, and depth was recorded using a dive computer (Suunto zoop) on the survey days and corrected to Chart Datum using tidal prediction software (POLTIPS v3, Bell, 2016). Wave energy index for each site was calculated using data taken from EMODnet (http://www. Emodnet. eu/en/seabed-habitats). For each site the three grid squares (0.3 km resolution) closest to the survey position that contained wave energy data were averaged to give an overall value.

At each site the mid-meadow and meadow edges were identified from previous site data collection and drop-down camera work (Brown, 2015; Burton et al., 2015; Nagle, 2013). Ten 50 cm x 50 cm quadrat were placed haphazardly through the middle of the meadow, perpendicular to the shore. Within each quadrat, 25 cm x 25cm area of seagrass was removed, with shoots being cut just at the level of the substrate and cut shoots placed in separate zip lock bags. Where visibility was good enough, a Go-Pro®Hero 4 camera attached to the top of the quadrat frame was used to video the quadrats. This allowed extra data to be collected including percentage coverage of seagrass and algae which were analysed from video footage. This was repeated at the edge of the meadow in order to get a good representation overall. At Pen-y-chain and Criccieth, the seagrass was found to be relatively patchy, and a distinct edge was not found owing to poor visibility, so only measurements through the middle of the meadow were possible.

All shoots collected were counted and each leaf measured. Shoot measurements included leaf length (taken from top of sheath to tip of leaf), leaf width, epiphyte and wasting disease cover. Leaf length was measured with a measuring tape to the nearest mm, and leaf width was measured using callipers to the nearest 0.1 mm. Canopy height was interpreted by taking the maximum leaf length of each shoot. Epiphyte and wasting disease cover was scored between 0-5 for each leaf (whereby 0= 0%, 1= >0% - 2%, 2=>2% - 25%, 3=>25% - 50%, 4=>50% to 75% and 5=>75 - 100%) based on the index developed for wasting disease (Burdick et al., 1993).

Shoot data for the Isles of Scilly site, Little Arthur, was obtained from Natural England annual surveys which follow a comparable method outlined in Bull *et al.,* (2016). This allowed for the inclusion of metric data from 2016 survey to be included into this study.

Leaf nutrient analysis

Samples of seagrass were taken from each of the sites and leaves were separated, scraped free of epiphytes, and dried. The dried seagrass was ground up with a pestle and mortar to a fine homogenous powder. Samples were sent to OEA laboratories Limited for analysis of the % composition of Carbon, Nitrogen and Phosphorus by weight using a continuous flow isotope ratio mass spectrometer (Sercon 20-20 IRMS coupled to Thermo EA1110 elemental analyser). The ratios of stable isotopes 13C to 12C ($\delta^{13}C$) and 15N to 14N ($\delta^{15}N$) were also determined to give values which can indicate light availability, nutrient availability, and anthropogenic sources of nutrients (Jennings et al., 1997; Lepoint et al., 2004). Leaf nutrient data for the Isles of Scilly was obtained from a previous study by Jones *et al.,* (2018).

5.2.2 Long-term data analysis

Four long-term monitoring datasets for Skomer (Burton et al., 2019), Littlewick (Hiscock, 1987; Irving and Worley, 2000; Nagle, 2013; Unsworth et al., 2017), Porthdinllaen (Project Seagrass, 2019) and Isles of Scilly (Alotaibi et al., 2019) were collated and standardised. All comparable data were extracted for analysis for temporal changes and trends.

5.2.3 Statistical analysis

All averages are reported ± Standard Deviation. Generalise Linear Modelling (GLM) is a flexible method of analysis that can be used on different types of data including count data (shoot density) and continuous data (leaf lengths) without being limited by the assumptions of normally distributed data (Crawley, 2005). For leaf lengths and widths, GLMs with Gamma errors were used which is most appropriate for continuous data such as measurements (Crawley, 2005; Zuur et al., 2009). For epiphyte, wasting disease, seagrass cover and algae cover, GLM with binomial errors was used for proportion data. All scores and percentages were converted to proportions (0-1). For over or underdispersed data whereby the residual deviance was higher or lower than the degree of freedom, quasi-binomial GLM was used instead to correct for this, making the models more conservative with lower chance of type 1 error (Crawley, 2005). For count data, shoot density and number of leaves, Poisson (or quasi-poisson for overdispersion) GLM with log link was used which ensures all fitted values are positive (Crawley, 2005). All GLM were carried out using R Studio (R version 4.0.2). Model comparisons were made using a likelihood ratios test with and without site as a factor to assess significance of site on the parameter. Where appropriate, Tukey pairwise comparisons between sites were undertaken using the ‘glht’ function in the ‘multcomp’ package in R studio. This analysis was also carried on long-term datasets using year as a factor.

Principal Component Analysis (PCA) was carried out using shoot level data for maximum leaf length, leaf width, epiphytes and wasting disease. All data were scaled before analysis. As not all data were collected at the same resolution separate PCA were conducted including shoot metric data, quadrat level data (to include shoot density), and meadow-scale data (to compare nutrient data). PCA was conducted on quadrat level data to include shoot density and leaves per shoot. Leaf nutrients and stable isotopes (C:N, %N, %P, $\delta^{15}N$, $\delta^{13}C$) were analysed using PCA separately alongside average shoot density to see if they were having an effect on shoot count as has been found in other studies. Owing to cost of nutrient analysis, sample number for nutrients was limited therefore a separate PCA was conducted to visualise similarities between meadows. Principal components with eigenvalues >1.0 were considered, and eigenfactors or variable coefficients ≤−0.3, or ≥ 0.3 were selected. All PCA was carried out using Primer-e (version 6).

5.3. Results

5.3.1 Seagrass condition in Wales

The morphological plasticity of seagrass throughout our six survey sites from 2016 was highly variable and likelihood ratios tests showed that site as a factor had a significant effect on all metrics (Table A.5.1). Leaf length was significantly longer in the Isles of Scilly (630.68 ± 162.71 mm, t=17.74, *p*=<0.001, d.f=677) than any other site (Fig. 5.2). Littlewick had the widest leaves than the remaining sites (450.79 ± 173.93 mm, 3.41 ± 0.78 mm respectively) although width data was not available for Isles of Scilly. Density was highest in Porthdinllaen (189.18 ± 109.43 shoots per m^2) along with Skomer and Isles of Scilly all of which were found to have significantly higher shoot densities than other sites. Criccieth and Pen-y-chain were found to have similar shoot densities to Littlewick albeit with shorter and narrower leaves (Fig. 5.2). Wasting disease was significantly higher in Littlewick than Porthdinllaen, Skomer and Isles of Scilly (1.29 ± 0.51, z=2.68, *p*=0.007, d.f=1741) with the lowest scores in Porthdinllaen (0.47 ±0.47). Pen-y-chain had the highest epiphyte score (2.12 ± 0.59) and the lowest scores were in the Isles of Scilly (0.67 ± 0.39, z=-5.55, *p*=0.001, d.f=1856) although most sites were not different from eachother. Number of leaves per shoot were highest on the Isles of Scilly (4.38 ± 0.86, z=2.54, *p*=0.011, d.f=673) and significantly higher than all sites except for Porthdinllaen.

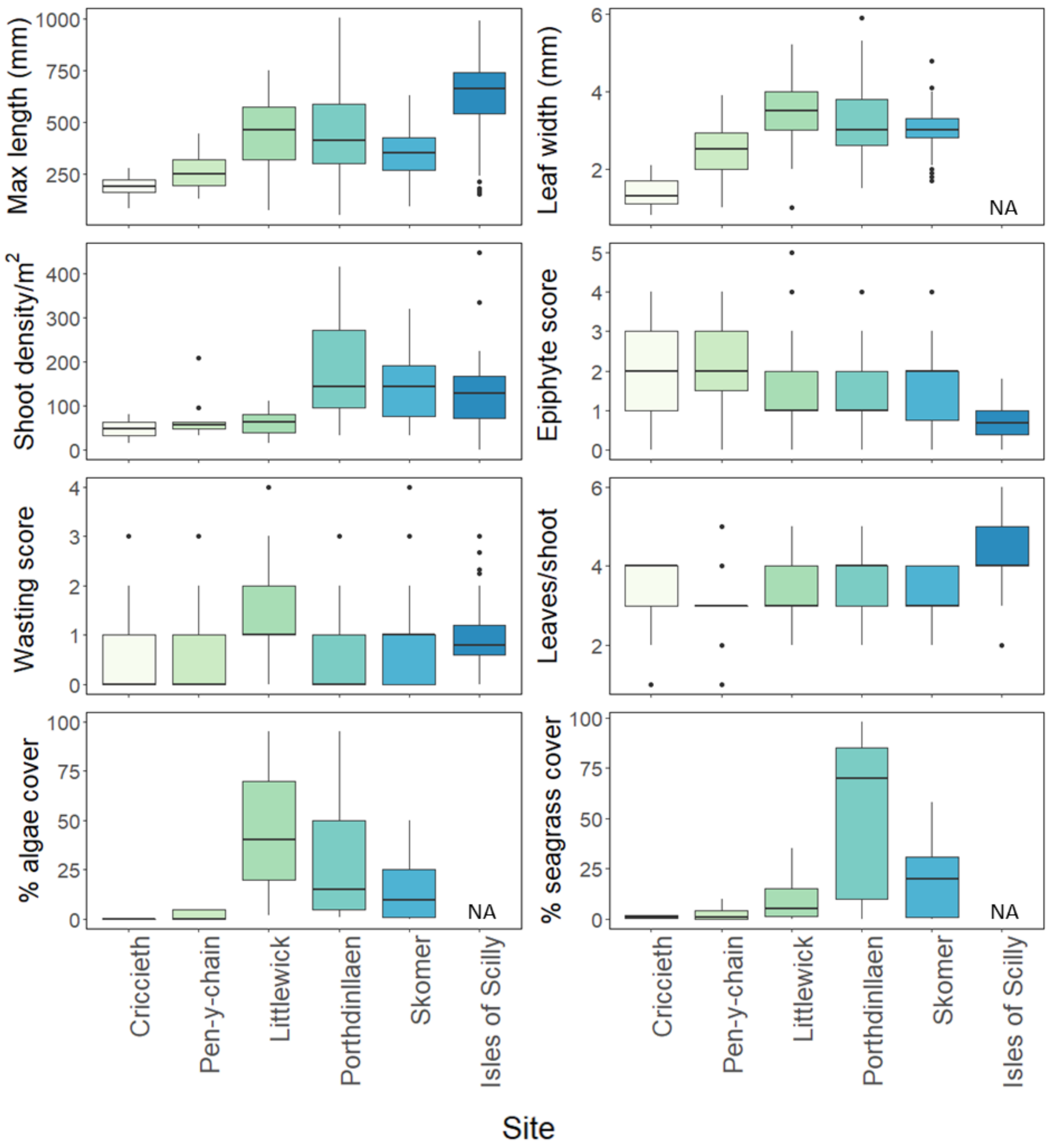

Figure 5.2. Boxplots showing different seagrass shoot and meadow characteristics measures at different seagrass sites. The box-whisker represents the median (line) and interquartile range (box) with additional 1.5 x interquartile range shown as whisker. Outliers are shown as points outside the box-whisker plots. Algae and seagrass cover taken from drop-down camera footage of quadrats taken at each site except Isles of Scilly (*n*=>40 per meadow except Criccieth where *n*=12 due to poor visibility).

Seagrass cover and algae percentage cover from the drop-down camera varied significantly between the sites surveyed (no data for Isles of Scilly). Model comparisons found that site as a factor was found to having a significant effect on seagrass and algae cover. Seagrass cover was significantly higher in Porthdinllaen (54.2 ± 37.69%) than all other sites (t=3.07, *p*=0.002, d.f=231). Algae cover was highest in Littlewick (44.8 ± 28.51%, Fig. 5.2). Littlewick which had significantly higher cover than Skomer and Pen-y-chain. The interaction between seagrass cover and algae cover was also found to be significant (t=-8.9, p=<0.001, d.f=231).

5.3.2. Nutrient analysis

Seagrass nutrient results show high levels of variability between sites (Table 5.1). Isles of Scilly had the lowest %P and $\delta^{15}N$ content showing little if any evidence of nutrient enrichment from anthropogenic sources at this site. These nutrient parameters were found to be highest in seagrass from Littlewick indicating nutrient enrichment. Skomer however had the lowest C:N, $\delta^{13}C$ and the highest %N suggesting light limitation and nutrient enrichment.

Table 5.1. Results from the elemental analysis of *Z. marina* leaf tissue taken from the study sites. The stable isotope values for δ15N indicate the deviation of the isotopic composition relative to air. The isotope values for δ13C indicate the deviation of the isotopic composition relative to the Vienna PeeDee Belemnite (VPDB) standard. All values are unitless.

Site	%N	%P	C:N	$\delta^{15}N$	$\delta^{13}C$
Criccieth	2.23 ± 0.23	0.24 ± 0.03	15.87 ± 0.46	6.37 ± 0.33	-14.71 ± 0.22
Littlewick	2.27 ± 0.24	0.40 ± 0.04	18.98 ± 0.18	10.17 ± 0.1	-14.36 ± 0.31
Pen-y-chain	2.26 ± 0.13	0.29 ± 0.03	19.41 ± 0.82	7.60 ± 0.63	-13.69 ± 0.57
Porthdinllaen	2.22 ± 0.38	0.33 ± 0.04	21.09 ± 0.59	7.72 ± 0.05	-13.65 ± 0.59
Skomer	3.04 ± 0.19	0.33 ± 0.02	14.71 ± 0.18	8.03 ± 0.1	-16.90 ± 0.28
Isles of Scilly	2.76 ± 0.29	0.14 ± 0.01	20.56 ± 2.55	4.47 ± 0.97	n/a
Study average	*2.46 ± 0.36*	*0.29 ± 0.09*	*18.44 ± 2.44*	*6.71 ± 3.06*	*-14.66 ± 1.28*

Principal component analysis (PCA) was carried out to compare shoot density, shoot metrics and shoot nutrient data for each of the sites in Wales and the Isles of Scilly (Fig. 5.3. Table 5.2). Data from a previous study (Jones et al., 2018) was provided for the Isles of Scilly included all parameters except for $\delta^{13}C$. Epiphytes, $\delta^{15}N$ and %P showed significant negative correlation with leaf length, width and leaves per shoot in PC1 (47% variation). Clustering of sites shown in fig. 5.3 shows the Isles of Scilly sharing no overlap with other sites particularly on PC1 axis, whereas Skomer, Pen-y-chain and Criccieth show more similarity.

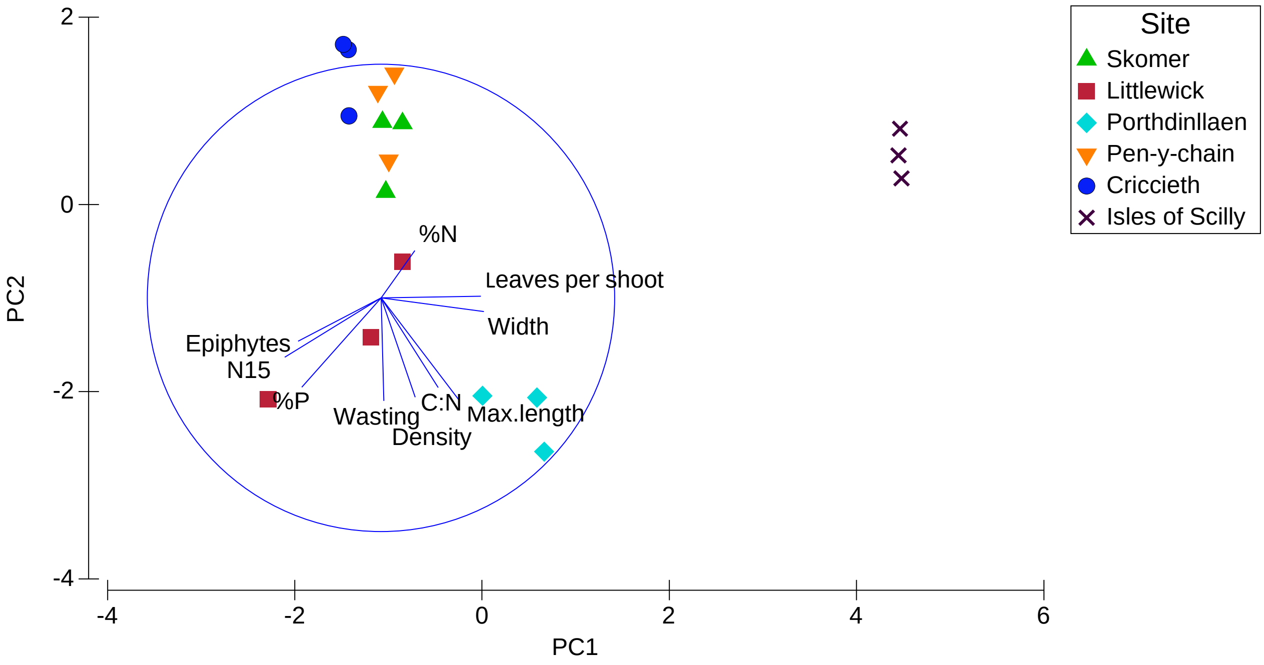

Figure 5.3. Graphs of Principal Component Analysis plots carried out on shoot nutrient and stable isotope data for each site, plotted with shoot density and metrics. Nutrient data for Isles of Scilly provided from Jones *et al.,* (2018).

Table 5.2. Results from the Principal Component Analysis carried out using available data from Welsh sites and Isles of Scilly for nutrient data, shoot metrics and density. Bold values show significant levels of eigenvalues (above 1 for principal component, and eigenfactors or variable coefficients ≤−0.3, or ≥ 0.3).

PCA1 – Shoot data	**PC1**	**PC2**	**PC3**
Summary Values			
Eigenvalues	**4.72**	**2.05**	**1.33**
Percent variation	47.2	20.5	13.3
Cumulative percent variation	47.2	67.7	80.9
Seagrass variables			
Max. leaf length	**0.333**	**-0.437**	0.074
Leaf width	**0.440**	-0.058	0.075
Epiphyte	**-0.355**	-0.186	0.145
Wasting	0.013	**-0.440**	0.163
Leaves per shoot	**0.428**	0.008	0.062
% N	0.145	0.204	**0.750**
% P	**-0.338**	**-0.381**	0.176
C:N	0.245	**-0.383**	**-0.493**
δ^{15}N	**-0.411**	-0.254	0.023
Density	0.147	**-0.424**	**0.317**

5.3.2 Environmental variables

Environmental variables are shown in Table 5.1. No data was available for the Isles of Scilly site. Pen-y-chain and Porthdinllaen were found to have the highest light availability based on PAR logger data, whereas light Criccieth had the lowest (Table 5.3). Temperature results showed little difference between sites so is likely having limited effect on the meadows that can be discerned from this short-term data (Table 5.3). Wave energy data shows the higher wave exposure effecting the seagrass at Criccieth and Pen-y-chain when compared to average results for Skomer, Porthdinllaen and Littlewick. Criccieth and Pen-y-chain were also found to be considerable shallower than other sites with higher turbidity.

Table 5.3. Abiotic and environmental data collected for each site collected in August-September, averages ± standard deviation. Light data for each site is a daily average of PAR logged every 10 mins. Temperature was also logged every 10 minutes. Depths were adjusted to Chart Datum. Wave energy was averaged from data taken from EMODnet https://www.emodnet-seabedhabitats.eu/access-data/launch-map-viewer/

Site	Light (PAR)	Temp (C°)	Wave energy ($N.m^2.s^{-1}$)	Turbidity - Secchi (m)	Max. depth (m)
Criccieth	391.42 ± 506.28	17.64 ± 0.31	160.45 ± 28.15	0.5 ± 0.01	2.5 ± 0.25
Littlewick	n/a	n/a	83.54 ± 46.49	1.65 ± 0.01	4 ± 0.45
Pen-y-chain	796.74 ± 875.16	17.76 ± 0.34	165.68 ± 39.1	1 ± 0.02	2.5 ±0.32
Porthdinllaen	779.84 ± 702.83	16.53 ± 0.25	19.18 ± 9.1	5 ± 0.02	5.2 ± 0.39
Skomer	420.49 ± 324.84	16.07 ± 0.34	24.20 ± 3.2	6 ± 0.025	8.2 ± 0.46
Study average	595.89 ± 656.68	16.99 ± 0.78	90.61 ± 70.45	2.83 ± 2.23	4.48 ± 2.12

5.3.3 Long-term changes

Long-term monitoring data was only available for shoot density, leaf length, wasting disease and epiphyte cover, and not at all sites. Analysis shows high variability between survey years with the likelihood ratios tests showing that year as a factor had a significant effect on all factors measured, except for shoot density at Porthdinllaen (Table A.5.3).

Shoot density

Significant changes in shoot density with year were found at all sites except for Porthdinllaen. For Littlewick, shoot density was found to be the highest in 1999 (141.39 ± 61.9, t=0.070, p=0.944, df=906). Shoot density has consistently decreased since surveys began (Fig.5.4) with the lowest density recorded in 2012 (t=-10.93, p<0.001). Pairwise comparisons show that all years measured have significantly lower shoot density than 1986 and 1999. Most recent surveys (2012, 2016 and 2018) are also significantly lower than in 2008 (Fig. 5.4). For Skomer, seagrass densities show a different pattern with densities significantly increasing between 1997 and 2006. The surveys in 2014 show the lowest overall density recorded (36.15 ± 22.04, t=-2.91, p=0.04, df=1986). Density was found to be highest in the 2016 survey (t=12.14, p=<0.001), although overall there appears to be some stability despite differences between years monitored (Fig. 5.4). Seagrass shoot density in Porthdinllaen has shown little variation with year having no effect on density for the years measured (deviance=125.78, p=0.41, df=4). For the annual Isles of Scilly surveys, year was found to be having a significant effect on density (p<0.001, deviance 4099.2, df=21). The highest average shoot counts overall were recorded in 2003 (256.64 ± 199.76 shoots m^2) and the lowest shoot density was in 2015 (106.24 ± 93.17 shoots m^2, t=-5.574, p=<0.001, df=495). The pairwise comparison showed that only the years 2002, 2003, and 2004 (with the highest densities recorded) were significantly higher than other years, with only 14 out of 231 pairwise comparisons showing significance. Most years did not show significant differences, and shoot density appears to be relatively stable over time (Fig.5.4). The lowest shoot densities for Isles of Scilly were found to correlate with historic sunshine hour data taken the closest weather station data (Metoffice.gov.uk)(Fig. A.5.1).

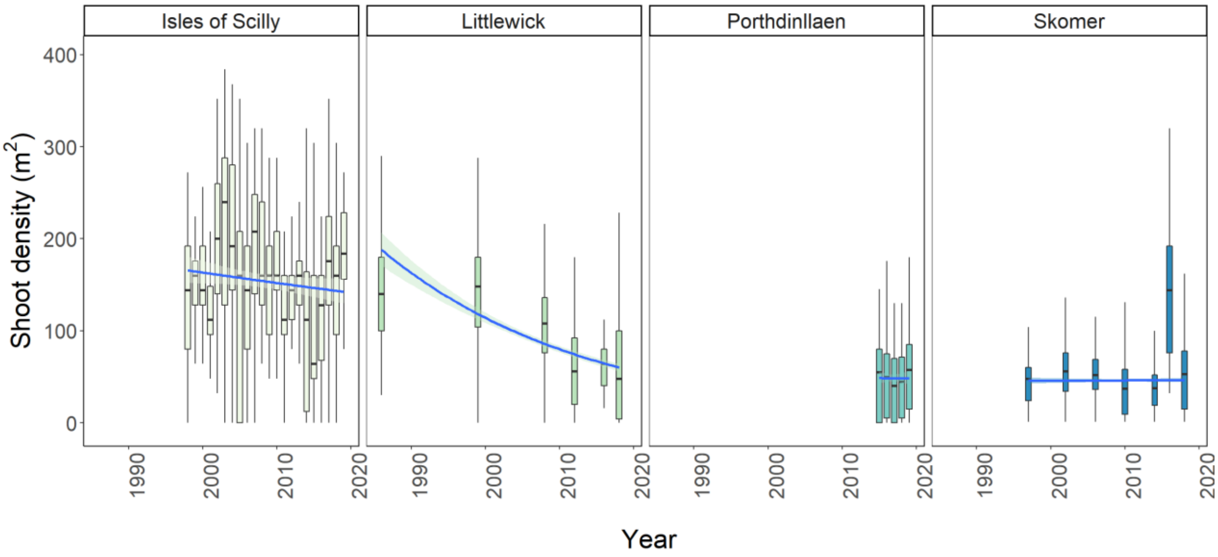

Figure 5.4. Boxplots showing change in average shoot density per m^2 over time for Isles of Scilly, Littlewick, Porthdinllaen and Skomer. The box-whisker represents the median (line) and interquartile range (box) with additional 1.5 x interquartile range shown as whiskers. Outliers not shown for clarity (data provided by NRW, Project Seagrass and Natural England respectively, with data from this study included for Skomer and Littlewick).

Leaf length

Leaf length data was the only other comparable metric monitored long-term, and only available for Littlewick and Porthdinllaen in Wales, and the Isles of Scilly whereby maximum leaf lengths are measured (Fig. 5.5). Model comparison demonstrated that leaf length at all three sites showed significant changes with year. Leaf length in Littlewick has changed significantly over time with the biggest overall increase in lengths recorded in 1999 (t=12.83, p=<0.001, df=7419), followed by the largest decline in 2012 (t=-21.96, p=<0.001). The survey in 2016 did not record a significant change in leaf length, but 2018 data shows a significant increase (372.9 ±192.02 mm, t=7.44, p=<0.001), back to similar lengths recorded in 1999. For Porthdinllaen, since 2015 there is some decline in leaf length, with the biggest decline in 2018 (t=-4.51, p=<0.001 df=1374), but lengths have increased somewhat by 2019 with pairwise comparisons showing a significant increase in length from 2015 to 2018 (z=-4.512, p= < 0.001). The seagrass in the Scilly Isles is showing significant fluctuations in leaf length with the longest records in increase since monitoring started in 2009 (994.16 ± 265.43mm, t=14.22, p=<0.001, df=5154), and the shortest in 2014 (534.63 ± 155.47mm, t=-15.655, p=< 0.001). Over time leaf length appears relatively stable (Fig. 5.5), however the results of the pairwise comparison showed significant differences between most years (156 out of 231 pairwise comparisons).

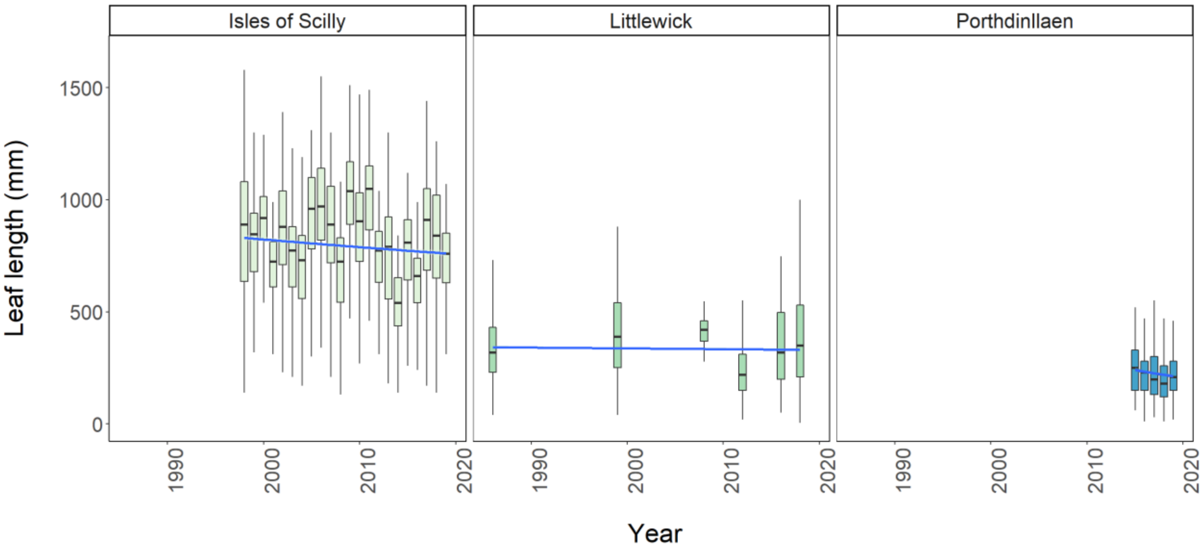

Figure 5.5. Boxplots showing change in average leaf length over time for Littlewick, Porthdinllaen and average maximum leaf length for Scilly Isles. The box-whisker represents the median (line) and interquartile range (box) with additional 1.5 x interquartile range shown as whisker and a temporal trendline in blue (GLM smooth with Gamma family), grey area shows 95% confidence. Outliers not shown for clarity (data provided by NRW, Project Seagrass and Natural England respectively, with data from this study included for Littlewick).

Leaf condition

Long-term shoot condition data was only available for Littlewick and the Isles of Scilly. For Littlewick, both epiphyte and wasting disease showed significant temporal changes, with a decrease in epiphytes and an increase in wasting disease cover over each year (Fig. 5.6). Changes in epiphyte cover between years for the Isles of Scilly site fluctuate but with a slight increase over time. Wasting disease shows little variation with the only significant increases shown in 2001 and 2015 (Fig. 5.6).

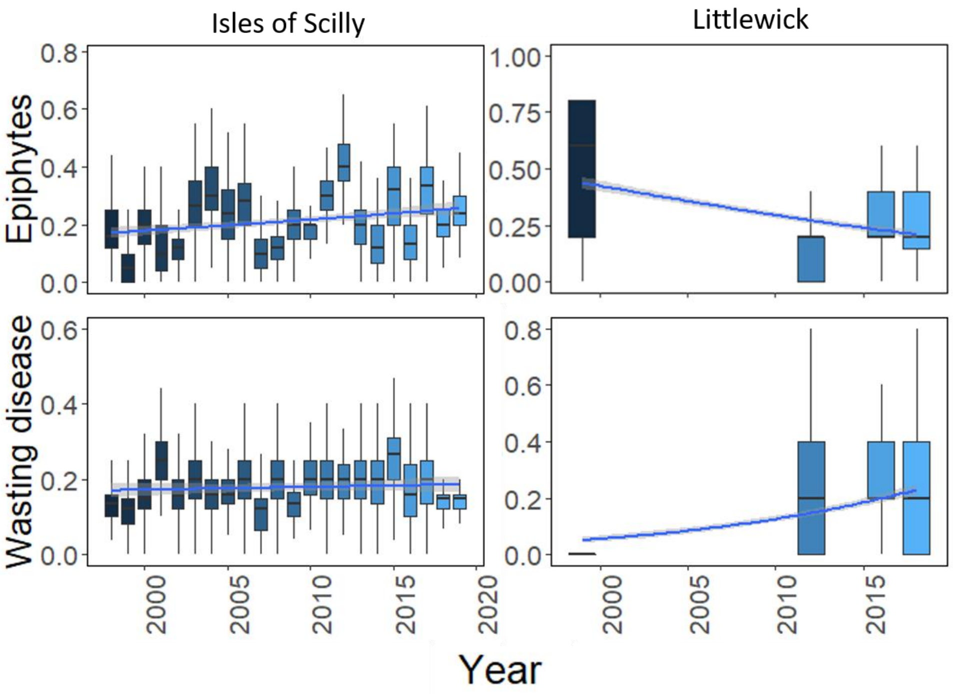

Figure 5.6. Boxplots showing change in leaf condition (epiphyte cover and wasting disease) over time for Littlewick and the Isles of Scilly. The box-whisker represents the median (line) and interquartile range (box) with additional 1.5 x interquartile range shown as whisker. Scale is as a proportion based on the original scores, with temporal trendline in blue (GLM smooth with binomial errors for proportion data) with 95% confidence in grey either side. Outliers have been taken out for clarity (data provided by NRW, and Natural England, with data from this study included for Littlewick for 2016).

5.4. Discussion

Here we provide a unique analysis of bioindicators of seagrass at spatial (short-term) and temporal (long-term) scales. The spatial study allowed for the measurement of a wide range of seagrass characteristics which can provide evidence of environmental drivers affecting the variation in seagrass plasticity and condition between different locations. The long-term study involving the analysis of data from monitored seagrass sites provides insight into the relative stability or instability of the meadows studied.

The plasticity of seagrasses enables them to adapt to changes in environmental conditions and to a degree withstand or recover from some level of anthropogenic disturbance (Maxwell et al., 2014; Short and Wyllie-Echeverria, 1996). At sites in Wales and SW England environmental and anthropogenic factors were found to influence this plasticity as demonstrated in the large variation found across a suite of seagrass of indicators.

All the bioindicators measured were found to describe significant amounts of variation between sites. The morphological and physiological bioindicators enabled differentiation in Wales between sites, with the extensive meadow at Porthdinllaen appearing to be the healthiest reflected by shoot morphology, condition and leaf biochemistry. This meadow was found to have the highest shoot density and cover, with leaf nutrient bioindicators indicating a higher light environment and lower nutrient loading. The long-term data and earlier studies validate this finding with the seagrass community found to be stable between years (Edwards et al., 2003; Morris et al., 2009). Although the temporal range of data for Porthdinllaen is limited, evidence exists that this site remains a stable eelgrass bed showing similar shoot density to the Isles of Scilly site.

Relatively high wave energy and turbidity were recorded as the principle drivers of the two shallowest meadows at Criccieth and Pen-y-chain. These meadows had the shortest and narrowest leaves and lowest shoot densities, a possible response to increased wave motion and risk of uprooting. Average temperatures measured over the survey period were over 1 °C higher in these two shallow meadows than the other sites surveyed which is likely to have an effect on the respiratory demand of the plants. Higher variability in temperature in shallower waters will be contributing to the dynamism of the localised environment. Eelgrass from Criccieth was found to be in the poorest condition due to low shoot C:N, $\delta^{13}C$, shoot density and high epiphyte cover. The PAR levels measured were found to be lowest in Criccieth presumably due to increased turbidity via the resuspension of sediments from high wave energy. However, shoot nutrient analysis indicates low nutrient input at this site suggesting natural processes are having the biggest impact on seagrass condition. Pen-y-chain was found to have the highest PAR levels most likely due to shallow depth and lower turbidity, reflected by high shoot C:N and $\delta^{13}C$. Criccieth has been previously recorded as a sparse meadow (Edwards et al., 2003), suggesting it is somewhat dynamic owing to its physical environment.

Our bioindicator approach found low light differentiated the meadow at Skomer from other localities (low PAR, C:N, $\delta^{13}C$) even though superficially shoot density was similar to Porthdinllaen and the Isles of Scilly. This prognosis is verified by the long-term instability in the system. Low light maybe a natural phenomenon driven by elevated nitrogen due to run-off from the colonies of breeding seabirds that nest on the surrounding cliffs from April to June (Wilkie et al., 2001). This regular seasonal input of nutrients appears to be causing periodic reductions in the local light environment, causing seagrass here to be relatively dense but with shorter and narrower leaves. The long-term data shows this meadow to be fluctuating significantly but there is no steady decrease which suggests these changes could be attributed to natural fluctuations in yearly sunshine hours and short-term, seasonal light limitation from plankton blooms and epiphyte growth caused by nutrient run-off from seabird colonies.

By comparison, the bioindicators measured show the meadow at Littlewick is showing strong signs of anthropogenic impact. The shelter from wave action suggests the area should be conducive to seagrass growth, yet shoot densities are comparable to sites where wave action is much higher. The leaf condition and nutrient biondicators suggest that nutrient loading is impacting this meadow (highest $\delta^{15}N$, %P and wasting score) despite leaf length and width being high. This meadow was also found to have the highest percentage cover of algae. Other studies looking at the effects of eutrophication in eelgrass beds have also found increases in leaf length and a reduction in shoot density as a response to increased shading from opportunistic algae (Moore et al., 1996; Schmidt et al., 2012; Short and Burdick, 1996). High inorganic nitrogen (N_i) in the water column can cause seagrasses to be more susceptible to infections from wasting disease as anti-microbial compounds are produced less to compensate for the synthesis of excess nitrogen in plant tissues (Burkholder et al., 2007; Short and Burdick, 1996). These factors combined strongly to imply that the seagrass meadow in Littlewick is under threat from eutrophic conditions and is undergoing a system shift from a seagrass dominated to macroalgae-dominated community. Long-term data for Littlewick supports this assumption, whereby leaf length has shown significant increases in most years, but shoot density is showing a steady significant decline. Wasting disease has also increased significantly since monitoring started.

Seagrass in Wales relative to the Isles of Scilly (IoS) as a reference site seagrass with limited anthropogenic impacts. Shoot densities and leaf widths in IoS are somewhat comparable with Skomer and Porthdinllaen, but the addition of shoot nutrient parameters (in this case C:N, $\delta^{15}N$ and %P) results in huge dissimilarities between meadows. Leaf length is significantly longer in Isles of Scilly which has been previously recognised as the longest eelgrass found in UK waters (Den Hartog, 1970; Jones and Unsworth, 2016). The increased water clarity of this archipelago is caused by the granite substrate and sediments that settle rapidly (Jackson et al., 2011) and the lack of large scale agriculture and urbanisation. This allows *Z. marina* to grow at greater depths with longer leaf lengths than other locations where turbidity reduces the maximum depth limit of seagrass growth (Nielsen et al., 2002). The lower impacts from terrestrial run-off are shown in the high C:N and lower %P and δ^{15}N. The long-term yearly monitoring of the eelgrass meadows in the Isles of Scilly allows for fine-scale temporal changes to be shown. The main threats to seagrass around these remote islands is physical damage caused by boat moorings, anchoring and storms (Bull and Kenyon, 2015; Jackson et al., 2013; Unsworth et al., 2017), not necessarily water quality issues. The data used for this study comes from the site that was found to be the least impacted and provided a good control site for comparison of status. The yearly monitoring of the Isles of Scilly allows for better evidence-based projections of long-term trends and changes, with shoot density showing much more stability than canopy height over time. It is likely that fluctuations are caused by changes in sunshine hours or other natural processes, with sunshine hours showing a positive correlation with shoot density for the Isles of Scilly. The slower response of shoot density to environmental stresses than other metrics raises the alarm for systems that are seeing continuous declines.

Density of the seagrass *Zostera marina* overall is showing some decline over the last two decades, providing evidence that seagrass in the UK is still somewhat degraded in state with no measurable upward trend of recovery as seen in some species such as *Z. noltii* (Bernard et al., 2007; Bertelli et al., 2018). The lowest densities appear to have been recorded between 2012 and 2015 which could be a UK wide response to natural processes such as significant changes in average recorded sunshine hours.

We also present strong evidence of significant and consistent long-term decline of one of Wales' largest seagrass meadows at Littlewick in the Milford Haven Waterway. The increase in leaf length together with the reduction in density strongly indicate that Littlewick Bay is suffering from frequent and/or prolonged nutrient loading, to the point that natural environmental processes, such as fluctuations in sunshine hours, could be hidden. Milford

Haven Waterway, which encompasses Littlewick, has been designated as being of moderate status and hypernutrified in terms of the WFD standards for nutrients (NRW, 2016). This is reflected in the high tissue nutrients found from the spatial study which explains this trend. By contrast, other sites have shown some increase in shoot density in the most recent years and an overall level of stability in density as seen in the Isles of Scilly, Porthdinllaen and Skomer.

Due to complexities of the factors influencing the resilience of seagrass meadows it is difficult to determine how close such a meadow is to a catastrophic tipping point, however considerable long-term seagrass monitoring evidence globally indicates that once such a point is reached complete degradation and loss can be rapid (Waycott et al., 2009).

Shoot density is affected by numerous disturbances, including light limitation, nutrient loading, physical damage, temperature, or natural storm events, and therefore is one of the most important parameters that can be implemented into monitoring programmes. Consistent monitoring methods between sites can enable the identification of naturally occurring temporal trends that could be affecting structural responses or where trends are not consistent, indicate localised anthropogenic disturbances. Significant changes to shoot density should then justify the use of other robust bioindicators of stress to determine the causes of decline.

5.5. Conclusion

This study demonstrates the high levels of plasticity exhibited by eelgrass to environmental conditions and the need for regular, consistent long-term monitoring of seagrass sites for significant declines to be detected. Structural bioindicators or responses such as shoot density, cover, biomass and extent are often included (one or all) in general seagrass monitoring programmes but do not integrate the use of bioindicators.

Our evidence indicates that where significant changes are detected such biochemical indicators can become powerful metrics for determining sources of declines. For sites where there is a lack of monitoring data, a suite of bioindicators and abiotic factors can be measured to interpret environmental conditions and provide meaningful understanding as to the status of those seagrasses that are potentially indicative of long-term trends. Left unchecked seagrass meadows are highly susceptible to degradation and loss, principally due to the development of a phase shift from seagrass to an algal dominated state. Our study provides a warning that such shifts may be likely at some, particularly as their resilience to future stressors is compromised by poor water quality. In conclusion we find that long-term monitoring of seagrasses is critical for helping inform management of such meadows to prevent catastrophic changes from occurring.

Acknowledgements

This study was made possible with the help of Max Robinson, whose help with the 2016 surveys was invaluable. Thanks to Blaise Bullimore and NRW for help with accessing of data from seagrass meadows around Wales, especially the Skomer MCZ team who also assisted with 2016 surveys providing use of their boat and dive team (Kate Lock, Jen Jones, Mark Burton and Phil Newman), and the Milford Haven Waterway Environmental Surveillance Group.

5.7 Appendix

Figures

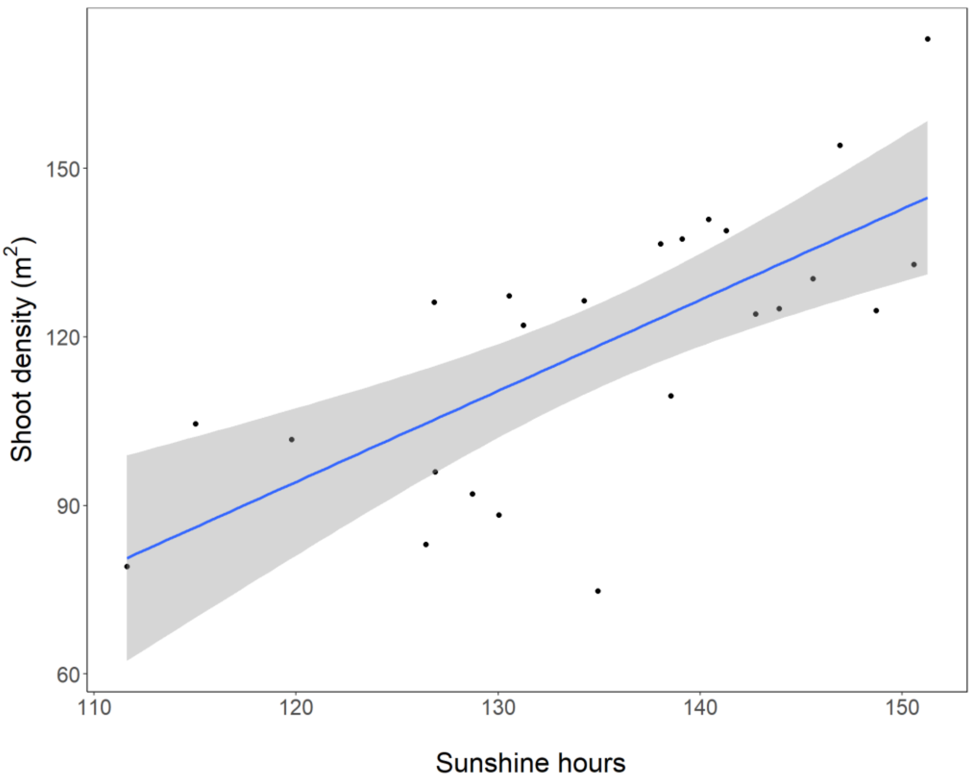

Figure A.5.1. Graph showing linear regression of yearly average shoot density (m2) from Isles of Scilly plotted against average sunshine hours per month for each year density data was available, taken from Met Office data recorded at nearby Cambourne weather station, Cornwall. The blue line shows linear trendline (linear model) with 95% confidence limits shaded in grey. Results from the linear regression shows a positive correlation (R2=0.505, F=22.4, p=<0.001).

Tables

Table A 5.1. Analysis of Deviance table showing results of the likelihood ratios test for comparing GLM models with and without 'Site' to assess significance of test.

<table>
<tr><td colspan="7">Shoot density</td></tr>
<tr><td colspan="7">Model 1: density ~ 1</td></tr>
<tr><td colspan="7">Model 2: density ~ site</td></tr>
<tr><td></td><td>Resid. Df</td><td>Resid. Dev</td><td>Df</td><td>Deviance</td><td>F</td><td>Pr(>F)</td></tr>
<tr><td>1</td><td>98</td><td>6322.6</td><td></td><td></td><td></td><td></td></tr>
<tr><td>2</td><td>93</td><td>4158.5</td><td>5</td><td>2164.1</td><td>9.6228</td><td>1.993e-07 ***</td></tr>
<tr><td colspan="7">No. of leaves</td></tr>
</table>

Model 1: no_lvs ~ 1						
Model 2: no_lvs ~ site						
	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	
1	678	145.04				
2	673	104.38	5	40.66	**1.099e-07** ***	
Leaf width						
Model 1:leaf_width ~ site						
Model 2: leaf_width ~ 1						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	1670	92.069				
2	1674	148.729	-4	-56.659	273.06	**< 2.2e-16** ***
Max. leaf length						
Model 1: max_length ~ 1						
Model 2: max_length ~ site						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	682	156.22				
2	677	91.637	5	64.582	111.93	**< 2.2e-16** ***
Wasting						
Model 1: wasting_prop ~ 1						
Model 2: wasting_prop ~ site						
	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	
1	1746	179.14				
2	1741	146.06	5	33.082	**3.626e-06** ***	
Epiphytes						
Model 1: epiphyte_prop ~ 1						
Model 2: epiphyte_prop ~ site						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	1861	496.29				
2	1856	477.21	5	19.078	14.395	**7.421e-14** ***
Seagrass cover						
Model 1: seagrass_cover ~ 1						

Model 2: seagrass_cover ~ site						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	235	125.944				
2	231	68.337	4	57.607	50.371	**<2.2e-16 *****
Algae cover						
Model 1: algae_cover ~ 1						
Model 2: algae_cover ~ site						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	235	129.78				
2	231	108.65	4	21.132	11.264	**2.291e-08 *****

Table A.5.2. Results from Generalized Linear Models (GLM) for shoot metrics (leaf length, width, epiphyte and wasting disease scores) and meadow data (shoot density and number of leaves per shoot). Gamma GLM was used for continuous measures (length and width), binomial GLM for proportion data (epiphyte and wasting scores, seagrass and algae % cover as proportion 0-1), and poisson or quasipoisson GLM for count data (shoot density and number of leaves).

Coefficients - Metric	**Estimate**	**Std. Error**	**t-value**	**Pr(>|t|)**
Max. leaf length formula=glm(max_leaf_length~site, family = Gamma (link="log"))				
(Intercept) Criccieth	5.25119	0.06308	83.243	< 2e-16 ***
Littlewick	0.85980	0.07428	11.575	< 2e-16 ***
Pen-y-chain	0.30616	0.08125	3.768	0.000179 ***
Porthdinllaen	0.85557	0.06846	12.497	< 2e-16 ***
Skomer	0.59197	0.06834	8.662	< 2e-16 ***
Isles of Scilly (Little Arthur)	1.19561	0.06740	17.740	< 2e-16 ***
Leaf width formula=glm(leaf_width~site, family = Gamma (link="log"))				
(Intercept) Criccieth	0.31316	0.02362	13.26	<2e-16 ***
Littlewick	0.93534	0.02749	34.02	<2e-16 ***
Pen-y-chain	0.59841	0.03065	19.53	<2e-16 ***
Porthdinllaen	0.82705	0.02531	32.68	<2e-16 ***
Skomer	0.78844	0.02551	30.90	<2e-16 ***
Epiphytes formula=glm(epiphyte~site, family = binomial)			**z-value**	
(Intercept) Criccieth	-1.0479	0.2365	-4.431	9.39e-06 ***
Littlewick	-0.8245	0.3133	-2.632	0.008501 **

Pen-y-chain	-0.6641	0.2922	-2.272	0.023062 *
Porthdinllaen	-0.2471	0.316	-0.782	0.434137
Skomer	-1.0085	0.2682	-3.761	0.000169 ***
Isles of Scilly (Little Arthur)	-0.6495	0.2646	-2.455	0.014092 *
Wasting disease formula=glm(wasting~site, family = binomial)				
(Intercept) Criccieth	-3.449	0.597	-5.777	7.59e-09 ***
Littlewick	-1.2557	0.9513	-1.32	0.1869
Pen-y-chain	1.1674	0.6339	1.842	0.0655 .
Porthdinllaen	-0.511	0.9089	-0.562	0.574
Skomer	-0.6738	0.6911	-0.975	0.3296
Isles of Scilly (Little Arthur)	-0.2895	0.6614	-0.438	0.6615
Shoot density formula=glm(shoot_density~site, family = quasipoisson)			**t-value**	
(Intercept) Criccieth	3.8373	0.3113	12.325	< 2e-16 ***
Littlewick	0.3216	0.3660	0.879	0.38180
Pen-y-Chain	0.4169	0.4010	1.040	0.30124
Porthdinllaen	1.4054	0.3330	4.220	5.68e-05 ***
Skomer	1.1100	0.3360	3.304	0.00136 **
Isles of Scilly (Little Arthur)	1.1228	0.3326	3.375	0.00108 **
Leaves per shoot formula=glm(leaves_per_shoot~site, family = poisson)			**z-value**	
(Intercept) Criccieth	1.20039	0.10370	11.576	<2e-16 ***
Littlewick	0.03722	0.12070	0.308	0.7578
Pen-y-chain	-0.07125	0.13517	-0.527	0.5981
Porthdinllaen	0.14680	0.11112	1.321	0.1865
Skomer	0.01944	0.11221	-0.173	0.8625
Isles of Scilly (Little Arthur)	0.27692	0.10901	2.540	0.0111 *
Seagrass cover formula=glm(seagrass_cover~site, family=quasibinomial)			**t-value**	
(Intercept) Criccieth	-4.426	1.492	-2.967	0.00332 **
Littlewick	2.208	1.510	1.462	0.14504
Pen-y-chain	1.228	1.530	0.802	0.42318
Porthdinllaen	4.596	1.498	3.068	0.00241 **
Skomer	3.085	1.506	2.048	0.04172 *
Algae cover formula=glm(algae_cover~site, family=quasibinomial)				
(Intercept) Criccieth	-17.57	816.92	-0.022	0.983
Littlewick	17.36	816.92	0.021	0.983

Pen-y-chain	16.02	816.92	0.020	0.984
Porthdinllaen	16.69	816.92	0.020	0.984
Skomer	15.96	816.92	0.020	0.984
Seagrass cover ~algae cover formula=glm(seagrass cover ~algae cover + as.factor (Site), family = quasibinomial)				
(Intercept) as.factor(Site)Criccieth	-4.4262	1.3038	-3.395	0.000808 ***
Algae cover	-4.1674	0.4684	-8.898	< 2e-16 ***
as.factor(Site)Littlewick	3.6014	1.3260	2.716	0.007108 **
as.factor(Site)Pen-y-chain	1.4919	1.3379	1.115	0.265969
as.factor(Site)Porthdinllaen	5.7542	1.3161	4.372	1.86e-05 ***
as.factor(Site)Skomer	3.6233	1.3179	2.749	0.006447 **

Table A.5.3. Analysis of Deviance table showing results of the likelihood ratios test for comparing GLM models with and without ‘Year’ to assess significance of test for shoot density and leaf lengths from long-term monitoring data.

Skomer						
Model 1: Z.marina_density ~ 1						
Model 2: Z.marina_density ~ as.factor(Year)						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	1992	51997				
2	1986	47325	6	4672	36.774	**< 2.2e-16 *****
Isles of Scilly - Little Arthur						
Model 1: density_m2 ~ 1						
Model 2: density_m2 ~ as.factor(Year)						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	516	33303				
2	495	29204	21	4099.2	3.7908	**5.013e-08 *****
Model 1: max_length ~ 1						
Model 2: max_length ~ as.factor(year)						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	5166	680.75				
2	5145	583.63	21	97.118	55.368	**< 2.2e-16 *****
Model 1: av_inf ~ 1						
Model 2: av_inf ~ as.factor(year)						
	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	

1	5207	240.85				
2	5186	196.69	21	44.152	**0.002234** **	
Model 1: prop_epiphytes ~ 1						
Model 2: prop_epiphytes ~ Year						
	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	
1	5207	588.53				
2	5186	359.5	21	229.03	**< 2.2e-16** ***	
Porthdinllaen						
Model 1: Z.marina_density ~ 1						
Model 2: Z.marina_density ~ as.factor(Year)						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	584	22633				
2	580	22507	4	125.78	0.9984	0.4078
Model 1: leaf_length ~ 1						
Model 2: leaf_length ~ as.factor(Year)						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	1378	357.09				
2	1374	351.57	4	5.5175	5.7486	**0.0001375** ***
Littlewick						
Model 1: Z.marina_density ~ as.factor(Year)						
Model 2: Z.marina_density ~ 1						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	906	33543				
2	911	44976	-5	-11433	71.322	**< 2.2e-16** ***
Model 1: leaf_length ~ 1						
Model 2: leaf_length ~ as.factor(Year)						
	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	7424	1990.9				
2	7419	1802.8	5	188.17	181.32	**< 2.2e-16** ***
Model 1: prop_wasting ~ 1						
Model 2: prop_wasting ~ as.factor(Year)						
	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	
1	4051	1016.51				
2	4048	858.51	3	158	**< 2.2e-16** ***	

<table>
<tr><td colspan="7">Model 1: prop_epiphytes ~ 1</td></tr>
<tr><td colspan="7">Model 2: prop_epiphytes ~ as.factor(Year)</td></tr>
<tr><td></td><td>Resid. Df</td><td>Resid. Dev</td><td>Df</td><td>Deviance</td><td>Pr(>Chi)</td><td></td></tr>
<tr><td>1</td><td>4051</td><td>1904.1</td><td></td><td></td><td></td><td></td></tr>
<tr><td>2</td><td>4048</td><td>1370.2</td><td>3</td><td>533.86</td><td colspan="2">< 2.2e-16 ***</td></tr>
</table>

Chapter 6. General Discussion

This thesis thoroughly explores the responses of Z. marina, Z. noltii and H. wrightii to a range of environmental drivers through experiments, field survey and utilizing existing long-term monitoring data. This study examined seagrass responses at shoot and meadow-scales across a range of Z. marina, Z. noltii and H. wrightii meadows and the environmental parameters that are modifying them, specifically related to water quality. The findings were then applied to long-term monitoring case studies to assess if current methods are picking up important changes. Declines in the status of seagrass meadows will affect the ecological benefits they provide, as described in Chapter 1. Decreases in structural properties including density, cover and extent will affect the stability and reduce resilience to future impacts that arise from localised pollution events and climate change.

At a meadow-scale, shoot density was consistently found to be a robust bioindicator of environmental disturbance within seagrass meadows, whilst other responses including leaf length, shoot nutrients and stable isotopes of nitrogen and carbon, provided evidence of potential sources of disturbance. This work explores the range of morphological and physiological responses that seagrasses can exhibit to a range of local environmental drivers, and the complexity of these relationships. Understanding these bioindicators and the rate at which they respond provides useful, applied information for identifying where seagrass meadows may be at risk and in need of strategic intervention. It also highlights the necessity for continued monitoring and the need for consistency in methods for successful conservation and management which will in turn help them adapt to effects of climate change.

One of the biggest threats to seagrasses worldwide is light limitation caused by deteriorating water quality (Hemminga, 1998; Unsworth et al., 2019). As primary producers, seagrasses need a minimum level of photosynthetically active radiation (PAR) to survive. The effects of light limitation on seagrass have been well studied, although responses can vary between species, within species and geographically due to local environmental conditions (Collier et al., 2012; Longstaff and Dennison, 1999). Only a few studies have investigated an extensive range of bioindicators for looking at light stress within a laboratory setting or *in situ*, therefore the experiment in Chapter 2 was designed to explore this in detail. This experiment allowed a wide range of bioindicator responses to be measured regularly and to assess the rate of change using conventional methods as well as PAM fluorescence. This study found the minimum light threshold for *Z. marina* was above 20 µmol photons $m^{-2}s^{-1}$ which is comparable to other studies (Lee et al., 2007). Photosynthesis rates, inferred from ETR_{max}, alpha and E_k, showed rapid responses to light limitation followed by shoot growth within the first week. Morphological factors took longer to be affected by light stress with responses dependent on previous condition, such as larger shoot surface area and rhizomal stores, which would affect the rate of the negative effects caused by reduced light. Monitoring of light levels within *Z. marina* meadows would enable potential risks to be foreseen if light is being attenuated to around 20 µmol photons $m^{-2}s^{-1}$ or below for prolonged periods of time. This would be especially important at sites that where seagrass has been known to be decreasing in density or extent or for sites where seagrass restoration is being considered. Using a combination of robust bioindicators that have been identified as particularly relevant to *Z. marina* (leaf length, width, leaf area, alpha, ETR_{max}/E_k), would make it possible to assess whether light limitation has or is occurring. If this study were to be repeated, it would be recommended to investigate the use of other parameters that can be measured using PAM fluorometry, such as relationship between photochemical quenching (NQ) and non-photochemical quenching (NPQ) which can provide more insight into the overall photosynthetic activity. As this was not considered to be a robust bioindicator in the review by McMahon et al. (2013) it was not used, however, this could be due to the limited use of it as a parameter in seagrass studies at the time. It has since been used to evidence photoacclimation to reduced light in *Z. marina* (Park et al., 2016). Other bioindicators such as shoot C:N, chlorophyll content and rhizome sugars were not found to exhibit such strong bioindicator responses in the relatively short time-scale of the laboratory experiment, however they could give longer-term responses to the light environment of a meadow and have been found to be consistent, robust bioindicators in other *in situ* studies.

Around the world, there are many seagrass meadows at risk from light limitation caused by known anthropogenic causes such as harbour dredging, nutrient loading and run-off from poorly managed catchments. Milford Haven (Wales, UK) provides an interesting case study for looking at the effects of a highly industrialised waterway on seagrass habitats within. Milford Haven has been subjected to serious pollution events in the past, the most significant being the grounding of the Sea Empress oil tanker in 1996 at the mouth of the Haven. The industrial nature of the Haven has resulted in the area being more regularly monitored than other locations. In Milford Haven there are several Z. noltii meadows, some of which have been monitored for many years. This provided an opportunity to look at changes over time to see whether the Z. noltii is surviving, improving or in decline as is so often the case when looking at the status of seagrasses globally (Unsworth et al., 2019). For this intertidal species of seagrass, despite being exposed to a highly nutrient enriched and industrial waterway, it was found to have been able to endure in a favourable state over a long timescale. The Z. noltii in Milford Haven was found to be increasing in extent over the past decade. The abundance data for the two largest meadows strengthens these findings although unfortunately, long-term abundance data were unavailable for the other sites. Reasons for this improvement could be that although Milford Haven has been found to have far from favourable nutrient levels, the Haven is well flushed owing to its size, depth and tidal range. Also, the fast-growing, dynamic properties of small seagrass species like Z. noltii lends itself to coping in perhaps more challenging conditions such as the intertidal within which it is found. Long-term monitoring data provides evidence for the status of this seagrass species in Milford Haven and highlights the importance of monitoring, particularly where risks are potentially higher. By including other metrics, such as shoot density, leaf length and shoot nutrients in monitoring strategies, evidence of the status of the seagrass meadows would be more conclusive. Measuring leaf length and shoot density, not just % cover, will provide a better idea of productivity of a seagrass meadow, whilst tissue nutrient analysis can provide further indication of environmental conditions including light availability (using C:N ratio and stable isotope of δ13C) and nutrient availability (%N, %P, δ15N). The very presence of seagrass in coastal waters is used as an indicator of water quality within Water Framework Directives (WFD). In the case of Z. noltii presence alone, Milford Haven Waterway appears to be improving but consistent monitoring needs to continue as it is critical for effective management (Griffiths et al., 2020).

Seagrasses grow in shallow coastal waters and estuaries, which are often subjected to the worst levels of anthropogenic impacts within the marine environment. *H. wrightii* is a species of seagrass commonly found along tropical to subtropical and warm temperate coasts, and is the most common seagrass species in Brazil (Copertino et al., 2016; Sordo et al., 2011). Like *Z. noltii*, it is a pioneering species well adapted to high levels of disturbance and grazing (Sordo et al., 2011). Around the coast of southern Brazil, *H. wrightii* meadows exist under various anthropogenic impacts including discharges from highly urbanised areas, industry, and untreated sewage. The prolonged intense rainfall in this area can exacerbate turbidity with run-off from surrounding catchments effected by soil erosion. This study tested a wide range of bioindicators, including those identified in Chapter 1, *in situ* in order to further explore the effects of anthropogenic stressors on seagrasses. An impact assessment index was created to establish a gradient of disturbance against which seagrass bioindicators could be assessed. Some bioindicators well reflected the level of impact, particularly in the most impacted site, which included shoot density, photophysiological responses and shoot nutrients. However, the least impacted site showed significant levels of disturbance indicated by low shoot density and biomass. This study highlights the complexity of the environmental drivers that affect seagrass meadows, especially when impacts are multi-faceted. The site perceived to be least impacted could have been subjected to increased storm event leading to burial, damage and reduced production shown in reduced density and biomass. However, this could not be determined from this study which assessed these meadows at one point in time. The lack of long-term data for most of these sites means only inferences can be made on their previous condition. Seagrass meadows, such as these *H. wrightii* beds, growing at their geographical limit will likely be significantly affected by the climate change. More storm events, and increased rainfall will have a severe impact on seagrasses that are already existing under levels of disturbance affecting their resilience. The perceived anthropogenic impacts were reflected in shoot density and leaf nutrients for the most impacted sites, but other bioindicators such as leaf length, did not. Leaf length as a metric on its own does not provide a clear enough reflection of environmental stress which stands for most metrics if taken in isolation. In many cases, leaf length has been found to reduce with light stress (Bertelli and Unsworth, 2018; Collier et al., 2012) but in some cases leaf length can increase as a response to competition for light (Schmidt et al., 2012; Shafer, 1999). When leaf length is measured alongside shoot density, it provides a much better idea of impacts that can be cause for concern. Decreases in density with increases in leaf length can be indicative of nutrient loading (Schmidt et al., 2012) which has been shown to be the case for the most impacted sites for *H. wrightii* in Brazil and which also rings true for the *Z. marina* meadow in Milford Haven (Chapter 5).

The plasticity of seagrasses enables them to adapt to a range of environmental drivers to some extent, making them excellent indicators of the conditions within which they exist. However, increasing human pressures are causing decreases in coastal environmental quality to a point where seagrasses are unable to cope resulting in significant declines in seagrass meadows globally (Marbà et al., 2013; Orth et al., 2006). The short-term spatial surveys conducted in Chapter 5 were found to be indicative of the long-term trends for the sites. When analysed alongside long-term monitoring data, shoot density, leaf length, leaf nutrients (C:N ratio, %N, %P) and stable isotope of $\delta^{13}C$ and $\delta^{15}N$, presented good insight into the longer-term status of the meadows studied and good indication of the causes of long-term decline. The Isles of Scilly presents a seagrass meadow with little evidence of impacts when compared to other sites and also uses thorough monitoring protocol carried out yearly. By contrast, Littlewick showed high levels of impacts, with bioindicators showing clear warning signs of nutrient loading reflected in the long-term decline. Littlewick is a relatively large *Z. marina* meadow located in a sheltered bay within Milford Haven. The meadow has been monitored every 4+ years since 1986 but has been declining in density since 1999. Littlewick is showing effects of eutrophic conditions which are causing a system shift from a seagrass dominated to macroalgae-dominated community. Without intervention, this meadow will not be able to recover and is at risk of being lost, along with all the ecological functions it provides.

As sentinel species, seagrasses have been integrated into water quality frameworks around the World. Nonetheless, not all seagrass meadows are monitored, and where they are, there are discrepancies in methodologies, occurrence and the chosen parameters, even at a national scale (Marbà et al., 2013). There is still the need for regular, consistent long-term monitoring of seagrass sites and careful consideration of the bioindicators measured so that any declines and their causes can be detected and acted upon. For this to become possible, the difficulty then arises with the decision to persist with the following of current, diverse methodologies that have been implemented for many years in some places, in order to continue to collect comparative datasets to determine long-term trends, or to change to a different approach, breaking the cycle. To avoid losing consistency in monitoring data, the addition of indicators to existing monitoring protocols is one way forward to avoid any disruption to data collection as is, although this will require extra funding. For example, in the UK, many seagrass sites are not regularly monitored and where they are, methods vary (Unsworth et al., 2014). There is a gap for the creation of a standardised seagrass monitoring programme that is specific to the species and the main environmental stressors that exist around the UK. Comprehensive seagrass monitoring protocols have been produced by SeagrassWatch (McKenzie et al., 2003) and SeagrassNet (Short et al., 2015), both of which are global networks which are focused on collecting comparable long-term datasets and have been employed at a few locations around the UK. These protocols are relatively thorough but may not be practical for monitoring at all seagrass sites. For example, SeagrassWatch uses seagrass cover not density as the main abundance parameter, whereas SeagrassNet uses both parameters. To have a broader general monitoring protocol that can be implemented at a wider range of sites more consistently, is arguably more achievable.

A global review of seagrass indicators of environmental stressors by Roca et al., (2016) describes a fit-for-purpose monitoring strategy that brings in finer-scale bioindicators for use depending upon the outcomes of general seagrass monitoring programme. Figure 6.1. shows a monitoring strategy adapted to the findings from this thesis including the main environmental stressors likely to be affecting seagrasses (*Zostera* genera) in the UK. The programme consists of a general monitoring protocol for assessing seagrass status using metrics such as shoot density, percentage cover, extent, maximum depth and biomass, although not necessarily all of these. Most of the monitoring protocols currently in use measure two or more of these already. If significant changes are detected, the use of bioindicators can be used to determine the cause of decline in status. The inclusion of abiotic measurements may allow stressors to be identified prior to the initial survey and justify the inclusion of extra bioindicators to be measured at this point. However, if a change has been detected after the initial assessment and the stressor remains unknown, a range of bioindicators can be used to determine the cause of decline. Instead of drastically changing the current monitoring practises in place, this strategy suggests the inclusion of extra bioindicators if a change/decline is detected. Some of the robust bioindicators measured in this study may need the use of highly specialised equipment such as the PAM fluorometer for gaining photosynthetic parameters, would prove to be costly to include in regular monitoring programmes but could be considered in when significant declines are observed. This strategy could be adapted even further for local areas or Marine Protected Areas (MPAs) to make it more applicable. The monitoring programme suggested (Fig. 6.1) is based on the major concerns for *Z. marina*, based upon the sites that have been covered in this study.

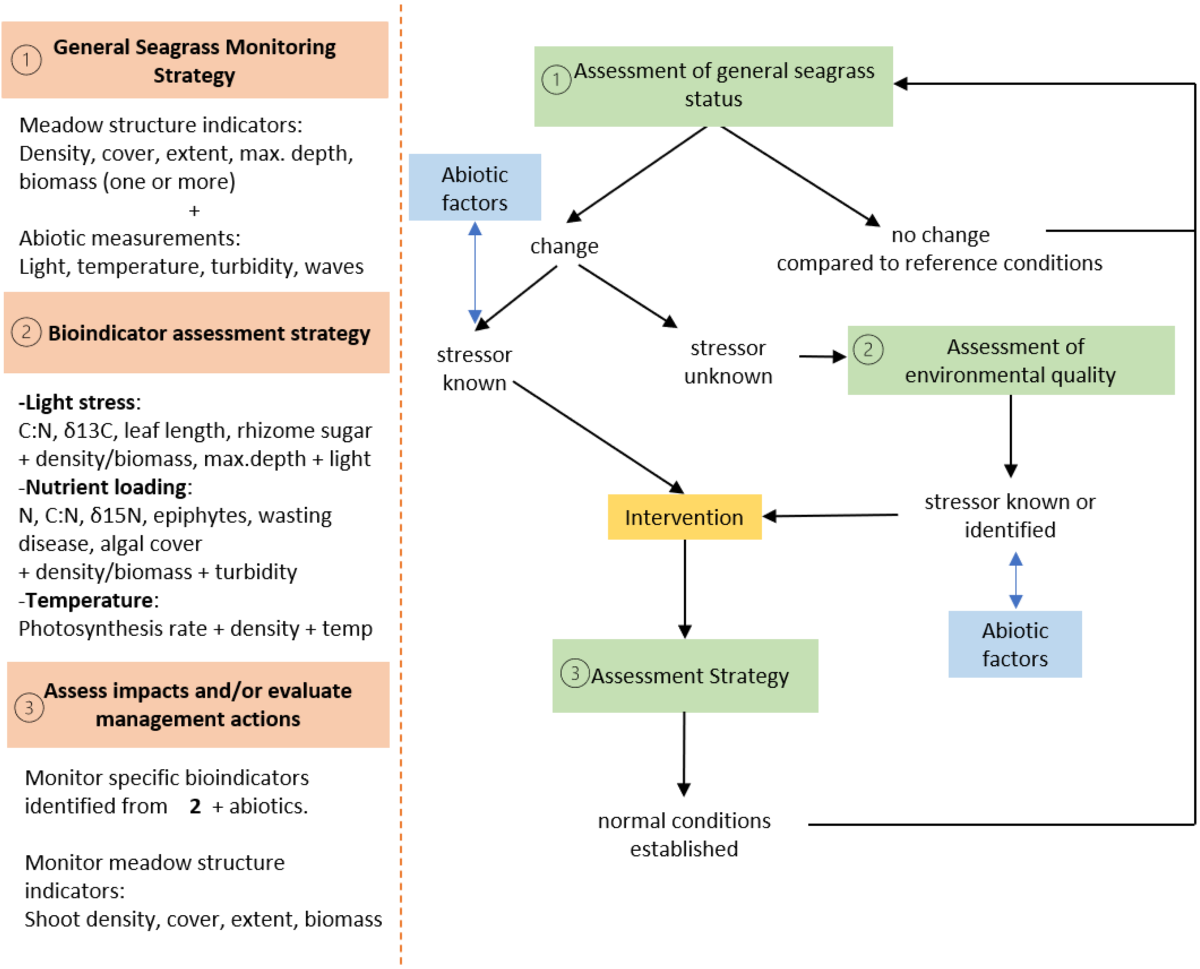

Figure 6.1. Suggested monitoring programme adapted from Roca et al., (2016) for UK seagrass assessments, modified for main environmental threats from water quality issues. In the UK, general seagrass status is measured using various methods but always include structural indicators such as density/cover. If significant change is measured, more detailed bioindicator analysis should then be undertaken, especially if the stressor is unknown. Abiotic measurements can help determine the stressor and can indicate when reference conditions have been achieved.

The programme only relies on the use of additional bioindicators if necessary - if significant changes are detected. This programme can be adapted for other species or to be more site specific. For example, intertidal species or meadows found in shallow lagoons or estuaries, are likely to be affected by fluctuations in temperature and salinity more so than subtidal sites. Therefore, salinity would be an extra abiotic factor to be considered. Some species are heavily affected by grazers so including those species in surveys may be more practical. In some locations, seagrass meadows overlap with physical modifiers, such as established moorings, jetties, harbour activities etc. so these factors would need to be considered within the general monitoring strategy. A monitoring programme of this kind needs a set of reference or baseline conditions to be established. For sites where there is a lack of monitoring data, an initial assessment covering a wide range of meadow-scale and shoot bioindicators would be recommended along with a range of abiotic factors. This would be useful in determining if the seagrass traits measured are being modified by naturally occurring pressures such as wave exposure, or from anthropogenic causes. Increasing the consistency in approach to seagrass monitoring and management will have positive consequences for the protection of these habitats and their adaptation to future shifts in environmental conditions caused by the effects of climate change.

Conclusion

The aims of this thesis were to investigate the biological responses of seagrasses to environmental drivers, specifically related to water quality issues which is one of the biggest threats to seagrasses worldwide. This study provides useful evidence of seagrass responses to a variety of environmental drivers and how these bioindicators can be utilised for assessing and monitoring the status of seagrass meadows. Shoot density is consistently shown to be indicative of environmental stresses at a meadow scale as it will decline with light limitation, nutrient loading and wave exposure. This is the reason why it is one of the most commonly measured metrics within monitoring programmes. However, only measuring one metric is not suitable for management purposes as different bioindicators have different response times and sensitivities to different stressors. However, measuring other metrics in conjunction will provide better insight into the possible causes of change in meadow density over time. Measuring shoot nutrients and stable isotopes can provide a clear indication of light limitation, nutrient loading, and its possible sources. The more data collected for different seagrass species will provide a better picture of what the typical ranges should be and at what point they are indicative of thresholds and the need for intervention. Leaf length, or canopy height, was found to respond rapidly to light limitation under experimental conditions, but this was not always found to be the case in the field. Some sites with lower light levels were found to have smaller, shorter shoots which will reduce the respiratory pressure on the plant. However, other sites, where light was found to be limited, the seagrass was found to have significantly longer leaves. Leaf length can also increase as a response to other factors such as competition, therefore the measurement of shoot density and algal cover, for example, would be important in determining the environmental pressures.

These findings highlight the need for regular, long-term monitoring of seagrass sites including a range of robust bioindicators of light stress and poor water quality if significant changes are detected. For sites that have not been surveyed before, initial monitoring should include as many applicable bioindicators as possible in order to provide a baseline. Shoot density, and shoot biochemistry can provide ample warning of water quality issues such as hyper-nutrification as was found to be the case with *H. wrightii* and *Z.marina.* If these impacts are not addressed, the possibility of a phase shift could result, leading to a loss of seagrass meadows and their ecosystem benefits. The monitoring programme suggested in Figure 6.1, adapted for seagrass monitoring around the UK, provides a useful strategy/framework that can aid the decision-making process for seagrass monitoring. The more that is known about these bioindicators the easier it will be to designate the status of a meadow. Due to the plasticity of seagrasses, it is difficult to determine what the current status of a seagrass meadow is without long-term monitoring data. Levels of change can then be used to establish if the status or health of a seagrass meadow is stable or in decline.

Seagrasses have been identified as significant contributors to climate change mitigation and adaptation (UNEP, 2020) as natural carbon sinks, by stabilising coastal sediments, reducing coastal erosion and buffering the effects of ocean acidification. Disturbance to seagrasses has been repeatedly shown to increase vulnerability to pressures and will decrease their ability to provide the ecosystem services upon which we depend. The continued failure to effectively manage pressures on seagrass meadows is effectively in breach of international conventions and our commitments for tackling climate change. If water quality issues are not addressed, it will be difficult to determine what the future for seagrass meadows entails.

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