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University of Plymouth

Ocean acidification and carbon limitation affect photosynthetic capacity of the seagrass (*Amphibolis antarctica*) and its calcifying epiphytes

By

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Author's declaration

At no time during the registration for the degree of Research Masters in Biological Sciences has the author been registered for any other University award without prior agreement of the Sub-Committee.

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Ocean acidification and carbon limitation affects photosynthetic capacity of the seagrass (*Amphibolis antarctica*) and its calcifying epiphytes

Abstract

Amphibolis antarctica seagrass meadows, and their associated calcifying epiphytes, are abundant on Australia's west coast, but have declined in recent years due to anthropogenic factors such as marine heatwaves, damaging fishing practices and increased turbidity resulting from eutrophication which causes light limitation. Burning fossil fuels has increased the flux of CO₂ in to the ocean, lowering surface seawater pH, and making more carbon available for photosynthetic life. There are benefits of increasing CO₂ for those seagrasses that are carbon limited, as this alleviates their energetic use of carbon concentrating mechanisms (CCM'S) which are less efficient, and more energy costly than passive diffusion of CO₂ across cell walls. This study used pulse amplitude modulation fluorometry to quantify relative electron transport rates (rETR) at a range of pH levels both above and below current ocean pH of 8.1, and found that *A. antarctica* has significantly decreased rETR at pH treatments of 7.81 and 7.61. Calcifying epiphytes on *A. antarctica* also had a significant drop in rETR at the lower pH treatments. There was also significantly lowered rETR at higher pH treatments, likely the result of carbon limitation. These results from the lower pH tests may have profound implications for *A. antarctica* meadows under ocean acidification. A decline in these meadows would cause the loss of ecosystem services provided by them, such as carbon storage and sequestration, commercial fisheries and a decline the abundance of biodiversity that they support.

Table of Contents

List of Tables	8
List of Figures	9
Introduction	10
Changing climate in the Anthropocene	12
Ocean Acidification	15
Carbon limitation and carbon concentrating mechanisms.....	21
1. Seagrasses and their global decline	22
2: <i>Amphibolis antarctica</i> and associated calcifying epiphytes	27
2.1 Seagrass – <i>Amphibolis antarctica</i>	27
2.2 Calcifying algae.....	30
3. Design, methods results.....	32
3.1 Methodology.....	32
3.1.1 Sampling site and collection of specimens	32
3.1.2 Pulse Amplitude Modulation (PAM) Fluorometry	33
3.1.3 Experimental set-up	34
3.2 Statistical analyses & hypotheses	36
3.3 Results.....	38
3.3.1 Seagrass results.....	38
3.3.2 Calcifying epiphytes results.....	41
3.3.3 Two-sample T-test	44
4. Discussion.....	45
4.1 <i>Amphibolis antarctica</i>	45
4.1.1 Reduced primary productivity	46
4.1.2 Feedback loops	48
4.1.3 Commercial fisheries and ecotourism	49
4.1.4 Sinks as sources.....	51
4.1.5 Fluid dynamics and coastal erosion	52
4.2 Calcifying epiphytes	53
4.2.1 Increased niche availability.....	55
4.2.2 Release of carbon stores.....	56

4.2.3 Loss of nutrition to grazing species.....	57
4.3 Consideration of multiple stressors	57
Conclusions	59
References	61

List of Tables

Table 1: A summary of the RCP scenarios created by IPCC to project the future of climate change. RCP numbers (i.e. 2.6 - 8.5) denote the radiative forcing in watts per square metre anticipated as a result of projected atmospheric CO ₂ concentrations for different emissions scenarios. Values shown below are for 2100 from: EAA, 2016; Genner et al., 2017, Van Woesik et al., 2015)	17
Table 2: A small selection of the projected outcomes of increased CO ₂ for marine primary productivity.	19
Table 3: Results of a two-way ANOVA (GLM) on the effects of pH, Irradiance and pH*Irradiance on electron transport rates in <i>A. antarctica</i> , showing that both pH and irradiance cause significant changes in electron transport rates, and that there is an interactive effect between the two	38
Table 4: Results of a Bonferroni pairwise analysis (95% confidence) of the two-way ANOVA (GLM) results shown in table 3. Means that do not share a letter are significantly different.....	38
Table 5: Results of a two-way ANOVA (GLM) on the effects of pH, Irradiance and pH*Irradiance on electron transport rates in calcifying epiphytes, showing that both pH and irradiance cause significant changes in electron transport rates, and that there is an interactive effect between the two.....	41
Table 6: Results of a Bonferroni pairwise analysis (95% confidence) of the two-way ANOVA (GLM) results shown in table 5. Means that do not share a letter are significantly different.....	41

List of Figures

Figure 1: A comparison of carbon burial rates of various naturally occurring carbon sinks, from (Mcleod et al., 2011)	14
Figure 2: Global mapping of seagrass distribution (darker regions with higher species diversity) shows the north and south-west coasts of Australia to be highly biodiverse in terms of seagrass distribution (from (Short et al., 2007)	23
Figure 4: CO ₂ absorbed in to the oceans forms carbonic acid, lowering the pH of seawater and reducing the availability of carbonate ions (Slipher, 2019)	16
Figure 5: Figure 3 (adapted from Honisch et al., 2012) shows diversity trajectories of calcareous plankton (black), calcareous benthos (blue) and organic fossils (green), with red lines indicating events associated with ocean acidification. Whilst calcareous species are not uniformly affected across these events, of note is the change in species richness (denoted by narrowing of the coloured lines) during the permo-triassic extinction which occurred approximately 252 mya.	18
Figure 6: Seedlings of <i>A. antarctica</i> , with the comb-like hooking structure (circled) clearly visible (Verduin et al., 2013)	29
Figure 7: Location of the sampling site (A&B), Mindarie (location: 31°41'42.4"S, 115°42'06.6"E), off the south-west coast of Australia (googlemaps, 2021), and the habitat where samples were taken for this study (C (Rasmusson, 2015)).....	33
Figure 8: Mean relative electron transport rate of seagrass samples for each pH treatment with standard error.	39
Figure 9: Polynomial linear regression including 95% confidence intervals, showing the relationship between seagrass electron transport rate (rETR) and pH	40
Figure 10: Mean electron transport rate of calcifying epiphytes for each pH treatment with standard error.	42
Figure 11: Polynomial linear regression including 95% confidence intervals, showing the relationship between calcifying epiphyte electron transport rate (rETR) and pH.....	43
Figure 12: Calcifying epiphytes exhibited significantly higher rETR than <i>A. antarctica</i> across the range of pH treatments, though with a greater degree of variation within the data. Error! Bookmark not defined.	

Introduction

The Anthropocene era has become characterised by rapid climate change and biodiversity loss (Bradshaw *et al.*, 2021). Atmospheric CO₂ concentrations are causing earth's climate to change at a rate unprecedented in the known geological history of the planet (IPCC, 2013; Hurd *et al.*, 2018), and this, in conjunction with other anthropogenic stressors has led to the commencement of the sixth mass extinction (Ceballos *et al.*, 2017; Ripple *et al.*, 2017).

Earth's ecosystems and the species that inhabit them are in decline in terms of both populations and trophodynamic complexity (Houk *et al.*, 2012). This is perhaps most visible in terrestrial environments, as witnessed in the recent bushfires in Australia, which destroyed more than 17 million hectares of forest and killed an estimated 1bn wild animals (Komesaroff *et al.*, 2020). In comparison, little light has been shone on the rapid trophic downgrading and climatic change taking place within earth's marine systems, despite the fact that marine biodiversity is critical for all life on earth in terms of oxygen production and energy flow. Whilst the pelagic realm is far from immune to such influences, the greatest damage thus far has occurred in coastal zones. Coastal development and damaging fishing practices such as trawling of benthic regions, are destroying the complex three-dimensional nature of coastal habitats such as coral reefs, seagrass meadows and oyster beds; nutrient runoff from agriculture and industry is causing eutrophication, leading to light limitation, toxic algal blooms and hypoxia; over-exploitation has significantly reduced global populations of commercial species such as oysters and cod (Jackson, 2009).

Anthropogenic carbon emissions are changing global climate and are both warming and acidifying the oceans (IPCC, 2013). The advent of research into the environmental consequences of climate change, initially focused largely on the impacts of warming in both terrestrial and aquatic habitats, with some researchers even suggesting that absorption of CO₂ by the oceans may be beneficial by limiting atmospheric CO₂. Whilst there is an element of truth in this, in that it means some CO₂ is prevented from entering the atmosphere, we now know that the ecological consequences of a continuing rise in ocean CO₂ has innumerable negative outcomes for the life inhabiting earth's oceans (Doney *et al.*, 2020). The discovery that ocean acidification (the result of anthropogenic CO₂ mixing with ocean water) was leading to a decline in calcification in coccolithophores (one of the most abundant phytoplankton in the oceans), led to a paradigm shift in scientific thinking about ocean acidification (Riebesell *et al.*, 2000). Two decades of research later, and threats to a variety of taxa around the globe via ocean acidification, have been

identified, from apex predators such as sharks (Dixon *et al.*, 2015) to microscopic diatoms that form the basis of many marine food webs (Petrou *et al.*, 2019). Part of that paradigm-shift has been a heavy focus on the impact of elevated ocean CO₂ on primary productivity in coastal habitats, such as seagrass meadows.

Seagrasses are rooted, flowering angiosperms which produce both seeds and flowers, and form large complex structural habitats for a wide variety of organisms, including many of commercial value (van Dijk *et al.*, 2018). Seagrass meadows are amongst the most productive and biodiverse habitats in the marine environment, contributing around 15% of global ocean net carbon production every year (Nayar *et al.*, 2009) and make a vital contribution to global carbon sinks (Mcleod *et al.*, 2011; Russell *et al.*, 2013; Macreadie *et al.*, 2019). Seagrass beds lock up carbon for millennia, at rates higher than those of tropical rainforests (Mcleod *et al.*, 2011), but much like tropical rainforests, they are in global decline (Orth *et al.*, 2006). This is due to a number of factors, primarily pollution leading to eutrophication which blocks out light for photosynthesis, though climate change is now also playing a role (van Katwijk *et al.*, 2016). During the 2010-11 marine heat wave off the west coast of Australia, large swathes of seagrass (particularly *A. antarctica*) died due to thermal stress (Strydom *et al.*, 2020). Such events are becoming more common and are likely to increase in frequency in the future (Thomson *et al.*, 2015; Oliver *et al.*, 2018) which is becoming a significant concern for the fate of seagrasses under ocean warming (Rasmusson *et al.*, 2019).

Some studies suggest that seagrasses may benefit from the increased availability of CO₂ under climate change, particularly given that seagrass meadows typically experience large diel fluctuations in carbon availability and pH (often regulated by seagrasses themselves via photosynthesis and respiration (Buapet *et al.*, 2013; Hendriks *et al.*, 2014)), they may be somewhat resilient to ocean acidification. Any species inhabiting these meadows would be exposed to pH fluctuations within the meadows too and may be well adapted to tolerate such temporal heterogeneity in the carbonate chemistry of the waters they inhabit. This is far from certain, however, particularly in the face of other environmental stressors such as marine heat waves and pollution which may act synergistically with acidification.

Gaining a better understanding of the response of seagrasses to the continuing rise in carbon emissions may help make the case for increased protection and restoration efforts for seagrass habitats, not just for the sake of biodiversity, but also as part of the “nature based solutions” approach to climate change (Nellemann *et al.*, 2009; Russell *et al.*, 2013; Turner, 2018). In marine environments, the nature based solutions approach focuses principally on primary productivity in coastal ecosystems where seagrass meadows, salt marshes, mangroves and forests of kelp lock up huge amounts of carbon. Protecting these

types of habitat also enhances ecosystem functioning and services such as the provision of nursery grounds for commercially important marine species, or natural flooding and tidal surge defences (Berke *et al.*, 2006; Blandon *et al.*, 2014). Protecting coastal habitats such as these, may prove key in helping to limit the ongoing rise of atmospheric CO₂ which is contributing to anthropogenic climate change (Macreadie *et al.*, 2019).

To date, few studies have investigated the influence of increased CO₂ on *A. antarctica*, yet this species of seagrass creates vast habitats for endangered marine species and commercial fisheries species (Bellchambers *et al.*, 2012; Kendrick *et al.*, 2019) and helps to create habitats by modifying fluid dynamics and sedimentation. This study seeks to fill some of the existing knowledge gaps on the photophysiological responses of *A. antarctica* and associated calcifying epiphytes to changes in carbon limitation.

Changing climate in the Anthropocene

Since the industrial revolution, the burning of fossil fuels has led to a continuing increase in greenhouse gases in earth's atmosphere, most notably, in carbon dioxide (CO₂) (IPCC, 2013). This increase has altered earth's climate, leading to higher average temperatures globally (IPCC, 2013; Cook *et al.*, 2016) and this trend is expected to continue (IPCC, 2013). The targets of international agreements, most notably the 2015 Paris accords have largely been missed and despite legislative efforts and increased global awareness, the latest CMIP6 climate models suggest greater future warming than previously projected (Forster *et al.*, 2020). Attempts to address climate change have been hampered by political stagnation and the rise of right wing populism (Lockwood, 2018; Bradshaw *et al.*, 2021). Division exists within the scientific community as to whether scientists should refrain from being overly vocal about climate change in order to preserve their scientific impartiality, though some researchers claim it is time for the community to "eschew reticence" in favour of promoting change (Bradshaw *et al.*, 2021). Whatever the reasons for the inaction on climate change, it has become apparent that unless an effective international response is mounted to tackle climate change, outcomes globally can be expected to be catastrophic for human life and the planet's ecosystems (Travis, 2003; Bradshaw *et al.*, 2021).

Given the scale of the threat, research into climate change has become one of the largest, and arguably most important branches of scientific research. As a result, despite political controversies surrounding climate change, the overwhelming weight of scientific evidence indicates that anthropogenic greenhouse

gas emissions are altering earth's climate (Cook *et al.*, 2013). The consequences of this change are already being witnessed, with the frequency and severity of tropical cyclones (Paerl *et al.*, 2012), El Niño events (Yeh *et al.*, 2009), flooding (IPCC, 2013), droughts (Paerl *et al.*, 2012) and wild fires (Williams *et al.*, 2019) all increasing, with climatologists projecting that these increases will both continue, and likely accelerate as a result of continued anthropogenic carbon emissions (IPCC, 2013).

In the marine environment, climate change has led to more frequent and severe marine heatwaves (Filbee-Dexter *et al.*, 2020). In fact, between 1925 and 2016, marine heatwaves increased in frequency by 34%, and increased in duration by 17% (Oliver *et al.*, 2018). This increase in severity and frequency is set to continue as atmospheric CO₂ levels rise, with marine heatwaves triggering mass mortalities amongst coral communities and the collapse of kelp forests off the coast of Australia (Head *et al.*, 2019; Filbee-Dexter *et al.*, 2020). Seagrass meadows are not immune to such heatwaves either. Off the coast of Western Australia during the 2010-11 marine heatwave, water temperatures during the austral summer rose as high as 5°C above average with some areas reaching 30°C, killing off large swathes of *Amphibolis antarctica* seagrass meadows (Strydom *et al.*, 2020). This impact of marine heatwaves on primary producers is particularly concerning, since massive losses of primary producers has the potential to trigger ecosystem collapse, and the loss of associated ecosystem services, as well as the release of vast natural carbon stores.

In addition to marine heatwaves, the increased flux of CO₂ in to the oceans is causing changes in ocean chemistry, by lowering the pH of the oceans. Since the industrial revolution, average ocean pH has increased in acidity by 0.1, a relative increase in acidity of approximately 25% (Foster *et al.*, 2016). This change has not been uniform however, and there is significant spatial variation in pH changes in the ocean, and whilst the pelagic realm will likely see a simple shift in mean pH, coastal areas are likely to be more variable, since they routinely experience pH fluctuations, making it difficult to determine future pH changes under ocean acidification for coastal areas. This means that whilst some areas may see profound changes in pH that may negatively impact local ecology, there may also be "refuge" zones where acidification is buffered by marine flora, making acidification less severe (Chan *et al.*, 2017). This may be particularly true of coastal areas of high primary productivity such as seagrass meadows, where significant diel variations in local pH exist as CO₂ is removed from the water during the day when photosynthetic activity is high, and CO₂ levels rise again at night, as seagrasses and the inhabitants of the meadows respire (Cyronak *et al.*, 2018).

Whilst many species are expected to suffer negative consequences due to climate change and ocean acidification, those that are able to adapt (whether genetically or via plasticity or acclimatisation) to the rapid environmental changes that have become characteristic of the Anthropocene, may benefit. An increased availability of CO₂ may benefit some photosynthetic organisms, though this may come at a cost to ecosystems, for example harmful algal blooms (HABs) are predicted to increase in frequency and density under climate change, negatively impacting marine life and disrupting food webs (McHugh *et al.*, 2011; Litz *et al.*, 2014; Riebesell *et al.*, 2018). Some primary producers that are more ecologically advantageous for biodiversity (particularly habitat-forming species), may benefit from increased availability of CO₂, such as seagrasses, which form expansive habitats of high productivity, biodiversity and carbon sequestration (Orth *et al.*, 2006; Waycott *et al.*, 2009). In fact, seagrass meadows have carbon burial rates which far exceed those of temperate, boreal and tropical forests (figure 1).

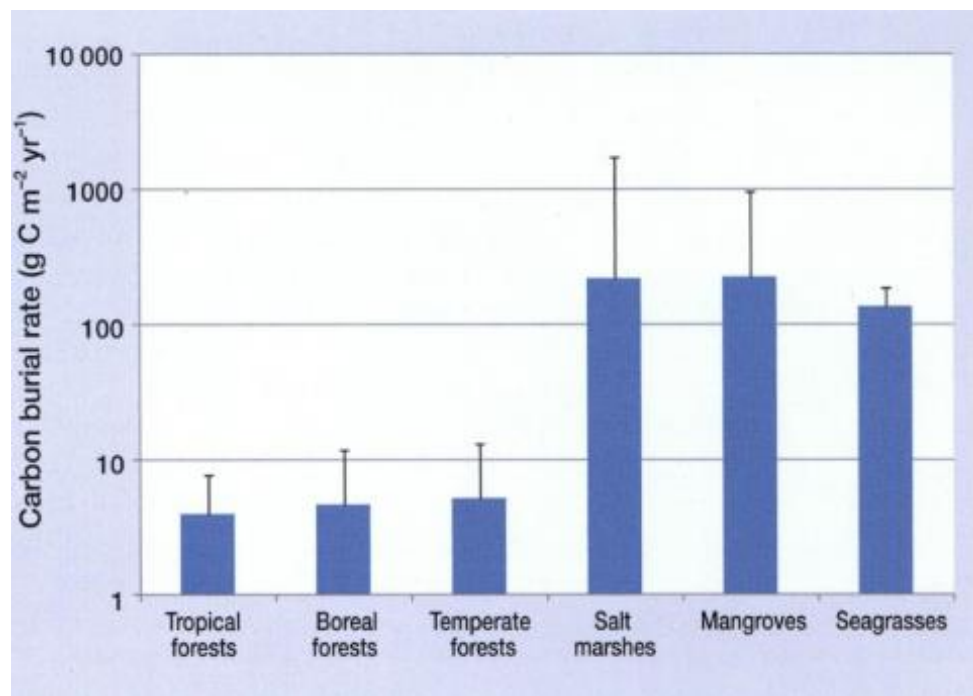


Figure 1: A comparison of carbon burial rates of various naturally occurring carbon sinks, from Mcleod *et al.*, 2011 (Permission to reproduce this figure has been granted by the copyright holder)

Additionally, seagrasses lock up carbon in their sediments for millennia, far longer than forest habitats do, making them a vital carbon sink in the face of climate change, thus adding weight to their importance as

part of the growing focus on the nature-based solutions approach to tackling climate change (Nellemann *et al.*, 2009; Mcleod *et al.*, 2011; Macreadie *et al.*, 2019). The recent discovery and quantifying of seagrass meadows as highly-effective carbon sinks, means that that nations aiming to comply with the Paris climate accords can include seagrass protection and restoration within their nationally determined contributions (NDCs) (McKenzie *et al.*, 2020). This means that the Paris Agreement may offer an effective regulatory framework for protection and restoration of seagrass meadows, which have been in global decline for decades. Such international agreements are, however, only as effective as the efforts made by each nation to achieve them, and given that the world has missed every single one of the Aichi biodiversity targets set out under the Convention on Biological Diversity (Vaughan, 2020), success in halting and reversing the global decline of seagrass, is far from certain.

Ocean Acidification

Approximately half of all carbon emissions from anthropogenic sources during the last 200 years, have been absorbed by the oceans (Turley *et al.*, 2007). When CO₂ is absorbed by seawater, it bonds with water molecules to form carbonic acid which disassociates in to HCO₃⁻ and H⁺, thus lowering the pH of the water (see figure 2). The result of this acidification process and increasing anthropogenic carbon emissions, has caused a reduction in ocean pH of 0.1, equivalent to ~25% increase in acidity (Foster *et al.*, 2016). The means that carbonate ions are less available for biological processes such as calcification in crustose algae species like maerl (Riosmena-Rodriguez *et al.*, 2017), and for skeletogenesis in both vertebrate and invertebrate species (Byrne, 2011; Pimentel *et al.*, 2014). At depth, ongoing acidification may result in seawater becoming corrosive to carbonates, potentially destroying deep-water coral reefs via dissolution of carbonate structures (Hall-Spencer *et al.*, 2019).

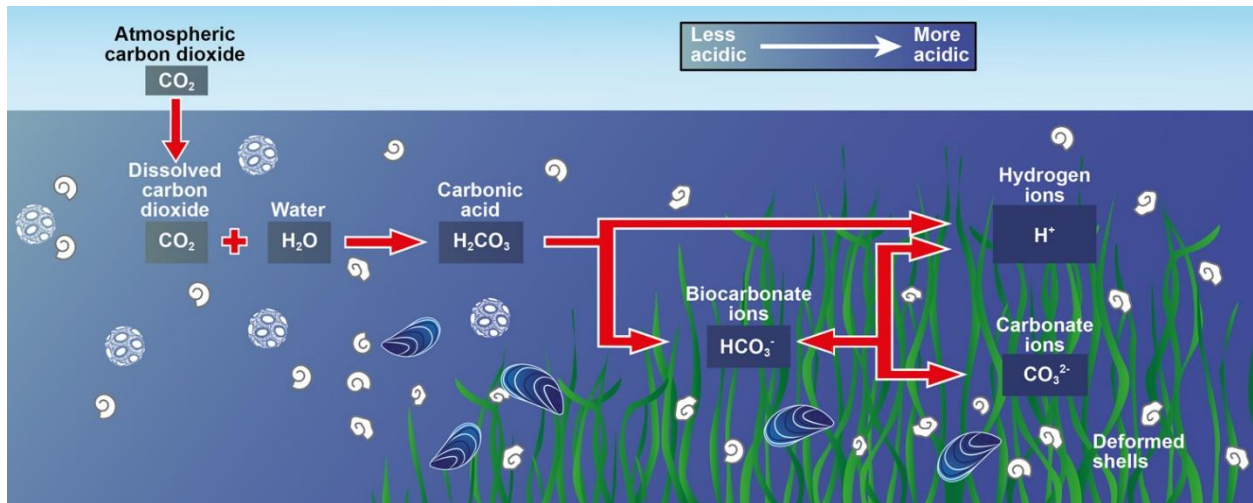


Figure 2: CO_2 absorbed by the oceans forms carbonic acid, lowering the pH of seawater and reducing the availability of carbonate ions (PML, 2021) (Permission to reproduce this figure has been granted by the copyright holder)

Ocean acidification was only recently recognised as a significant threat to marine life, following the discovery in 2000 that coccolithophores, one of the most abundant phytoplankton in the oceans, exhibited significant damage to their calcified shells under elevated levels of CO_2 (Riebesell *et al.*, 2000). The implications of this go beyond the role of coccolithophores as a food source at the bottom of marine food chains. Coccolithophores are an abundant calcifying phytoplankton, a group of primary producers who are one of the main sources of biogenic ocean sediments, as well as being a major component of “marine snow”, pivotal in the vertical flux of carbon and biogenic material to the deep ocean (Jackson, 2009). As such, reduced calcification of coccolithophores may have a profound impact not only on zooplankton within the photic zone which supports huge swathes of marine life, but could also lead to stoichiometric mismatch in deep sea benthic habitats, disrupting the ecology of the deep ocean (Moreno *et al.*, 2017).

The change in ocean pH brought about by CO_2 emissions is not occurring uniformly, and there is significant temporal and spatial variation in the change of pH (Hurd *et al.*, 2018). In the open ocean, pH fluctuates very little, and there is little difference in pH across temporal and spatial scales in pelagic waters (Cornwall *et al.*, 2013). This is not true in coastal areas, however, where diel and seasonal changes in pH and carbon flux occur, sometimes by as much as one pH unit per day (Cornwall *et al.*, 2013). In seagrass meadows, pH levels are in a near-constant state of flux due to numerous environmental variables that influence photosynthetic rates, such as variations in light intensity due to weather conditions, variations in currents,

and variations in nutrient flow. All of these variables influence the rate of photosynthesis in seagrasses, and thus, the rate at which CO₂ is taken up by them. During the night when photosynthesis ceases, respiration releases CO₂ back in to the water, causing pH levels to drop, until photosynthesis begins again in the morning (Cyronak *et al.*, 2018). Seagrasses typically hold a heavy epiphytic load, which similarly contribute to these pH fluctuations (Larkum *et al.*, 2005).

The influence of ocean acidification on the ecology of the planet’s oceans represents a significant environmental threat, particularly given that the likelihood of meeting the emissions targets laid out in the Paris agreement is rapidly diminishing (FEU-US, 2019), making the higher-end climate change scenarios laid out by IPCC, increasingly likely (see table 1). The pH values reported by IPCC for various future climate change scenarios are global averages, and whilst they are likely accurate for pelagic regions, coastal habitats are likely to acidify more than pelagic waters (Duarte *et al.*, 2013).

Table 1: A summary of the RCP scenarios created by IPCC to project the future of climate change. RCP numbers (i.e. 2.6 - 8.5) denote the radiative forcing in watts per square metre anticipated as a result of projected atmospheric CO₂ concentrations for different emissions scenarios. Values shown below are for 2100 from: EAA, 2016; Genner *et al.*, 2017, Van Woesik *et al.*, 2015)

RCP	Summary	Projected CO ₂ (ppm)	Projected global mean surface temperature increase (°C)	Projected ocean surface temperature increase (°C)	Projected ocean pH
8.5	GHG emissions increase continually	1370	4.9	3.7	7.76
6.0	GHG emissions increase but stabilise shortly after 2100	850	3.0	2.2	7.89
4.5	GHG emissions increase but stabilise by 2100	650	2.4	1.8	7.96
2.6	GHG emissions peak in 2050 and then gradually decline	490	1.5	1.2	8.05

The oceans play a major role in supporting life on our planet. Roughly half of global primary productivity and more than half of global oxygen production, comes from the oceans (Boyd, 2014; Witman, 2017). The oceans also play a critical role in regulating climate and weather patterns through exchange and transport

of heat and gases (Bigg *et al.*, 2003; Yeh *et al.*, 2009; Hurrell *et al.*, 2010; Hu *et al.*, 2015), thus, the impact of climate change on the oceans is of paramount concern for all life on earth.

Historically, major extinction events have been characterised by large shifts in ocean chemistry, particularly ocean acidification (Honisch *et al.*, 2012) (Figure 3). Approximately 252 million years ago, a major extinction event known as the permo-triassic extinction, eradicated more than 90% of life on earth (Sahney *et al.*, 2008). The cause of this extinction is believed to be changes in global climate as a result of the release of CO₂ from volcanoes. This event occurred in two phases, and a 2015 study that analysed boron isotope data as a proxy for ocean pH, concluded that the second phase of the extinction can most likely be attributed to the acidification of oceans as a result of increases in atmospheric CO₂ (Clarkson *et al.*, 2015). The mass extinction following the Chicxulub impact ~66mya has also been linked to ocean acidification, with research indicating that a drop in ocean pH of 0.25 (smaller than that projected in RCP 8.5) triggered the global collapse of marine ecosystems.

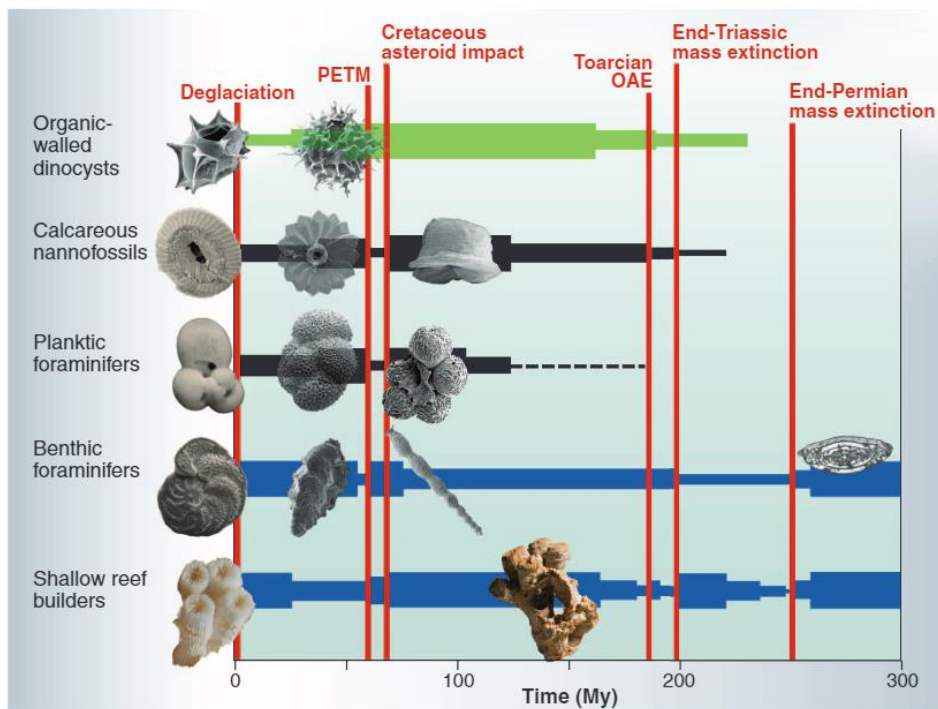


Figure 3: Figure 3 (adapted from Honisch *et al.*, 2012) shows diversity trajectories of calcareous plankton (black), calcareous benthos (blue) and organic fossils (green), with red lines indicating events associated with ocean acidification. Whilst calcareous species are not uniformly affected across these events, of note is the change in species richness (denoted by narrowing of the coloured lines) during the permo-triassic extinction which occurred approximately 252 mya. (Permission to reproduce this figure has been granted by the copyright holder)

Contemporary research has already identified myriad threats to life in the oceans that is posed by the process of acidification through increased levels of carbon dioxide. From increased harmful algal blooms (HAB's) (Riebesell *et al.*, 2018), inhibition of skeletogenesis in marine arthropods (Dupont *et al.*, 2008), to disruption in neurotransmitters in marine apex predators (Doney *et al.*, 2020), a broad taxonomic spectrum appears to be threatened by ocean acidification, though within vegetated coastal habitats and different forms of marine primary production, the outcomes of ocean acidification are expected to vary greatly (see table 2).

Table 2: Examples of the projected outcomes of increased CO₂ for marine primary productivity.

Species affected	Summary	Supporting literature
Giant Kelp (<i>Macrocystis pyrifera</i>)	<i>M. pyrifera</i> is expected experience an increase in growth rates with increased CO ₂ , though the associated microbiome may be disrupted.	(Minich <i>et al.</i> , 2018)
Diatoms	Antarctic diatoms will experience reduced silicification under projected future ocean acidification, disrupting the silicon cycle.	(Petrou <i>et al.</i> , 2019)
Cyanobacteria	Greater availability of CO ₂ will result in increased toxic cyanobacteria, disrupting pelagic foodwebs.	(Riebesell <i>et al.</i> , 2018), (Errera <i>et al.</i> , 2014)
Maerl	Ocean acidification reduces availability of carbonate ions, which will result in the loss of maerl beds across the northeast Atlantic.	(Brodie <i>et al.</i> , 2014)
Seagrass (<i>Posidonia oceanica</i>)	<i>P. oceanica</i> net productivity is expected to increase at lower pH levels.	(Cox <i>et al.</i> , 2015) (Hall-Spencer <i>et al.</i> , 2008)
Seagrass (<i>Zostera marina</i>)	<i>Z. marina</i> net productivity is expected to increase at lower pH levels.	(Zimmerman <i>et al.</i> , 2017)
Calcifying epiphytes	Calcifying epiphytes are expected to see reduced productivity as pH levels lower, and will eventually completely dissolve.	(Martin <i>et al.</i> , 2008)

Clearly, ocean acidification impacts a broad spectrum of species, and this is borne out in evidence linking ocean acidification to widespread extinctions resulting from historic shifts in atmospheric CO₂ concentrations (Clarkson *et al.*, 2015; Foster *et al.*, 2016; Henehan *et al.*, 2019; Smith, 2019).

Amongst invertebrate calcifiers, increased CO₂ concentrations in seawater has been shown to hinder calcification – the process by which marine organisms such as coccolithophores and crustaceans secrete calcium carbonate (CaCO₃) to form skeletons and other calcified structures, thus reducing survival rates (Dupont *et al.*, 2008; Long *et al.*, 2013; Foster *et al.*, 2016). It may be that calcification is driven by bicarbonate rather than carbonate (at least in some species), and thus declining carbonate which is anticipated due to acidification may not be the primary pressure exerted on many invertebrates, but rather the increased concentration of H⁺. This would alter the proton gradient between seawater and the internal reservoirs of cells, making maintenance of pH homeostasis more difficult, thus disrupting membrane transport and lowering the saturation states of both calcite and aragonite (Roleda *et al.*, 2012; Cyronak *et al.*, 2016).

In terms of primary production, the influence of ocean acidification is likely to vary widely, as demonstrated in table 2. Those species that are heavily calcified such as maerl, are likely to be significantly negatively impacted (Brodie *et al.*, 2014). There may, however, be some benefit of increased CO₂ for potentially carbon-limited species such as seagrasses (Hall-Spencer *et al.*, 2008; Brodie *et al.*, 2014), as well as many species of non-calcifying macroalgae (Cornwall *et al.*, 2017), though only if the balance between this and incurred physiological costs, are balanced in their favour. For example, it has been shown that ocean acidification may lead to the loss of phenolic substances in seagrass species, which seagrasses use as a chemical defence against grazers (Arnold *et al.*, 2012). This highlights the fact that ocean acidification will likely have combined positive and negative influences over photosynthetic organisms, meaning that their ability to thrive by utilising the additional CO₂ available to them, will depend at least in part, on the balance between the two.

The future of ocean acidification remains uncertain, though projections have been made by IPCC for a number of scenarios (table 1), which largely depend on the capacity of humankind to make large cuts in global carbon emissions (IPCC, 2013). The loss of important global carbon sinks such as seagrass meadows (Russell *et al.*, 2013), the threat of climate tipping points (Lenton *et al.*, 2019) and the limitations and failures of carbon capture and storage (CCS) and other negative emissions technologies (Anderson *et al.*, 2016), are making the likelihood of effectively decreasing carbon emissions, more remote. As such, the higher-end projections for future climate change scenarios appear likely, making protection of habitats of high carbon-sequestration and storage (such as seagrass meadows), of paramount concern. Understanding the physiological responses of species such as seagrass to changes in carbon limitation anticipated due to climate change, is therefore, a vital area of research.

Carbon limitation and carbon concentrating mechanisms

Coastal ecosystems that are dense in submerged vegetation such as seagrass meadows, experience large fluctuations in pH as a result of changes in inorganic carbon (C_i) concentrations within the waters of the meadows caused by photosynthesis and respiration. All organisms inhabiting seagrass meadows (including the seagrass itself) release CO_2 via respiration 24/7, but during the day photosynthesis of seagrasses and epiphytes results in a net decrease in CO_2 which causes pH to increase. At night when photosynthesis ceases, pH begins to drop once again (Larkum *et al.*, 2005). Rates of photosynthesis can be so high, that ambient CO_2 becomes exhausted, potentially causing photosynthesis to cease altogether, but many aquatic plants have evolved physiology to overcome carbon limitation.

For terrestrial plants, the only form of C_i is atmospheric CO_2 (Beer *et al.*, 2015), but in seawater, C_i exists in four forms: carbon dioxide (CO_2), bicarbonate (HCO_3^-), carbonic acid (H_2CO_3) and carbonate (CO_3^{2-}). HCO_3^- is approximately 100 times more abundant in seawater than CO_2 (Beer *et al.*, 2015), which diffuses far slower into aquatic plants than terrestrial plants (Nayar *et al.*, 2009). Despite the abundance of these other forms of C_i , only CO_2 can be reduced via the Calvin cycle, and so aquatic plants have evolved mechanisms to capitalise on this abundance of inorganic carbon through carbon concentrating mechanisms (CCM's), which break down HCO_3^- into CO_2 (Beer *et al.*, 2015). This is an energy-costly process (Burnell *et al.*, 2014), but it ensures a continued supply of CO_2 to the site of Ribulose -1,5-biphosphate carboxylase-oxygenase (Rubisco) carboxylation, allowing photosynthesis to continue when ambient CO_2 is low or absent (Larkum *et al.*, 2017).

CCM's are prevalent and highly efficient amongst algae, but less so amongst seagrasses (Arnold *et al.*, 2012), likely the result of algae having evolved several hundred million years before seagrasses, giving them an evolutionary "head-start" (Beer *et al.*, 2015). Seagrasses evolved from land plants, which have no need of powerful CCM's since they only utilise one form of inorganic carbon (i.e. CO_2) and diffusional gas exchange via stoma is fast and efficient. Algae evolved in aquatic environments, and thus evolved CCM's much earlier than seagrasses, which likely explains why they typically have highly effective CCM's.

Unlike CO_2 , HCO_3^- cannot diffuse across cell membranes in plants, and therefore must first be broken down to CO_2 to allow for uptake of C_i for photosynthesis. There are three ways in which this can be accomplished by aquatic plants, depending on the type of CCM that they possess (see figure 4). Firstly, the enzyme carbonic anhydrase can be secreted to the diffusion boundary layer (DBL), and then used to catalyse the breakdown of HCO_3^- into CO_2 . Secondly, H^+ can be released by the plant leaves to the DBL resulting in

very localised pH reductions which allows HCO_3^- to be broken down in to CO_2 and H_2O . Finally, some plants may have the capacity to utilise active pumps that move HCO_3^- across the cell membrane.

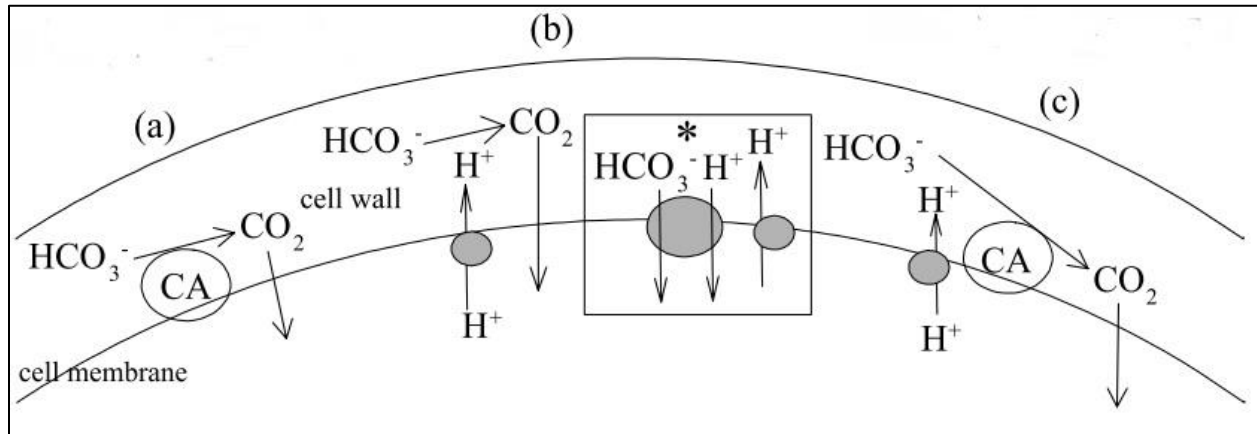


Figure 4: Carbon concentrating mechanisms for seagrasses have been theorised to function one of three ways: (a) catalysing conversion of HCO_3^- to CO_2 by use of the enzyme carbonic anhydrase; (b) H^+ being released via cellular membranes to the DBL, creating acid zones that break down HCO_3^- which then releases CO_2 ; (c) a combination of both acidic zones in the DBL and the use of carbonic anhydrase. CO_2 that is released as a result of CCM's then diffuses across cell membranes to rubisco sites for photosynthesis (Beer *et al.*, 2006). (Permission to reproduce this figure has been granted by the copyright holder)

Thanks to the presence of CCM's, seagrass meadows and their epiphytic communities are able to continue to photosynthesise, even if ambient CO_2 is exhausted. In fact this process may be highly beneficial for calcifying algae species that grow as epiphytes on seagrass, which have been shown to calcify and photosynthesise at higher rates in the presence of seagrasses (Semesi *et al.*, 2009). The process of calcification in calcifying algae releases CO_2 which is then utilised by seagrasses for photosynthesis. This means that even when pH is high and ambient CO_2 is low, seagrasses have a source of CO_2 from increased calcification rates in calcifying epiphytes.

Undoubtedly, there are other complex ecological interactions between seagrasses and the epiphytic communities that they support, which form part of the dynamic and biodiverse ecosystems of which they are the basis, but these ecosystems are currently in global decline.

1. Seagrasses and their global decline

Seagrasses are found in every continent around the globe with the exception of Antarctica, and form expansive meadows that support high biodiversity in shallow coastal waters (see figure 5). These meadows typically occur down to around 20m in depth as they require sufficient light for photosynthesis,

though some species tolerate depths of up to 70m (Avery *et al.*, 2014). Seagrasses are well adapted to varying light conditions that exist in marine environments, and are capable of maintaining photosynthetic activity across a broad range of light conditions. They are aided in this by various mechanisms which allow them to optimise interception of light via canopy architecture, chloroplast distribution, and thylakoid membrane organisation (Ralph *et al.*, 2005). Seagrasses also require soft sediments for anchoring in, with enough wave energy to prevent them from becoming buried, and either no, or very brief periods of exposure to prevent damage (Avery *et al.*, 2014).

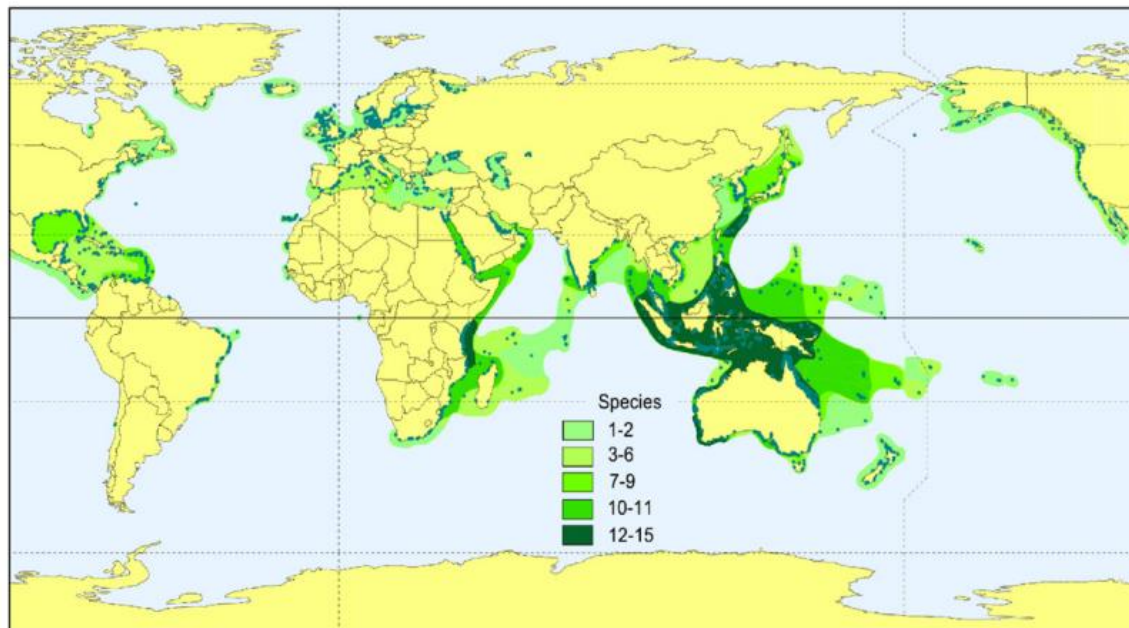


Figure 5: Global mapping of seagrass distribution (darker regions with higher species diversity) shows the north and south-west coasts of Australia to be highly biodiverse in terms of seagrass distribution (from Short *et al.*, 2007) (Permission to reproduce this figure has been granted by the copyright holder)

Seagrass habitats are amongst the most productive habitats in marine ecosystems with high rates of photosynthesis and primary productivity, with a significant contribution (in some meadows, >50%) made by the epiphytic communities that grow on seagrasses (Larkum *et al.*, 2005). Seagrass meadows support a wide diversity of life in their varied roles as nursery grounds for fish, substrate for some anemone species, grazing habitats for turtles, dugongs and manatees, and hunting grounds for many species of shark (Aragones *et al.*, 2006; Heithaus *et al.*, 2013; Brooker *et al.*, 2019). In addition, seagrass beds help to dissipate wave force, reduce turbidity and sequester and store vast amounts of carbon (Orth *et al.*, 2006), as well as cycling nutrients and stabilising sediments (Avery *et al.*, 2014). As such, seagrasses have

a profound influence over the community structure and composition of coastal ecosystems in which they occur (Avery *et al.*, 2014).

The evolutionary “roots” of terrestrial plants lay with marine algae, from which they evolved ~850 million years ago (Knauth *et al.*, 2009). Seagrasses evolved from terrestrial plants that migrated back in to the oceans around 70 million years ago, and vary widely in their biology (Les *et al.*, 1997). For example, seagrasses from the *Amphibolis* genera reproduce by growing seedlings on adult plants which are released, and hook on to rhizomes and substrates upon which they anchor, whereas *Ruppia* genera release flowers on a spiraled stalk, which are pollinated above the water’s surface (Avery *et al.*, 2014).

Globally, seagrass meadows are in decline, with annual losses of approximately 110 km² per year since 1980 (Waycott *et al.*, 2009), and the UK may have lost more than 90% of historic seagrass meadows due to anthropogenic disturbance (Green *et al.*, 2021). This decline is the result of a combination of anthropogenic factors such as pollution from land runoff, coastal development, fishing practices that disturb and damage benthic habitats, and the wasting disease *Labyrinthula sp.* (Jackson, 2001; Orth *et al.*, 2006; Waycott *et al.*, 2009). Whilst wasting disease is not directly caused by anthropogenic activity, it has been shown that the disease usually infects parts of seagrass blades that are typically fed on by large grazers such as turtles, dugongs and manatees, all of which have significantly declined across the globe due to historic exploitation and habitat loss. As such, it is likely the global impact of wasting disease was exacerbated by the loss of these species (Jackson, 2001).

Climate change is beginning to play a significant role in seagrass decline globally, particularly when marine heatwaves result in water temperatures beyond thermal tolerance thresholds of seagrasses. This was observed during the 2010-11 marine heatwave off the west coast of Australia (Kendrick *et al.*, 2019; Strydom *et al.*, 2020). Prior to the 2010-11 heatwave, seagrass cover had, in fact, been increasing in some areas of the western coast of Australia. In Shark Bay, approximately 800km north of Perth, seagrass cover had increased by ~313km² in the 8 years prior to the heatwave, but reduced by approximately 1,069km² as a result of die-off from thermal stress experienced during the heatwave (Strydom *et al.*, 2020). The reduction in seagrass area following the heatwave also resulted in a change of seagrass density, with total seagrass cover consisting of 70% dense meadows and 29% sparse prior to the heatwave, and 53% dense, 46% sparse following the heatwave (Strydom *et al.*, 2020). It’s noteworthy that this occurred in a world heritage area that is relatively remote and well protected from many of the anthropogenic stressors that other seagrass meadows are frequently exposed to.

Orth *et al.*, (2006) discussed the lack of public awareness and focus within marine biological research on seagrass beds, which seemed at odds with their global importance. Since this time, recognition of the significance of seagrass beds as hotspots of biodiversity and carbon sequestration has led to a surge in research and restoration efforts focused on halting and reversing the decline of seagrass meadows (Tanner, 2014; van Katwijk *et al.*, 2016), though there still appears to be a great disparity within news media on the focus of terrestrial habitats over marine habitats for climate change mitigation. Recognition of this decline and the value of seagrass habitats for both biodiversity and carbon storage, have led to better protection and restoration efforts, but this has been both selective and limited. For example, restoration efforts are well-documented in the scientific literature in wealthy western nations such as Sweden, Australia and the UK (Tanner, 2014; Unsworth *et al.*, 2019), though projects in poorer countries to restore meadows are in place, but are likely under-represented in the literature. In many parts of the world, however, particularly where seagrass meadows are poorly mapped and environmental protection is limited, the rate of change in seagrass habitat area size remains unknown (Waycott *et al.*, 2009). It is worth noting however, that even in western countries, protection of marine habitats is often inadequate. In the UK, it was hoped that the creation of marine protected areas (MPA's) would create safe zones where marine habitats are protected from harmful fishing practices, but 97% of the UK's MPA's are still being dredged and bottom-trawled (McVeigh, 2020), leading some environmental commentators to refer to MPA's as "paper parks", that offer no significant protection (Monbiot, 2014). Similarly, in the Gulf of California, the creation of >23,000km² of MPA's has largely failed to meet sustainability or conservation goals (Rife *et al.*, 2013). Marine environments have long suffered from the old adage, "out of sight, out of mind" (Braid, 2005), and whilst the goals of MPA's may be admirable, lack of enforcement of regulations, poor management and budget shortfalls have the potential to create a false sense of security via a system of protections that falls significantly short of achieving what is needed (Rife *et al.*, 2013).

Global seagrass coverage began to decline long before the creation of MPA's, though it is difficult to determine by exactly how much. Seagrass meadows have declined since exploitation of the oceans began, yet mapping of seagrass meadows is, even now, limited. It is likely however, that as much as 51,000 km² of seagrass habitat has been lost in the last 127 years (Waycott *et al.*, 2009), an area more than twice the size of the nation of Wales. Seagrass meadows may have already declined significantly prior to this time, since their health is intrinsically linked to species that have been harvested en-masse for centuries. Grazing of seagrass beds by species that are now endangered such as green turtles (*Chelonia mydas*) (Seminoff, 2004) helps maintain the health of seagrass by altering the flow of nitrogen and detritus within seagrass meadows (Jackson, 2001). This alters the microbiology of seagrass beds, preventing over-

abundance of epibiontic species such as cyanobacteria, which can smother seagrass beds in eutrophic environments (Paerl *et al.*, 2013). Accurately measuring what impact widespread ecological change may have had on seagrass meadows in the past is challenging, particularly given that seagrass does not fossilise well (Jackson, 2001), but it may be that the current global decline, may significantly predate the 51,000 km² lost in the last 127 years, particularly given that grazing species instrumental to the health of seagrass meadows, have been exploited for at least 800 years (Van Houtan *et al.*, 2014).

As such, restoring and protecting seagrass habitats is a significant global challenge, and whilst restoration efforts for seagrass have shown that success is possible, it is also slow, costly (as much as \$3m per hectare in some instances (Tanner, 2014)), and comes with a set of challenges unique to working in marine environments such as costly equipment, and violent seas. Almost half of seagrass restoration efforts fail completely with 0% recruitment of new seagrass by the end of the study period. Many of the reported successes do not monitor survival of seedlings long enough to be conclusive, and many of these successful experiments are conducted on very small scales, <10m² (Cunha *et al.*, 2012). Viewed in isolation, failure at this scale is discouraging, but in the broader context of the scientific process, these failures have helped to create a large pool of knowledge on what influences success and failure of seagrass restoration attempts, and restoration efforts are now more successful and cost-effective, as has been seen in several studies in recent years.

Off the coast of Adelaide in South Australia, seagrass beds of *Amphibolis antarctica* have declined by approximately 5,000 ha due to a decline in water quality (Tanner, 2014). Attempts to restore this bed by transplantation failed, but recent success in using hessian sacks for seedlings to attach to, has offered one possible method for restoring seagrass beds of varied species in other parts of the world. This has the benefit of being low-cost and highly effective in recruitment and long term survival of *A. antarctica* seedlings, whose comb-like root structure evolved specifically to help them anchor to substrates after detaching from the parent plant. In Chesapeake bay, an 11-year restoration effort for eelgrass (*Zostera marina*) meadows that had been absent for 70 years, exceeded expectations, after attempts to create plots of 125ha, resulted in the restoration of seagrass plots totaling 1,700ha (Orth *et al.*, 2012). Whilst this is encouraging, restored habitats seldom if ever, have the same ecological value in terms of productivity biodiversity and nutrient cycling as well-established, natural habitats that have been created under centuries or millennia of natural ecological interactions and processes (Hilderbrand *et al.*, 2005). Natural systems are inherently complex, and there are risks in presuming that ecological restoration can create habitats that are as complex and well-functioning, or that any researchers, no matter how knowledgeable,

can accurately decide on what was natural, and should therefore be the goal of a restoration project. Additionally, any loss of habitat and subsequent recovery, will naturally carry with it a recovery debt due to the loss of ecological functioning and interactions between the destruction of that habitat, and the complete recovery (López-López *et al.*, 2017). Ecological restoration is effectively an attempt to create something on short timescales that would naturally take centuries or millennia, and as such, there is an inevitable loss of natural complexity in restored ecosystems. For example, some species of coral can live for more than 4,000 years (Girard *et al.*, 2018), and the loss of one individual has incalculable repercussions in terms of the genetic contribution of that individual to the species gene pool. This means that protection of existing habitats is always preferential over allowing habitats to be lost and subsequently restoring them, or offsetting the destruction of a habitat by creating a new one nearby.

Whilst restoration methodology for seagrass is improving, seagrass is still in global decline, and without concerted efforts to halt this decline, restoration efforts are ultimately doomed to the same fate as Sisyphus (Hilderbrand *et al.*, 2005). Thus, protecting seagrass habitats is of key concern, and understanding the plight of these habitats in light of global change, particularly ocean warming and acidification, is of critical importance.

2: *Amphibolis antarctica* and associated calcifying epiphytes

2.1 Seagrass – *Amphibolis antarctica*

The coastlines of Australia are characterised by highly productive coastal ecosystems which support high levels of biodiversity (Wernberg *et al.*, 2011; Evans *et al.*, 2017). They are also one of the most abundant and biodiverse regions in terms of seagrass distribution, particularly along the northern and south-western coasts.

Almost half of all seagrasses are found in southern temperate oceans, with the seagrass diversity on the south-western coast of Australia, being amongst the highest in the world. 36 out of the world's 72 species of seagrass occur there, encompassing all but one genera of seagrass (Avery *et al.*, 2014). The seagrass beds of south-west Australia are environmentally and commercially important habitats that support both ecotourism and commercial fisheries (Blandon *et al.*, 2014), and provide breeding and feeding grounds

for huge assemblages of marine fauna (Evans *et al.*, 2017). Seagrasses typically exhibit high rates of photosynthesis, and can alter the carbonate chemistry of the waters in which they grow, reducing CO₂ concentrations and increasing pH via photosynthesis during the day (Larkum *et al.*, 2005). This can be highly beneficial for calcifying organisms, and is likely one reason why calcifying algae are typically abundant as epiphytes in seagrass meadows, particularly in *Amphibolis* meadows (Larkum *et al.*, 2005), and the capacity of seagrass meadows to increase local pH has been shown to mitigate the influence of ocean acidification for calcifying algae species (Bergstrom *et al.*, 2019).

Amphibolis antarctica is endemic to southern and western temperate coasts of Australia, forming both mono-specific and mixed-species meadows (though predominantly mono-specific (James *et al.*, 2009)), often in environments of high tidal energy with sandy substrates (Avery *et al.*, 2014), where the wiry tough stems and leaf clusters help to reduce water motion and stabilise the sediments in which they grow (van Dijk *et al.*, 2018). Meadows of *Amphibolis* genera seagrass, may support in excess of 100 species of invertebrates, and more than 70 species of fish, making them particularly rich in biodiversity (Avery *et al.*, 2014). *Amphibolis* species (of which there are only two extant species) are exclusively temperate-water species. They are dioecious, and reproduce by vivipary, with seedlings growing on adult plants, eventually detaching and using comb-like hooking structures at the basal end (see figure 6), to snag rhizomes or substrate upon which they settle (Avery *et al.*, 2014; Tanner, 2014). Given that *A. antarctica* meadows typically occur in high tidal-energy zones, this comb-like structure is likely to be critical in settlement and survival of seedlings, and may have evolved in response to this pressure (Verduin *et al.*, 2013).



Figure 6: Seedlings of *A. antarctica*, with the comb-like hooking structure (circled) clearly visible (Verduin *et al.*, 2013) (Permission to reproduce this figure has been granted by the copyright holder)

Amongst seagrass species, this form of reproduction is unique to *Amphibolis* and *Thalassodendron* genera species, and this characteristic has aided some restoration efforts, through the use of sand-filled hessian sacks as substrate upon which seedlings hook and settle, and has shown to be effective and cost-efficient. This is a significant finding, given the cost and ineffectiveness of many seagrass restoration efforts (Cunha *et al.*, 2012; Tanner, 2014).

Amphibolis antarctica, like most species of seagrass, has declined significantly over the past 127 years (Waycott *et al.*, 2009), with more than 5,000ha of seagrass (predominantly *Amphibolis* species) having been lost off the coast of Adelaide since the 1930's, largely due to a decline in water quality caused by increased nutrient loading and sediment flow which led to increased turbidity and limited light availability for photosynthesis (Tanner, 2014).

Whilst declining water quality and subsequent turbidity is a significant threat to seagrasses globally, this is by no means the only threat that seagrasses face. Coastal waters are becoming warmer and more acidic as a result of climate change, and a great deal of uncertainty exists as to how seagrasses will respond to this continued change. Research in to the responses of species such as *A. antarctica* which form vital carbon sinks and hotspots of biodiversity is critical, in order to understand what influence climate change

is likely to exert on seagrass meadows. It is also important to consider that within seagrass meadows, seagrass itself is only one facet of a complex and dynamic system which includes significant contributions to primary production (sometimes 80% of total) from a diverse community of phytoplankton and epiphytes (Borowitzka *et al.*, 2005).

2.2 Calcifying algae

Calcifying algae are species of macroalgae that deposit calcium carbonate (CaCO_3) as a means of protecting themselves against intense tidal energy and many grazing species. Due to their hard calcified structure, they are prevalent in the fossil record, which indicates that they date back to Precambrian times (>600mya) (Denny, 2008). They are widespread across subtidal and intertidal areas (generally the lower intertidal area, since they are susceptible to desiccation (Denny, 2008)), from arctic coasts, to tropical reefs, where they act as ecosystem engineers, creating large areas of substrate for a vast diversity of hard and soft corals as well as anemones, hydroids and other reef-dwelling species (Denny, 2008). Calcifying algae live as long as 1,000 years, and grow on a wide diversity of substrata, both natural and anthropogenic, making the most of the abundance of different surfaces available for settlement and colonisation in marine ecosystems (Larkum *et al.*, 2005; Beer *et al.*, 2015).

In marine habitats, any available surfaces are colonised rapidly by a wide diversity of organisms from sponges and hydroids (Larkum *et al.*, 2005), to biofilms that are ubiquitous in marine environments (Haynes *et al.*, 2007; Sawall *et al.*, 2012). In the case of biofilms, it takes as little as an hour for settlement to begin when a new surface is available. This has been demonstrated in the case of plastic pollution, leading researchers to coin the term “the plastisphere” for life that has been shown to thrive upon the massive quantities of plastic waste in the ocean (Amaral-Zettler *et al.*, 2020).

Seagrasses provide an excellent substrate available for colonisation by a multitude of organisms including non-geniculate calcifying algae species that grow as epiphytes. Although seagrass blades die off over time, seagrasses are constantly growing and renewing, providing an ever-refreshing surface for colonisation (Larkum *et al.*, 2005). Photosynthetic organisms growing on plants are broadly termed “epiphytes”. In seagrass meadows, these epiphytes are a crucial and dynamic component of the ecosystem, and play a number of critical ecological roles such as primary production, stoichiometric flow, the storing of nutrients and in the case of bacteria within the rhizosphere, nitrogen fixation (Borowitzka *et al.*, 2005). In kelp

forests, microbiomes have been shown to provide micronutrients such as B12, and to release antibiotic compounds that inhibit the settlement of detrimental bacteria (Minich *et al.*, 2018). It is likely that these benefits are not unique to the microbiomes of kelp, and similar benefits may exist for the microbiome of seagrasses (Wilkins *et al.*, 2019). Calcifying epiphytes may also assist seagrasses by releasing CO₂ during the process of calcification, meaning that they provide localised releases of CO₂ that may benefit seagrasses, particularly when ambient CO₂ has been exhausted, which can be the case in dense seagrass meadows when environmental conditions are optimal for high rates of photosynthesis (Beran, 1995).

Within healthy seagrass systems, epiphytes have no detrimental effect and may in fact confer many benefits, but the opposite can be true in unhealthy systems, such as polluted meadows that have become eutrophic, causing epiphytes (particularly cyanobacteria) to bloom, overgrowing seagrass and inhibiting photosynthesis (Larkum *et al.*, 2005). This is of particular concern within climate change research, since increased CO₂ is likely to benefit microbial primary producers that typically bloom and smother other species (e.g. cyanobacteria) and can be exacerbated by the type of trophic downgrading that is being seen globally as biodiversity continues to decline (Houk *et al.*, 2012; Harvey *et al.*, 2021)

For calcifying epiphytes, a reduction in availability of carbonate ions and an increase in acidity, is likely to be highly detrimental, and it has been suggested that the likely outcome will be a shift from calcified epiphytes within seagrass meadows to diatoms and filamentous algae species (Brodie *et al.*, 2014).

This study seeks to assess the influence of acidification and carbon limitation on the photophysiology of calcifying epiphytes and the host plant *Amphibolis antarctica*, to improve upon existing knowledge of carbon concentrating mechanisms, and the influence that climate change and increasing levels of ocean CO₂ are likely to exert on both species.

3. Design, methods results

3.1 Methodology

3.1.1 Sampling site and collection of specimens

A total of 45 healthy specimens of *Amphibolis antarctica* (those exhibiting strong colour, no broken leaves or spots, with intact roots and rhizomes) and approximately 200g of *Ulva* sp. (for pH manipulations) were collected at Mindarie, Perth SW Australia (figure 6) on two occasions (2015-03-05 and 2015-03-17 between 10:00 and 11:00 am) by snorkelling. The collection meadow was at approximately 2 metres depth. Photosynthetically active radiation (PAR) was approximately $50 \text{ umol photons m}^{-2} \text{ s}^{-2}$ (measured on a bright day with low cloud cover) at the growth site, established by the light sensor of a pulse amplitude modulated (PAM) fluorometer (Diving PAM, Walz Germany (Walz - Germany, 2021)). The specimens were transported to the laboratory at Edith Cowan University, in buckets filled with seawater collected at the site. Upon arrival at the university, specimens were cleaned and placed in aerated aquaria with filtered seawater. Temperature in the aquaria was set at 22.5°C (the average in-situ temperature at the collection site). Lights were provided by LED luminous tubes with a PAR of $50 \text{ umol photons m}^{-2} \text{ s}^{-2}$, with a light/dark cycle of 12:12 hours, as per the sampling site.



Figure 7: Location of the sampling sites (A&B), Mindarie (location: $31^{\circ}41'42.4''S$, $115^{\circ}42'06.6''E$), off the south-west coast of Australia (googlemaps, 2021), and the habitat where samples were taken for this study (C (Rasmusson, 2015)).

3.1.2 Pulse Amplitude Modulation (PAM) Fluorometry

Electron transport rates were measured using an Imaging PAM ((Walz - Germany, 2021)), a methodology which is recommended under the European Commission's best practice guidelines for research on changes in photosynthesis resulting from ocean acidification (Riebesell *et al.*, 2011). This methodology is both non-invasive and highly reliable for assessing relative changes in photosynthesis, by analysing quantum yield (carbon assimilated : photons of light absorbed) and electron transport rates in plants (Schreiber, 2007; Iluz *et al.*, 2013).

When a photon of light energy strikes a photosynthetic organism, there are three possible outcomes for that photon. Firstly, it may be absorbed and used as energy for photosynthesis; secondly it may be emitted as heat, and lastly, the photon may be absorbed by chlorophyll molecules and re-emitted at a longer

wavelength – a phenomenon known as fluorescence. Chlorophyll fluorescence carries with it, reliable quantitative data, which can provide information on the physiological state (i.e. health) of any photosynthetic organism, such as higher plants, algae (including zooxanthellae in corals) and phytoplankton (Schreiber, 2007). PAM fluorometry utilises a 1µs pulse of light, synchronised to a lock-in amplifier. The amplifier removes any signals not associated with the lock-in signal, which means that PAM fluorometry can be conducted without interference from ambient light sources. Photodiodes are used to detect and measure fluorescence, data which the fluorometer uses to measure the status of photosystem II (PSII) and the flow of electrons from PSII to photosystem I (PSI) (Ralph *et al.*, 2005).

PAM fluorometry can be used to run rapid light curves (RLC's) on plants, a technique which uses a series of short irradiances, each at a different intensity lasting approximately 10s each. RLC's measure the effective quantum yield of a plant over different irradiances, which can be used to calculate rETR (the rate at which electrons are pumped through the electron transport chain from PSII to PSI (Ralph *et al.*, 2005). Irradiances used for RLC's are typically determined by what is realistic for the environment that a plant is adapted to. For example, there would be little use in running a rapid light curve with irradiances as high as PAR 2000, for plants that grow exclusively in shaded environments. The data collected from rapid light curves can be used to assess photosynthetic capacity over a wide range of light intensities, emulating the fluctuations in irradiance that a plant typically experiences, and gives insight in to how well a plant can tolerate fluctuations in light intensity (Ralph *et al.*, 2005). RLC's are a particularly powerful tool to assess photosynthesis in seagrasses, as seagrass meadows experience wide fluctuations in light intensity on both short and long term temporal scales (Ralph *et al.*, 2005).

3.1.3 Experimental set-up

Leaves of approximately the same size and epiphyte cover (encrusting calcifiers) were used. For pH 7.6, 8.18 and the two first leaves of pH 8.8, the first batch of collected seagrass were used and the rest with the latter collection. pH was established using a hand held multimeter with a pH probe (pH probe: SenTix 940 connected to WTWMulti3430; WTW, Weilheim, Germany (WTW, 2021)). pH was adjusted using the photosynthesis and respiration of *Ulva* sp. placed in glass bottles filled with seawater. Increased pH was achieved by CO₂ removal via the high photosynthetic activity of the algae. Light sources were placed around the sample bottle to enhance photosynthesis and fans were used to cool the samples, preventing boiling of algae. To achieve lower pH the bottles with *Ulva* were kept in darkness and left to respire and

thus release CO₂. When desired pH was reached, the samples were sieved to remove plant material and then cooled off to 22.5° C, ambient mean temperature at the collection site used throughout the experiments.

A glass container filled to the rim with seawater of adjusted pH, enclosed by a lid to prevent CO₂ exchange with the air, was used during the experiments. A whole leaf was fixed with thread on plastic mesh kept in place by blue tac over the measuring head of the Imaging PAM. Constant water movement to reduce diffusive boundary layers was provided by a magnetic stirrer. In the Win Control computer program (Walz - Germany, 2021) six areas of interest (AOI) were chosen for areas with encrusting epiphytes and six AOI for pure seagrass on the fixed leaf (see figure 8). Encrusting epiphytes were species of Rhodophyta, but could not be identified to species level without molecular analysis, which was beyond the scope of this study.

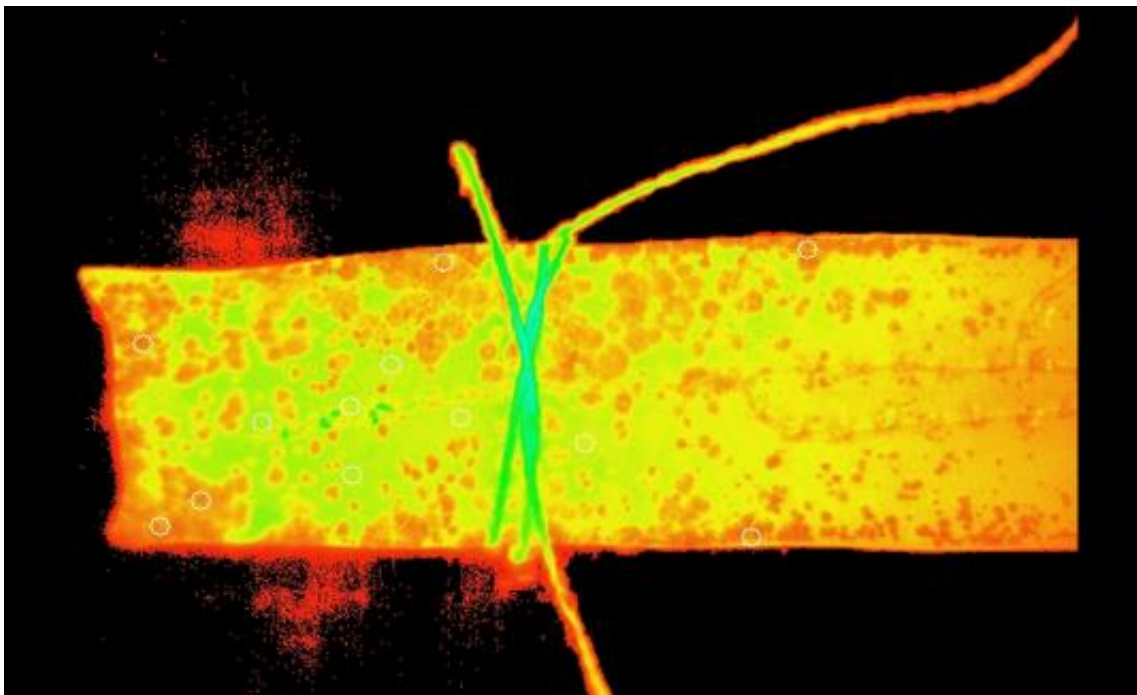


Figure 8: One of the *Amphibolis antarctica* leaves used during this study, with areas of interest (AOI's) denoted by white circles

Rapid light curves were then conducted using the following light intensities: 1, 21, 41, 76, 134, 205, 249, 298, 371, 456, 581 and 726 mmol photons m⁻² s⁻². Each intensity was used for 20 s before

saturating pulse was provided = 240s in total. For each pH, six randomised leaves were examined. pH, temperature and O₂ were measured before and after the experiments with handheld Multimeter. Mean values of the six AOI was calculated, in order to ensure that the photosynthetic variability in the different parts of the leaf was covered. The mean relative electron transport rate (rETR) for each leaf was then calculated for each irradiance (n=6, however only 5 leaves were used for pH 7.61 due to an experimental error). The rETR, a measure of the efficiency of electron transport through photosystem II (PSII) to photosystem I (PSI), was calculated as:

$$Y \times PAR \times 0.5$$

where Y is the effective quantum yield, indicating the working capacity of the photosynthetic apparatus, PAR is the photosynthetic active radiation and 0.5 is an assumption that half of the photons absorbed are going to PSII and half is directed towards PSI. In ETR measurements an absorption factor (AF) is usually used (an indication on how much of the incoming light is absorbed by the leaf and how much is shining through). Due to the difficulty to establish AF in the highly encrusted calcifying epiphytes, AF was omitted and relative electron transport rates are hence presented.

3.2 Statistical analyses & hypotheses

Three tests were performed on the data. First, data were tested for normality using the Ryan-Joiner method, and then via a two way ANOVA general linear model (GLM) and Bonferroni post-hoc pairwise analysis (95% confidence) for both seagrass and calcifying epiphytes, to test for significant differences in electron transport rates at different pH levels. Data for these tests were log¹⁰ transformed as the data were not normally distributed.

Secondly, data were tested via a polynomial linear regression to determine whether or not there was a statistically significant relationship between pH and electron transport rates for both seagrass and calcifying epiphytes. For this analysis, data for PAR at 41 mmol photons m⁻² s⁻¹ was used, since this most closely matched that of the site where samples were collected (50 mmol photons m⁻² s⁻¹ at sampling site).

Finally, the data were tested using a two-sample t-test to assess whether or not electron transport rates were significantly different between *A. antarctica* and calcifying epiphytes. All statistical analyses were performed in Minitab version 19 (©2020 Minitab).

The following hypotheses were made:

H1: Seagrass electron transport rate will increase at lower pH values with more CO₂ available and decrease at high pH values as CO₂ availability declines, thus inhibiting photosynthesis.

H2: Calcifying epiphyte electron transport rate will decrease at lower pH values with more CO₂ available as calcification is inhibited and metabolic costs incurred will have a negative impact on photosynthetic efficiency, and electron transport rate will increase initially towards higher pH then decline as CO₂ availability limits photosynthesis.

H3: Seagrass will demonstrate higher tolerance of lowered pH than calcifying epiphytes, since they are not a calcifying species.

H4: Calcifying epiphytes will demonstrate higher rETR than seagrass at higher pH treatments, given their differences in evolutionary history with calcifying epiphytes having evolved CCM's long before seagrasses.

3.3 Results

3.3.1 Seagrass results

Statistical analysis of the data revealed that electron transport rates of *A. antarctica* were significantly different across a variety of pH treatments ($p < 0.01$, Tables 3 and 4, and figure 9). Electron transport rates were highest at pH 8.18, and lowest at pH 9.0 and 9.65. Reduced pH levels of 7.81 and 7.61 resulted in significantly lower electron transport rates than those at current ocean pH of 8.18.

Table 3: Results of a two-way ANOVA (GLM) on the effects of pH, Irradiance and pH*Irradiance on electron transport rates in *A. antarctica*, showing that both pH and irradiance cause significant changes in electron transport rates, and that there is an interactive effect between the two

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	6	12.284	2.04735	29.43	0.000
Irradiance	11	57.415	5.21953	75.03	0.000
pH*Irradiance	66	8.758	0.13270	1.91	0.000
Error	408	28.381	0.06956		
Total	491	106.715			

Table 4: Results of a Bonferroni pairwise analysis (95% confidence) of the two-way ANOVA (GLM) results shown in table 3. Means that do not share a letter are significantly different.

pH	N	Mean	Grouping
8.18	72	0.742898	A
8.48	72	0.565936	B
7.81	72	0.552804	B
7.61	60	0.516447	B
8.80	72	0.467874	B C
9.00	72	0.338688	C D
9.65	72	0.219612	D

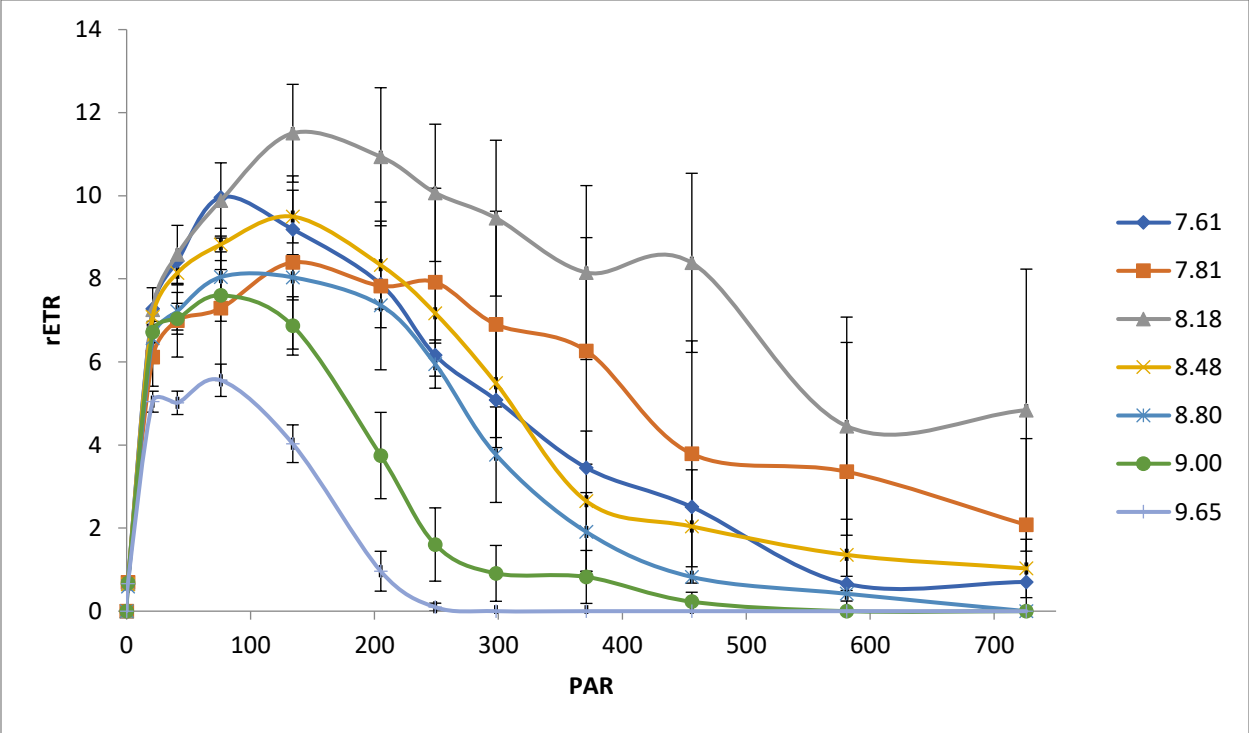


Figure 9: Mean relative electron transport rate of seagrass samples for each pH treatment with standard error.

A polynomial linear regression analysis confirmed a statistically significant relationship between pH and relative electron transport rates (rETR) for *A. antarctica* ($p < 0.05$), with the model showing that rETR declines as pH increases above current ocean pH, and as pH falls below current ocean pH levels (figure 10).

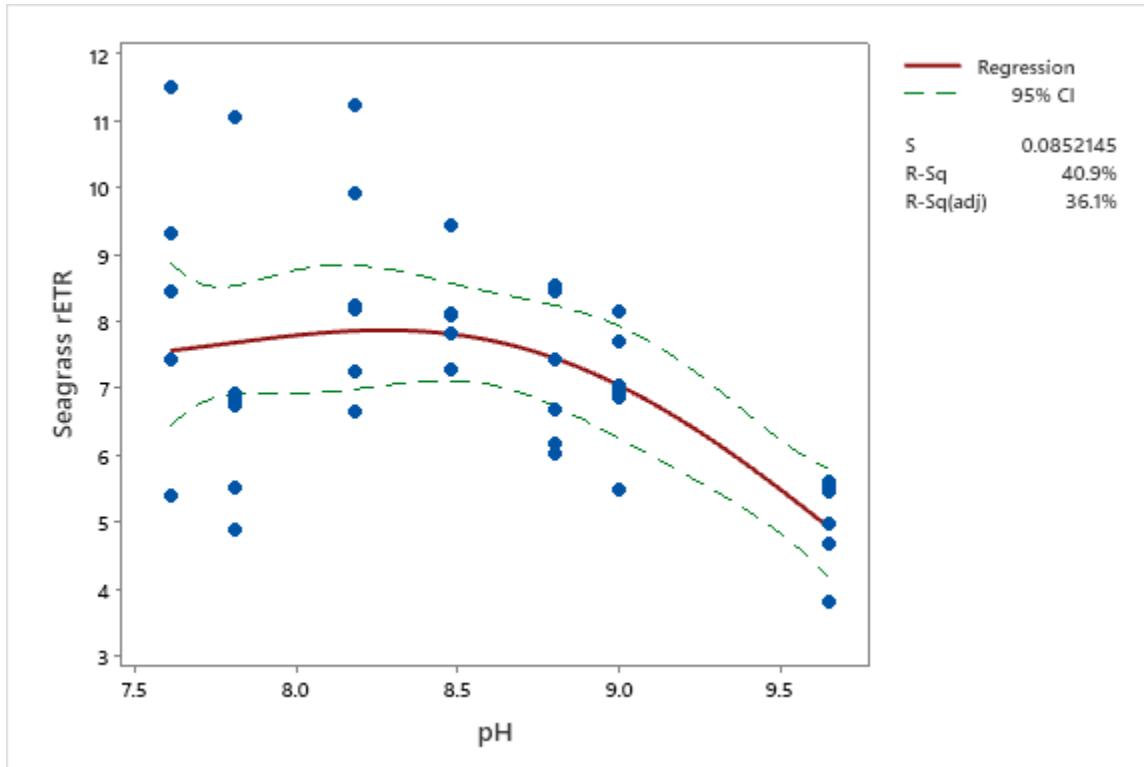


Figure 10: Polynomial linear regression utilising PAR 41 data including 95% confidence intervals, showing the relationship between seagrass electron transport rate (rETR) and pH.

3.3.2 Calcifying epiphytes results

Relative electron transport rates of calcifying epiphytes were significantly different across a variety of pH treatments ($p < 0.01$) (tables 5 and 6, and figure 11). The highest mean electron transport rates occurred at pH 8.18, 8.80 and 8.48, and the lowest rates occurred at 7.61, though electron transport rates at 7.81 were significantly lower than those for current ocean pH 8.18.

Table 5: Results of a two-way ANOVA (GLM) on the effects of pH, Irradiance and pH*Irradiance on electron transport rates in calcifying epiphytes, showing that both pH and irradiance cause significant changes in electron transport rates, and that there is an interactive effect between the two.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	6	71.619	11.9365	230.69	0.000
Irradiance	11	2.335	0.2122	4.10	0.000
pH*Irradiance	66	57.567	0.8722	16.86	0.000
Error	408	21.111	0.0517		
Total	491	152.532			

Table 6: Results of a Bonferroni pairwise analysis (95% confidence) of the two-way ANOVA (GLM) results shown in table 5. Means that do not share a letter are significantly different.

pH	N	Mean	Grouping
8.48	72	1.39624	A
8.18	72	1.31450	A
8.80	72	1.29506	A
7.81	72	1.07457	B
9.00	72	1.02857	B
9.65	72	0.81164	C
7.61	60	0.14025	D

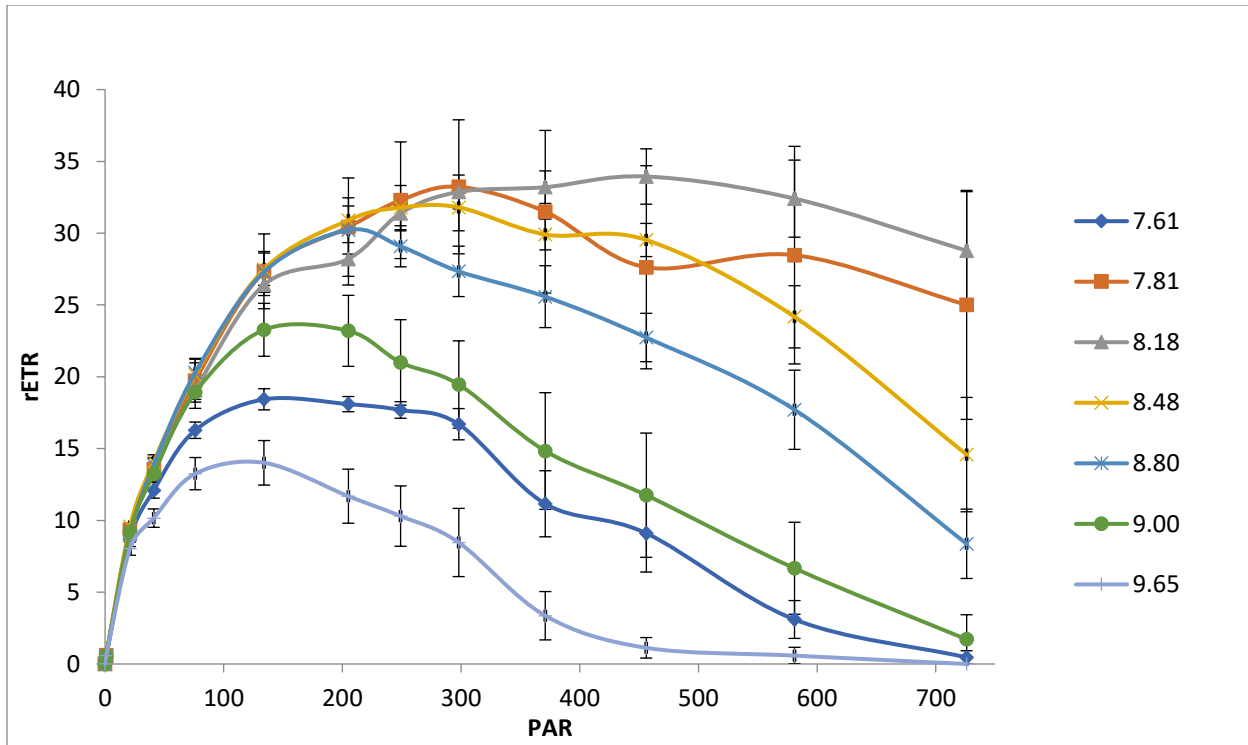


Figure 11: Mean electron transport rate of calcifying epiphytes for each pH treatment with standard error.

A polynomial linear regression analysis confirmed a statistically significant relationship between pH and relative electron transport rates (rETR) for calcifying epiphytes ($p < 0.01$), with the model identifying a significant drop in rETR as pH values either decrease from current mean ocean pH levels (8.18), or increase beyond pH 8.5 (figure 12).

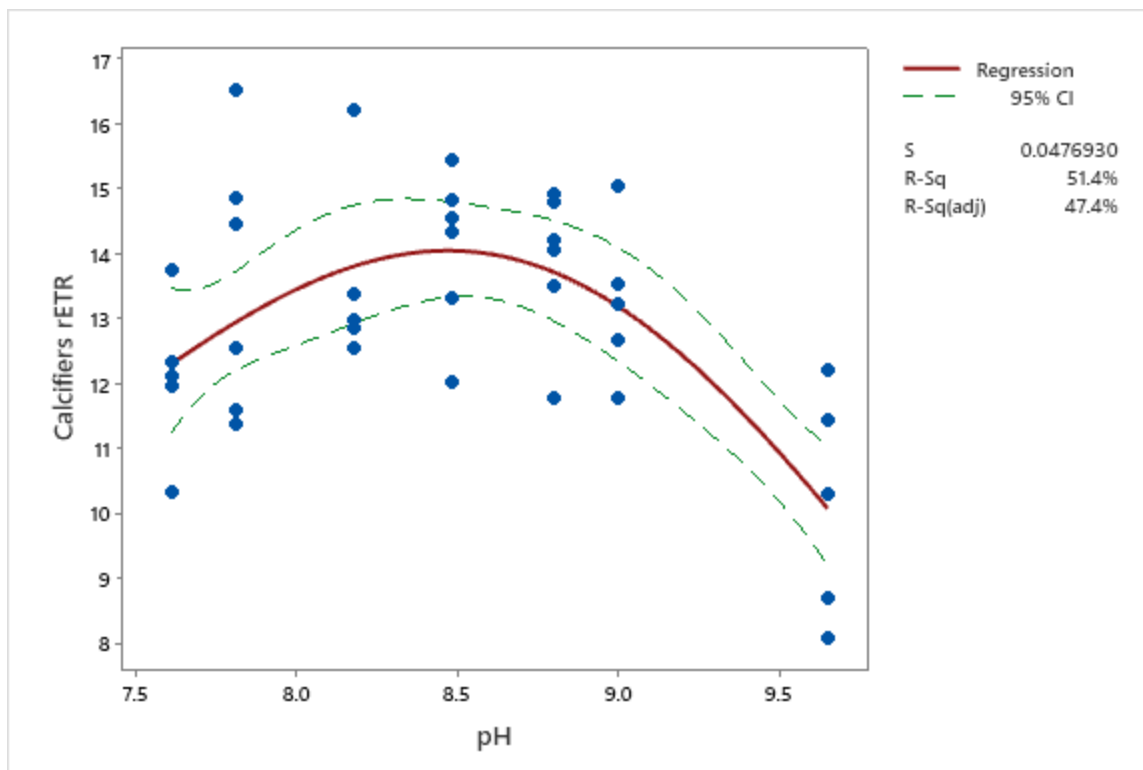


Figure 12: Polynomial linear regression including 95% confidence intervals, showing the relationship between calcifying epiphyte electron transport rate (rETR) and pH.

3.3.3 Two-sample T-test

The results of a two-sample T-test confirmed a significant difference ($P < 0.01$, $T = 6.63$) between electron transport rates of *A. antarctica* and calcifying epiphytes across the range of treatments. Calcifying epiphytes showed higher electron transport rates than *A. antarctica*, particularly at higher pH treatments where rETR of epiphytes was more three times that of *A. antarctica* at some light intensities, though with greater variation in rETR. Calcifying epiphytes also exhibited higher light intensity tolerance than *A. antarctica* at the highest pH treatment (9.65) where rETR crashed for *A. antarctica* at PAR 249, whereas epiphytes continued to photosynthesise up until PAR 581, albeit at very low rates (mean rETR = 0.58) (figures 9 & 11).

4. Discussion

4.1 *Amphibolis antarctica*

There were significant differences in rETR for *A. antarctica* across the range of pH treatments, with rETR being highest at pH 8.18 and significantly lower at all other pH treatments. This was expected for higher pH treatments, but unexpected for low pH treatments since other studies (Koch *et al.*, 2013) have shown that ocean acidification can increase seagrass photosynthesis.

The lowest rETR were identified at pH levels of 9.0 and 9.65, likely the result of carbon limitation, inhibiting photosynthetic activity (Burnell *et al.*, 2014; Beer *et al.*, 2015). Electron transport rates were low at the highest pH treatments, but photosynthetic activity continued despite the absence of CO₂ which would be the case at pH's >8.5 (Beer *et al.*, 2015). This indicates the presence of carbon concentrating mechanisms (CCM's) in seagrasses (summarised in Larkum *et al.*, 2017), facilitating the breakdown of HCO₃ to CO₂, providing a continued supply of CO₂ to rubisco sites thus allowing photosynthesis to continue. CCM's in seagrasses, are an energetically costly method of acquiring CO₂ for photosynthesis, since HCO₃ cannot passively diffuse across cell membranes like CO₂, but must first be broken down via extracellular mechanisms (Beer *et al.*, 2006). In *A. antarctica*, this is likely achieved via extracellular carbonic anhydrase (Larkum *et al.*, 2017). Considering that *A. antarctica* continues to photosynthesise when CO₂ is low / absent, it seems likely that *A. antarctica* utilise CCM's allowing photosynthesis to continue (albeit at lower rates) via the breakdown of HCO₃.

In the results shown here, rETR for *A. antarctica* at 7.81 and 7.61 were not significantly different from one another, but both were significantly lower than at current ocean pH of 8.18. Under RCP 8.5, future ocean pH is projected to be approximately 7.76 (IPCC, 2013), and the results of this study suggest that under this scenario, photosynthetic efficiency of this seagrass species will significantly decline.

The results of this study also confirmed that *A. antarctica* exhibits significantly lower rETR than calcifying epiphytes, particularly at higher pH levels, likely the result of significantly more efficient CCM's in calcifying epiphytes due to differences in evolutionary history. Calcifying algae species have existed in marine environments for hundreds of millions of years, whereas seagrasses evolved from terrestrial plants much later (Larkum *et al.*, 2005). Terrestrial plants have no need for CCM's since gas diffusion of CO₂ is relatively quick and efficient, so seagrasses evolved CCM's far later than calcifying algae, meaning that calcifying algae have had a significant evolutionary "head start", making their CCM's far more efficient.

The data also indicate that there may be a difference in light intensity tolerance across pH treatments for both seagrass and calcifying epiphytes, particularly at higher pH levels where CO₂ is absent and both species become dependent on HCO₃ and carbon concentrating mechanisms. At very low levels of light, rETR is similar across all pH treatments for both species, so it may be that at low irradiances, the effects of carbon limitation and ocean acidification are reduced, but as light increases, high and low pH treatments appear to cause a crash in rETR as PAR increases. If lowered pH is causing a reduction in light intensity tolerance, this could be a significant problem for *Amphibolis antarctica* as ocean pH continues to drop, since *A. antarctica* grows in waters where light intensity can be as high as 1,000 PAR (Van Keulen, 2021).

At high pH treatments, a reduction in rETR can be explained by carbon limitation and inefficiency of CCM's in comparison to calcifying algae, but the mechanism(s) behind this drop in rETR at lower pH treatments are unclear, given that carbon limitation would not be an issue. Evidently, a physiological cost exists when CO₂ levels are too high, which causes rETR to significantly decline, and since rETR is a measure of the efficiency of PSII (Ralph *et al.*, 2005), it is likely that the answer lies here. Under thermal stress, seagrasses experience a reduction in the ability of PSII to supply electrons to the electron transport chain, leading to high rates of mortality (Repolho *et al.*, 2017), and it may be that elevated CO₂ causes similar disruption in PSII, for example by lipid peroxidation. Whatever the mechanism, the outcome may have significant implications for meadows of *A. antarctica*.

A. antarctica meadows are areas of high productivity and diversity that function as highly effective natural carbon sinks. As such, a decline in photosynthetic efficiency has economic and ecological consequences too numerous to discuss here, but can be broadly placed within four main categories.

4.1.1 Reduced primary productivity

A reduction in rETR would mean reduced rates of primary production, since the Calvin cycle is driven by electron transport, meaning that rETR rate has a significant influence over rates of carbon assimilation (Beer *et al.*, 2015). A drop in rETR would, therefore, lead to reduced energy and stoichiometric flow to trophic levels above. This may be particularly significant in meadows of *A. antarctica* as their large and convoluted leaf structure creates a complex three-dimensional habitat with abundant space for epiphyte cover, which can account for more than 50% of primary productivity within seagrass meadows (Borowitzka *et al.*, 2005; Strydom *et al.*, 2020). *A. antarctica* is also one of the most productive species of seagrass forming dense meadows with 300-500 erect shoots / m² and as much as 4,500 leaf clusters / m²,

forming an above-ground biomass of 1-2kg dry wt / m² (Abbott, 1990) so a reduction in primary productivity would be disproportionately large in *A. amphibolis* meadows in comparison to many other seagrass species. In fact, the loss of *A. antarctica* may negatively impact other seagrass species. *A. antarctica* meadows typically contain abundant open sandy patches (1-30m in diameter), inside of which, multi-species micro-meadows of up to 9 species of seagrasses grow, apparently with neither the *A. antarctica* or multi-species micro-meadow encroaching upon one another (Abbott, 1990). It may be that the sediments *A. antarctica* meadows form, create optimum settlement sites for these other seagrass species, and other symbioses between *A. amphibolis* and seagrass species that form these micro-meadows may also exist.

Ecosystems are inherently complex and dynamic natural systems, and any change brought about by anthropogenic influences can have profound negative impacts, as seen in the results of this study. Seagrass meadows are dependent on grazers to crop the grasses to maintain healthy growth, the absence of which due to overexploitation has, in the past, triggered or exacerbated catastrophic disease outbreaks (Jackson, 2001, 2009). Seagrass meadows are also dependent the diverse assemblage of life that inhabit the meadows, as sources of “fertilisers” as well as vectors of transportation for seeds, helping meadows spread and grow (Kendrick *et al.*, 2012; McMahon *et al.*, 2014). Any changes in these populations due to lowered productivity, would negatively impact the health of *A. antarctica* meadows. Given that seagrass meadows function as nursery grounds for many fish species that are pelagic or migratory in adulthood, there could be a knock-on effect in other interconnected ecosystems that depend on productivity of seagrass meadows. King George Whiting (*Sillaginodes punctatus*) frequently utilise *A. amphibolis* meadows along the south and south-western coast of Australia as nursery grounds during the juvenile phase of their life-cycle, but migrate distances up to 200km to offshore coastal reefs and continental shelves as they approach adulthood (Jenkins *et al.*, 2006). As adults in these habitats, they can reach sizes of 70cm, and are an important food source for sharks, rays and dolphins (Government of South Australia, 1995).

Many ecologists are already predicting a global shift towards microbial primary productivity within the oceans due to climate change and trophic downgrading of ecosystems that are subject to multiple anthropogenic stressors (Jackson, 2001, 2009; Houk *et al.*, 2012). Under such circumstances, seagrasses often yield dominance to fast growing and opportunistic species such as turf-forming and filamentous algae and cyanobacteria (Duffy *et al.*, 2019). These species are not able to provide complex structural habitats of the scale formed by seagrasses such as *A. antarctica*, and thus many ecological interactions would be lost if seagrass meadows were to be replaced by large areas of microbial primary productivity.

The world is already in the midst of a biodiversity crisis, which is likely more damaging and severe than previously thought, even by expert commentators within the scientific community (Bradshaw *et al.*, 2021). Seagrass meadows are areas of high biodiversity, so a drop in productivity, or a total ecosystem crash that causes meadows to die off would feed in to an already dire situation for global biodiversity and the ecosystem services provided by them. Efforts are already being made to implement a global observing system for seagrass to improve mapping resolution globally (Duffy *et al.*, 2019), and this is likely to be of paramount importance in monitoring global and localised changes in seagrass cover.

4.1.2 Feedback loops

As previously discussed, lower photosynthetic rates would result in lower rates of carbon assimilation via the Calvin cycle, and thus less carbon storage, at a time when natural marine carbon sinks are of critical importance in the face of climate change as part of the “blue carbon” approach to nature-based solutions for climate change (Nellemann *et al.*, 2009; Fourqurean *et al.*, 2012). This could result in a negative feedback loop where *A. antarctica* photosynthetic efficiency drops as seawater CO₂ concentration increases, thus assimilating less carbon, resulting in a net decrease in carbon storage within *A. antarctica* meadows, and more carbon available to be released from the oceans back in to the atmosphere. Similar outcomes have already been recorded in terrestrial habitats, as rising temperatures and changes in rainfall have reduced the efficiency of tropical forests as carbon sinks (Hubau *et al.*, 2020), or turned them in to carbon sources instead of sinks (Wang *et al.*, 2018). Whilst the results of this study do not indicate that *A. antarctica* meadows would become sources instead of sinks, any drop in carbon sink efficiency is of significant concern, given that blue carbon sinks have historically outperformed most, if not all, terrestrial sinks in terms of carbon sequestration rates and long-term storage (Mcleod *et al.*, 2011).

Seagrass meadows may make significant contributions to deep-sea carbon sinks that are far distant from coastal habitats via export of carbon-rich particulate matter to deeper waters (Duffy *et al.*, 2019). This contribution has yet to be estimated, but the loss of this export of carbon to deep sea sinks could contribute to a feedback loop.

4.1.3 Commercial fisheries and ecotourism

Coastal ecosystems are estimated to provide ecosystem services worth more than \$25,000bn annually through commercial fishing, reducing pollution, protecting coastlines from erosion and extreme weather events, and other forms of industry such as off-shore wind farms (Nellemann *et al.*, 2009). Coastal waters account for 50% of the world's fisheries, providing vital nutrition for more than 3bn people globally (Nellemann *et al.*, 2009), and act as a major source of income and employment globally. The results of this study suggest a significant decrease in productivity of *A. antarctica* meadows, which would have a profound impact on commercial species that use the meadows as nursery and feeding grounds. Perhaps the biggest commercial fishery off the west coast of Australia utilising these meadows, is the western rock lobster (*Panulirus cygnus*), which contributes an estimated annual economic value of AUS\$505 million, and forms part of the social fabric of coastal communities (Acil Allen Consulting, 2017). *P. cygnus* use *A. amphibolis* as a nocturnal foraging ground, and as den-sites for juveniles during the day. *P. cygnus* feed on *A. antarctica* itself as well as associated calcifying epiphytes and an abundance of invertebrate species. In seagrass meadows, invertebrate prey species can comprise as much 98% of *P. cygnus* diet (Bellchambers *et al.*, 2012), likely supporting higher growth rates than in areas where seagrass habitat is limited, thus demonstrating the exceptional value of *A. antarctica* meadows as a feeding habitat. Due to this abundance and diversity of prey species, *A. antarctica* meadows help to provide high variation in nutritional intake for *P. cygnus* (Bellchambers *et al.*, 2012) (and therefore likely high nutritional value for predators and commercial harvests), so the loss of *A. antarctica* meadows could cause a crash in *P. cygnus* populations, triggering an economic cascade amongst coastal communities due to loss of jobs directly and indirectly associated with the fishery.

The results of this study suggest a decline in productivity of *A. antarctica* under future acidification, with the possibility of areas of *A. antarctica* dying off as a result. Insight into the possible consequences of this can be seen in those of the 2010-11 heatwave which killed off large swathes of *A. antarctica* meadows. It's likely that this die off was the result of *A. antarctica* reaching a thermal tolerance threshold where PSII (which has lower thermostability than PSI) becomes less capable of supplying energy to the electron transport chain during light harvesting (Repolho *et al.*, 2017). This thermal stress affect led to widespread mortality in *A. antarctica*, with consequences for iconic marine species that draw ecotourism to the west coast of Australia, such as the endangered green turtle (*Chelonia mydas*) (Seminoff, 2004). Following the heatwave, green turtles captured and assessed for health status saw a continuing decline in health status following the loss of seagrass meadows during the 2010-11 heatwave, with, only 6% of green turtles

surveyed in 2012, falling in to the highest health categories, compared to 35% prior to the heatwave (Thomson *et al.*, 2015). Green turtles are a migratory species, travelling thousands of kilometres between feeding grounds and natal shores which they return to, to nest (Hays *et al.*, 2014). As such, the loss of seagrass habitats off the coast of Western Australia could negatively impact ecotourism thousands of kilometres away. Green turtles are a popular species for “voluntourism” projects which support conservation efforts directly through people-power and funding, support employment of local people and businesses that benefit from the influx of tourists (Senko *et al.*, 2011).

Historically, Shark Bay held one of the largest populations of dugongs (*Dugong dugong*), an iconic species for ecotourism which relies heavily on seagrasses such as *A. antarctica* meadows as pastures for grazing (Marsh *et al.*, 2018). Following the 2010-11 marine heatwave in this region, dugong populations are believed to have remained stable, contrary to expectations (Bayliss *et al.*, 2019), though this is based on aerial survey data, which has limitations, particularly in the ability to detect subtle changes in regional population change. Other studies have shown localised dugong population declines of as much as 67.5% within Shark bay (Nowicki *et al.*, 2019), which could suggest that whilst the Shark Bay dugong population as a whole has remained stable, there has been spatiotemporal changes in dugong distribution within the region. The findings of Bayliss *et al.* (2019) are nonetheless encouraging for dugongs that are classed as “vulnerable” on the IUCN redlist (IUCN, 2019), but whilst this may be good news for dugong conservation, it may suggest a higher degree of top-down pressure on *A. antarctica* meadows. If dugong populations have remained stable but meadow coverage significantly declined as a result of the heatwave and has not yet fully recovered, then it is possible that dugong density on seagrass meadows within Shark bay has increased. There may be some benefit to this given that dugongs aid in seed dispersal over distances of 100’s of kilometres (Duffy *et al.*, 2019), but this would depend on the density of dugongs in the meadows and the amount of grazing pressure exerted.

Long term, this lack of recovery of *A. antarctica* meadows in combination with increased top-down pressure from marine mega-herbivores and the cumulative effect of other factors such as ocean warming and the drop in productivity due to acidification identified in this study, may place overwhelming pressure on meadows of *A. antarctica*. Alternatively, bottom-up limitation could occur, whereby the seagrass meadows are no longer able to support the dugong population.

4.1.4 Sinks as sources

Vegetated coastal habitats such as seagrass meadows, mangrove forests and salt marshes only cover ~0.5% of the seabed, but these habitats account for as much as 71% of all ocean sediment carbon storage (Nellemann *et al.*, 2009), and lock up significant quantities of methane (CH₄) and nitrous oxide (N₂O), (Macreadie *et al.*, 2019). The results of this study indicate a significant reduction in rates of photosynthesis of *A. antarctica* and associated calcifying epiphytes, which would result in a lower rates of carbon fixation via rubisco sites. Other studies have clearly demonstrated the potential for thermal stress to disrupt PSII and lead to die off *A. antarctica* meadows (Kendrick *et al.*, 2019; Nowicki *et al.*, 2019; Strydom *et al.*, 2020), and combined with the results of this study, are likely indicative of a synergistic or antagonistic influence of acidification and warming that at the least would lower carbon fixation and sequestration, and at worst, kill off meadows entirely. If this occurs, it would lead to the release of substantial carbon and greenhouse gas stores not just from the seagrass itself, but from carbon stored in sediments (Mcleod *et al.*, 2011).

Protecting vegetated coastal ecosystems could help prevent as much as 1.02 petagrams (pg) of carbon emissions per year (Pendleton *et al.*, 2012; Macreadie *et al.*, 2019), and globally, seagrass ecosystems may currently be storing as much as 20pg of carbon in their sediments alone (Fourqurean *et al.*, 2012), the equivalent to almost forty times the annual carbon emissions of Australia (Parliament of Australia, 2020). Estimates of how much more carbon can be “safely” emitted in to the atmosphere before the planet suffers catastrophic warming are difficult to predict accurately, particularly given that it could be reasonably argued that catastrophic warming has already taken place. The lower end of the estimate is believed to be ~1pg and the higher ~5pg (IPCC, 2013), thus, even the higher estimate of what can be “safely” emitted, equates to one quarter of what may currently be locked away in seagrass sediments. Climate tipping points in the earth system have been discussed at length within the scientific literature in recent years in relation to terrestrial carbon sinks such as tropical forests and permafrost (Lenton *et al.*, 2013; Lenton *et al.*, 2019). Little attention has been given to climate tipping points for seagrass meadows, despite the vast stores of carbon they hold, and their sensitivity to thermal stress, and this may represent an important knowledge gap in contemporary understanding of climate change.

Clearly, seagrass meadows and other vegetated coastal habitats have the capacity to play a major role as part of the nature-based solutions approach to climate change, and require urgent protection. Terrestrial carbon sinks often only have limited opportunity for spatial expansion, typically only vertically due to

anthropogenic encroachment (Nellemann *et al.*, 2009), but seagrass meadows have the capacity and space for vertical and, critically, massive horizontal expansion if they are effectively protected (Nellemann *et al.*, 2009). Global seagrass area is estimated at somewhere between 0.3-0.6 million km², though many areas lack extensive mapping, but the total potential habitable area for seagrasses globally is ~4.3 million

4.1.5 Fluid dynamics and coastal erosion

Fluid dynamics exert a major influence over seagrass ecosystems, affecting factors such as nutrient flow, pollination, import and export of organic matter and the amount of available photosynthetically active radiation reaching seagrass leaves (Larkum *et al.*, 2005). The epiphytic communities of seagrass meadows have also been shown to be heavily influenced by fluid dynamics due to variability within tolerance limits of wave energy for different species of epiphytes and the grazers that feed on them (Larkum *et al.*, 2005). This means that significant changes in local or regional fluid dynamics, for example from the degradation of coral reefs (already occurring extensively due to ocean warming and acidification) have the potential to trigger the total collapse of seagrass meadows if wave height and energy are increased (Macreadie *et al.*, 2019).

Seagrass ecosystems in turn, can exert a heavy influence over the fluid and sedimentary dynamics of coastal regions, altering currents, waves and turbulence, forming sand and mud banks and thus playing an important role in preventing coastal erosion. Rhizome networks are well established in mature, healthy seagrass meadows and aid in coastal sediment stability. The decline of *Posidonia* meadows off the coast of Adelaide has resulted in the loss of extensive beach-lines due to coastal erosion, which was previously protected against by the complex rhizome network of *Posidonia* meadows via the dissipation of hydrodynamic forces and the stabilising and raising of sediments (Seddon *et al.*, 2016). The loss of such beaches has come with a significant economic and cultural loss to communities in Adelaide who rely on the beaches for recreation, tourism and associated businesses (Seddon *et al.*, 2016).

Similar outcomes could be seen on the Western Australian coast if *A. antarctica* meadows are lost to climate change. Seagrass meadows are depositional environments, and the Shark bay area off the western coast of Australia was largely created by seagrass meadows (particularly *Amphibolis* genera) that aided in the build-up of huge sandbanks and the subsequent changes in hydrodynamic regimes that this caused (Van Keulen *et al.*, 2003). Even in high current regimes, *A. antarctica* grows so densely that it has the ability to reduce sediment transport by absorbing hydrodynamic forces, and a well-formed meadow is capable of nullifying currents as strong as 2 knots (Abbott, 1990). In fact seagrass meadows in Shark Bay

have sediment accretion rates higher than those of coral reefs, due to both the density of *A. antarctica* and the high epiphyte load that it typically carries (Abbott, 1990). The result of this, is the accumulation of fine mud particles and detritus within and beyond the meadows (Abbott, 1990). The extensive rhizosphere that seagrass meadows form helps to bind these sediments and protect coastlines from erosion. As a result, seagrasses such as *A. antarctica* have made a major contribution to the development of their own and neighbouring ecosystems that support massive biodiversity and associated ecosystem services such as carbon storage, ecotourism and commercial fisheries. The loss of the sediment-stabilising rhizosphere and sedimentary dynamics that *A. antarctica* provides could have catastrophic outcomes for these ecosystems and associated services, particularly given the projected increase in frequency and severity of storm systems.

4.2 Calcifying epiphytes

Results show that there is a significant difference in rETR for calcifying epiphytes of *A. antarctica* across the range of pH treatments, with rETR being highest at pH 8.18 through to 8.80 and significantly lower at all other pH treatments. The lowest rETR were identified at pH of 7.61 the most acidic treatment used in this study. rETR at 7.81 was significantly lower than at natural ocean pH of 8.18, but significantly higher than at 7.61. The results of this study suggest that under RCP 8.5, photosynthetic efficiency of calcifying epiphytes that associate with *A. antarctica* will significantly decline. This is in line with the findings other studies which show significant declines in calcifying epiphyte productivity, and total dissolution at some lower pH estimates (Martin *et al.*, 2008; Cox *et al.*, 2015). Given the results of similar studies, it is likely that the epiphytes studied here would have eventually completely dissolved over longer-term experiments at the lowest pH treatment.

The results at higher pH levels indicate that carbon concentrating mechanisms in the calcifying epiphytes tested here are significantly more efficient than those in *A. antarctica*. (mean 10.15 rETR at PAR 41, pH 9.65 compared to 5.01 for *A. antarctica*). This is unsurprising given that calcifying algae species extend back at least 600m years with evolutionary origins in the ocean, whereas seagrass species evolved on land and migrated to the oceans (Les *et al.*, 1997). With the anticipated continued rise in CO₂ saturation of the oceans however, the historic advantage of species with highly efficient carbon concentrating mechanisms may begin to fade. This is likely to also depend on biological responses to cumulative stressors, given that

some macroalgae species have shown to be negatively impacted by the antagonistic interactive effect of acidification and warming depending on future climate change outcomes (Kübler *et al.*, 2015).

Calcification rates in calcifying algae species typically follow photosynthetic rates (Beer *et al.*, 2015), and since the results here indicate a drop in photosynthetic rates at lower pH treatments, it is likely that calcifying epiphytes that grow on *A. antarctica* will be negatively impacted via reduced photosynthetic and calcification rates, under all future ocean acidification scenarios for pH values of 7.81 or below laid out by IPCC in comparison to current ocean pH. The mechanisms behind this likely lie in the impact of acidification on the processes within PSII, given that rETR is a measure of the efficiency of PSII in supplying energy to the electron transport chain (Ralph *et al.*, 2005). In calcifying algae, reduced pH has been theorised to: disrupt CO₂ accumulation pathways that feed CO₂ to rubisco sites, disrupt the transport of electrons via thylakoid proton gradients (Sinutok *et al.*, 2012) and reduce the repair rate of proteins in calcifying algae that become damaged by high light intensities (Briggs *et al.*, 2019). The results here also suggest changes in light-intensity tolerance thresholds for calcifying epiphytes, which may be explained by decreased efficiency of CCM's under the synergistic impact of high-light and increased acidification, making PSII more susceptible to photoinhibition (Briggs *et al.*, 2019). Other studies conducted on acidification and coralline algae have noted that lowered pH may reduce photosynthetic rates by reducing light capturing pigments, specifically chlorophyll and phycobiliproteins, and similar effects could be present here (Gao *et al.*, 2010).

The impact of acidification is likely to be highly dependent on the capacity of *A. antarctica* meadows to buffer themselves and associated species against acidification via localised pH alteration through photosynthesis (Bergstrom *et al.*, 2019). Seagrass meadows are known to mitigate the effects of ocean acidification for calcifying algae by taking up CO₂ and increasing pH which helps promote high rates of photosynthesis and calcification in calcifying algae (Bergstrom *et al.*, 2019). These metabolic interactions between non-calcifying organisms such as seagrass and calcifying organisms such as calcifying epiphytes, are critical in providing refuge for calcifiers against the influence of ocean acidification, but the results of this study, suggest that *A. antarctica* may be less capable of providing this buffering effect than other species of seagrass, and therefore may not act as refuge sites for the calcifying epiphytes that settle on them.

The epiphytic load and microbiome of seagrasses is a vital component of the seagrass ecosystem, and in *A. amphibolis* meadows on the west Australian coast, epiphytic species may account for the same biomass

as the seagrass itself (Larkum *et al.*, 2005; Van Keulen, 2021), and the loss of such species may have important ecological consequences for *A. antarctica* meadows and the species that inhabit them.

4.2.1 Increased niche availability

Calcifying epiphytes often act as pioneer colonisation species on seagrasses, and help to make habitat on blade surfaces more hospitable for settlement of other species such as diatoms or sponges (Martin *et al.*, 2008). This means that a loss of calcifying epiphytes on seagrass blades could result in niche availability beyond just the well-lit niche regions of the complex three dimensional structure of *A. antarctica* leaves that calcifying epiphytes would typically inhabit. As a result, an abundance of space may become available on *A. antarctica* leaves for settlement of other species. It is likely that symbiotic relationships exist between *A. antarctica* and associated calcifying epiphytes, for example CO₂ being released by calcifiers which may aid seagrass photosynthesis during carbon limitation (Semesei *et al.*, 2009), but it's likely that other symbioses exist that are as yet, unknown. As such, the loss of calcifying epiphytes may have unexpected consequences, particularly if there is a lack of ecological redundancy where other calcifiers are unable to capitalise on available space to fulfil similar roles.

In the Mediterranean, seagrasses growing in close proximity to volcanic vents are constantly exposed to lower than average pH levels, and studies have shown that amongst these communities, calcifying epiphytes are replaced by bryozoans as the only calcifying species to grow on seagrass blades (Hall-Spencer *et al.*, 2008). This is likely the result of differences in mineralogy, with bryozoans having lower magnesium (Mg) content than calcifying epiphytes, allowing them to tolerate lower pH levels (Martin *et al.*, 2008). In *A. antarctica* meadows, bryozoans are found almost exclusively on the woody stalks, and not on the leaves (James *et al.*, 2009). The reasons for this are unclear, however, *A. antarctica* grows very densely with 300-500 erect shoots / m² and 4,500 leaf clusters / m², thus limiting light below the leaves. This means that the leaves themselves form an optimum environment for calcifying algae species that benefit from high levels of light, whilst the bryozoans preferentially settle in areas of lower light intensity either because they are unable to compete with the calcifying algae for space, or because there is a negative relationship between light intensity and ability to settle. *A. Antarctica* have ~60 different bryozoan species recorded as growing on them either on the grass blades or stalks (Di Martino *et al.*, 2014), and should any of these species prove resistant to acidification as seen in the Mediterranean, there may be some degree of ecological redundancy if they are able to tolerate the higher light intensities on

the leaves, and thus replace calcifying epiphytes, but some complex ecological interactions will almost certainly be lost.

Many studies anticipate an increase in “smothering” species such as turf algae and cyanobacterial biofilms under future climate change, due to CO₂ enrichment of coastal waters (Jackson, 2009; Russell *et al.*, 2013; Johnson *et al.*, 2015). The space made available on seagrass leaves by the absence of calcifying epiphytes could be taken up by such species, particularly given that *A. antarctica* leaves have a very rough surface providing an optimum site for settlement of such species (James *et al.*, 2009). If this occurs, then smothering of *A. antarctica* meadows could become widespread, killing them off. It has already been observed that *A. antarctica* is one of the first species to decline in eutrophic environments due to smothering, since smothering algae settle and grow far more rapidly than leaves can be replaced (James *et al.*, 2009). There is precedent for this in other seagrass species’ meadows in the Gulf of Mexico (Paerl *et al.*, 2013), and Moreton Australia, (Watkinson *et al.*, 2005), which have experienced die-off due to cyanobacterial smothering. Given that most research points towards increases in cyanobacterial biofilms and other forms of microbially driven primary production under ocean acidification (Jackson, 2009; Houk *et al.*, 2012), it is likely that available space on *A. antarctica* leaves will be taken up by such species, and given that more than 200 species of epiphytes have been recorded in seagrass meadows in the Perth region alone (Van Keulen, 2021), there is a lot of potential for high competition for space on *A. antarctica* leaves.

It may be that such a shift in seagrass epiphytic communities will have neutral outcomes for the seagrass species itself, but a drop in diversity amongst the seagrass microbiome, given that bryozoans would not fulfil the same role of calcifying algae in helping to create settlement opportunities for other species.

4.2.2 Release of carbon stores

The epiphytic community of seagrass meadows can amount to the same biomass as seagrasses themselves, and as such, may constitute a significant carbon store. The dissolution of calcifying epiphytes at low pH levels would create a temporary abundance of CO₂, which could potentially be buffered by uptake of this by seagrass in some species, though since calcifiers also release CO₂ during calcification, a mutualistic symbiosis likely exists in terms of providing CO₂ to seagrasses which would be lost in the long term. The results of this study however, suggest a drop in photosynthesis of *A. antarctica* under ocean acidification, so this buffering may not take place in *A. antarctica* meadows, meaning that CO₂ released from dissolving calcifying epiphytes would contribute to atmospheric CO₂ concentrations.

Calcifying algae and the species that graze on them such as amphipods and gastropods make a significant contribution to the production of biogenic calcareous sediments via carbonate production (Borowitzka *et al.*, 2005). Amongst *A. antarctica* meadows, this rate of production is particularly high, in the region of 50-526g CaCO₃ m⁻² year⁻¹ (Larkum *et al.*, 2005), and such sediments form the basis of almost all coastal ecosystems off the western coast of Australia, which has few silica-based sediments.

4.2.3 Loss of nutrition to grazing species

Calcifying algae are an important source of nutrition for many grazing species such as amphipods and gastropods which preferentially graze on different calcifying algae species (Borowitzka *et al.*, 2005). In addition to these species, *P. cygnus* grazes heavily on the calcifying epiphytes that grow on *A. antarctica* (Bellchambers *et al.*, 2012). The CaCO₃ content of the algae provides important nutrition for skeletogenesis in *P. cygnus*, particularly for inter-moult juveniles, and given that ocean acidification is already expected to inhibit skeletogenesis in calcifying invertebrates such as lobsters (Arnold *et al.*, 2009), the loss of CaCO₃ rich food sources such as calcifying algae could become an additional stressor on species of high economic value. Calcifying epiphytes also form an important element of muscle nutrition for *P. cygnus* (Bellchambers *et al.*, 2012), so a lack of availability as a food source (if calcifying epiphytes were to be lost either partially or completely due to acidification), could have major detrimental impacts on the physiology of *P. cygnus* but also perhaps, upon their nutritional profile for both natural predators and commercial fisheries.

As previously discussed, *P. cygnus* fisheries are estimated to contribute \$505m to the economy of Western Australia, and the species relies heavily on both *A. antarctica* and the calcifying epiphytes that grow upon them, so a loss of both of these species could lead to a catastrophic crash in *P. cygnus* populations.

4.3 Consideration of multiple stressors

In the anthropocene era, environmental change and degradation has become commonplace, thus any studies designed to assess biological responses to any one stressor, must consider cumulative impacts of multiple stressors on both the study species, and that species' community as a whole.

The 2010-11 marine heatwave off the West coast of Australia resulted in mass die off of seagrasses, most notably *A. antarctica* (Strydom *et al.*, 2020). Combined with the results of this study, it is clear that the

two primary direct threats of climate change within the marine environment (ocean acidification and ocean warming) represent significant threats to the future of *A. antarctica* meadows and associated species and ecosystem services. Unexpected outcomes of climate change on species associated with *A. antarctica* meadows may also pose unforeseen threats. For example, *A. antarctica* meadows are home to a number of species that graze directly on seagrass such as the urchin *Amblypneustes pallidus*. Such species are typically expected to increase per capita grazing under ocean warming as metabolic rates and thus energy requirements increase in line with metabolic theory. Experiments have shown that *A. pallidus* also exhibit increased per capita grazing on *A. amphibolis* under ocean acidification (Burnell *et al.*, 2013) – this may at first appear counterintuitive, given that urchins are expected to suffer under ocean acidification (O’Donnell *et al.*, 2009; Carey *et al.*, 2016), though it is likely the result of increased nutritional demand to compensate for stresses imposed by acidification. This means that acidification as well as ocean warming may act in concert to exert additional top-down pressure on *A. antarctica* by herbivores.

Increased ocean temperatures may cause enhanced stratification, particularly in tropical and temperate waters, potentially inhibiting the movement of nutrients across the thermocline, therefore resulting in primary producers in these areas becoming nutrient limited, decreasing photosynthesis and primary productivity (Beer *et al.*, 2015). Additionally, adjacent ecosystems may influence one another by their responses to climate change. The degradation of coral reefs that are already profoundly threatened by climate change (Wisshak *et al.*, 2012; Head *et al.*, 2019) could trigger the collapse of seagrass meadows due to an increase in wave height and energy, altering turbidity and therefore light availability (Macreadie *et al.*, 2019).

The cumulative impact of increased marine heatwaves killing off large swathes of *A. antarctica*, a drop in primary productivity caused either by nutrient limitation, or as the results of this study suggest reduced rates of photosynthesis, and the threat of over-grazing means that the future for one of the most biodiverse and structurally complex seagrass meadows looks bleak.

Conclusions

Seagrasses are key species in coastal trophodynamics, and are amongst the most productive and biodiverse habitats on the planet. *Amphibolis antarctica* meadows are no exception to this, and the findings of this study indicate that *A. antarctica* meadows will see a drop in primary productivity as a result of lowered photosynthetic rates under future ocean acidification. This is contrary to most seagrass species that are expected to benefit from increased availability of CO₂ for photosynthesis, particularly those that experience frequent carbon limitation and have limited-to-no carbon concentrating mechanisms available to convert HCO₃ to CO₂. The mechanisms behind this drop in *A. antarctica* photosynthesis are unclear, but may related to a disruption of PSII supplying electrons to the electron transport chain, but continued laboratory experiments may yield answers, particularly where samples are isolated from other environmental variables.

Calcifying epiphytes have been reported to suffer under ocean acidification in many studies, and that finding is borne out in the results of this study as well. The results at higher pH treatments indicate highly effective carbon concentrating mechanisms, but in high CO₂ oceans where inorganic carbon is abundant, CCM's may become less important. A great deal depends on the capacity of *A. antarctica* meadows to buffer against ocean acidification, thereby providing "refuge" to a number of calcifying organisms, and larger-scale mesocosm or in-situ studies would help in determining whether this capacity exists in *A. antarctica* meadows.

Any studies conducted on specimens in laboratory settings are inherently simplified versions of the complex biological processes that occur in natural systems, but therein lies the value of such studies. Isolating organisms from other environmental variables helps to determine biological responses to specific environmental change such as ocean acidification which would not be possible in the field. This forms the basis for other broader studies on the responses of seagrasses and calcifying epiphytes to acidification either in situ, particularly where natural CO₂ events provide such opportunities, or via mesocosm experiments. To date, no volcanic vents have been identified that provide suitable in-situ field sites to study *A. antarctica* and associated calcifying epiphytes in terms of their response to acidification. As such, mesocosm experiments may prove useful.

Global observing systems using multispectral satellite imagery are already being developed to monitor and map seagrasses to aid conservation efforts, and this is likely to be pivotal in detecting changes in both global and local seagrass coverage. Such remote sensing technologies come with limitations, particularly in optically challenging environments such as the highly turbid waters of estuaries, but the rapid pace of development in remote sensing technology and analysis of extensive datasets via deep neural networks may help to address these limitations. Regions where mapping is limited to non-existent should be particular areas of concern given that they often have limited legal protections and may currently be in states of rapid decline that has thus far gone unnoticed. A combined approach using climate change-induced physiological responses of seagrass species globally, as well as remote sensing to monitor spatial distribution of meadows, could allow for meta-analyses to be made to project both the loss of meadows due to climate change, and estimate the release of carbon that this would lead to.

Ultimately, the loss of *A. antarctica* meadows would prove catastrophic in terms of all the ecosystem services that both they and their associated epiphytes provide such as carbon storage, nursery grounds for commercial fisheries species, and protection against coastal erosion in the face of increased storm intensity and frequency under what appears to be inevitable climate change. Complex ecosystem processes would be lost, many of which still remain undiscovered or poorly understood. Protecting seagrass meadows globally through improved remote sensing monitoring and both international and local environmental legislation, is therefore of the utmost importance.

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