SEAGRASS ECOLOGY AND PHYSIOLOGY IN A LOCALLY AND GLOBALLY CHANGING ENVIRONMENT

Owen W. Burnell

Presented for the degree of Doctor of Philosophy

School of Earth and Environmental Sciences

The University of Adelaide, South Australia

January 2014

Cover Image: A short-spined sea-urchin (*Amblypneustes pallidus*) within a seagrass meadow (*Posidonia* sp.). Photo credit: Owen Burnell

THESIS DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (of which permission to reproduce is listed in Appendix C) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Owen Burnell

January 2014

CONTENTS

THESIS ABSTRACT

Human beings can modify the abiotic conditions and biotic interactions that shape natural ecosystems. The primary aim of my thesis was to elucidate local- and global-scale anthropogenic changes of importance in shaping seagrass habitats, by examining how a number of both established and forecasted conditions can modify biotic interactions between seagrass and their consumers (i.e. grazing herbivores), as well as interactions between seagrass and their competitors (i.e. algal epiphytes). I approached this from both a top-down (i.e. consumer driven) and bottom-up (i.e. resource limitation) perspective.

I begin by examining how urchin-epiphyte-seagrass interactions can modify habitat loss in local seagrass beds. This was motivated by the observation that the seagrass species *Amphibolis antarctica* appeared to suffer disproportionately under the influence of herbivore expansions. To this end, I found the recovery rate of the seagrass *Amphibolis antarctica* was much slower following grazing damage than contiguous meadows of *Posidonia sinuosa*, which appeared to result from the distinct morphological differences between these two seagrasses (i.e. elevated vs. basal meristems, respectively). Given these emerging urchin effects and the legacy of seagrass loss from nutrient enrichment, next I investigated how these processes (i.e. top-down vs. bottom-up) might interact to shape the maintenance or loss of seagrass habitats. I found that the independent negative effects of urchins and nutrients do not combine in an intuitive manner, but rather eutrophic conditions reduce the *per capita* grazing of urchins on seagrass, as they appear to acquire greater nutrition from increasing food quality.

After documenting these emerging local changes to seagrass, I examined how forecasted global changes, in particular increases in dissolved CO_2 could modify future seagrass meadows. From a resource limitation perspective I examined the carbon physiology of three seagrass species, consistently finding they were reliant on energetically costly bicarbonate acquisition under contemporary CO_2 conditions. Subsequent growth experiments at enriched CO_2 uncovered this contemporary limitation could translate to greater growth for seagrass under future CO_2 due to lower energetic requirements for photosynthesis. However, I also examined the implications of changing CO_2 resources on epiphyte-seagrass interactions, finding under certain abiotic conditions, such as high light, opportunistic epiphytes are strong competitors that could inhibit future seagrass growth. Finally using urchin-seagrass interactions as a simplified model ecosystem, I found global increases in both temperature and CO_2 could increase top-down grazing control on seagrass meadows, as it appears the physiological demands on some grazing herbivores (i.e. ectotherms) could be greater in a high CO_2 world.

In conclusion, I add to the growing evidence for top-down grazer effects on seagrass, highlighting population expansions of urchins can drive strong, but variable, effects on seagrass, dependent upon species vulnerability and nutrient status of meadows. In reference to global change, I highlight the importance of biotic interactions in modifying the response of seagrass habitats to forecasted CO_2 levels. Thus, the future of habitat-forming producers should not be inferred or assumed *a priori*, but requires rigorous tests of how anthropogenic change modifies the interactions between organisms across all trophic levels.

ACKNOWLEDGEMENTS

Thank you to all my supervisors for their guidance, expertise and enthusiasm, your willingness to contribute advice and invest your limited time was always appreciated. Prof. Sean Connell, for your honest and critical opinion, which always helped to develop my work. Dr Bayden Russell, for your attention to detail and thoughtful advice on all aspects of my research. Dr Andrew Irving, for your contribution of countless hours to assist with experimental ideas and manuscript writing, making the experience a truly collaborative one. A special thanks also to A/Prof. Jennifer Watling whom offered up her time and physiology expertise.

Members past and present of the Southern Seas Ecology Laboratories; especially Laura Falkenberg, who has been a great friend since day one, you're always the first and only person who understands, but also laughs, at my frustrations and failures. Sam Owen, Kingsley Griffin and Eric Drew for your help and friendship during field work, those days help maintain resolve when the water seems very far away. To all of those I have shared an office, lab or field site with over the years, especially Tom Barnes, Kayla Gilmore, Pete Fraser, Skye Woodcock and Chloe McSkimming.

My family; you support me in whatever I choose to do, for which I am truly grateful. In particular my father for providing extra incentive the day he declared the 1992 bottle of Grange Hermitage wine he was gifted would be consumed either the day I handed up or on his 60th birthday in November 2013. A challenge I narrowly lost.

CHAPTER ACKNOWLEDGEMENTS

CHAPTER 2

Thanks to Eric Drew, Samuel Owen and Kingsley Griffin for the many hours of diving assistance. The Field Naturalists Society of South Australia provided two Lirabenda Endowment Fund Grants to O.W.B. that helped fund the research. S.D.C. and B.D.R. were funded by an ARC grant and S.D.C. an ARC Future Fellowship.

CHAPTER 3

We thank Eric Drew, Sam Owen and Kingsley Griffin for assistance in the field. Thanks also to Nenah Mackenzie from The University of Adelaide for operating the Mass Spectrometer. O.W.B. was awarded two Lirabenda Endowment Fund Grants from the Field Naturalists Society of South Australia that supported the research. S.D.C. and B.D.R. were funded by an ARC grant and S.D.C. an ARC Future Fellowship.

CHAPTER 4

We would like to thank members of the Plant Ecophysiology Laboratory at The University of Adelaide for welcoming us into their laboratory, as well as Julie Francis for assistance with oxygen electrodes. S.D.C. and B.D.R. were funded by an ARC grant and S.D.C. an ARC Future Fellowship.

CHAPTER 5

We would like to thank Nenah Mackenzie from The University of Adelaide for operating the Mass Spectrometer. S.D.C. and B.D.R. were funded by an ARC grant and S.D.C. an ARC Future Fellowship. We would also like to thank two anonymous reviewers and the Associate Editor whose comments improved an early draft of the manuscript.

CHAPTER 6

Thanks to four anonymous reviewers, whose comments significantly improved the manuscript. We would also like to thank the Nature Foundation of South Australia for a grant awarded to O.W.B. that contributed funding for the project, as well as L. Falkenberg for assistance with analytical methods. S.D.C. and B.D.R. were funded by an ARC grant, and S.D.C. was funded by an ARC Future Fellowship.

CHAPTER ONE

GENERAL INTRODUCTION

Anthropogenic impacts on natural environments continue to challenge our theoretical and applied understanding of ecosystem dynamics (Jackson, 2001, Vitousek et al., 1997). Identifying key drivers of habitat maintenance and loss is of critical importance for ecologists (Hughes et al., 2013), especially when widespread declines of primary producers cause temporary or permanent shifts to less productive states (Scheffer et al., 2001). Habitat-forming primary producers, such as mangroves, coral reefs, seagrasses and kelp-forests are the foundations of coastal productivity, with their degradation and loss impacting the host of ecosystem services they provide (Barbier et al., 2011, Costanza et al., 1997, Orth et al., 2006). Importantly, once these habitats are gone, feedbacks and the legacy of anthropogenic impacts often prohibit their recovery to pre-disturbance states (Scheffer et al., 2001, Schiel & Lilley, 2011).

Anthropogenic factors that drive widespread habitat loss in shallow near-shore environments transcend different marine biomes, such that benthic primary producers (i.e. coral reefs, algal-forests and seagrass meadows) all suffer from similar stressors. For example, local-scale changes such as land derived nutrient subsides and sedimentation from altered land use can shade primary producers, promote algal blooms, and inhibit the recovery of these sub-tidal habitats (Connell et al., 2008, Fabricius, 2005, Orth et al., 2006). Likewise, alterations to human harvesting of large mammalian, reptilian, fish and invertebrate predators commonly modifies trophic food-web dynamics, often culminating in overgrowth of habitats by opportunistic algae or overgrazing by first-order consumers (Eklöf et al., 2008, Estes & Palmisan.Jf, 1974, Hughes, 1994). Greater uncertainty surrounds the effects of emerging global-scale changes, such as those associated with anthropogenic CO_2 emissions. Frequently, opposing predictions range from negative to positive across different taxa. For example, increasing dissolved CO_2 concentrations that acidify the ocean have created concern regarding the direct negative effects on calcifying organisms (Hoegh-Guldberg et al., 2007, Orr et al., 2005), but might also benefit some habitat-forming marine primary producers that are carbon limited in contemporary oceans (Harley et al., 2012, Koch et al., 2013). Similarly, warming of global oceans can drive bleaching and die-back of habitats with limited thermal tolerance ranges (Hughes et al., 2003, Jorda et al., 2012, Short & Neckles, 1999), but such loss will not be ubiquitous to all taxa, as those species living within tolerance limits or that have the ability to adapt might undergo increases in productivity (Short & Neckles, 1999). Importantly, such changes all reflect the dominance that humans now exert over natural ecosystems (Vitousek et al., 1997).

1.1 SEAGRASSES AND ANTHROPOGENIC LOSS

Seagrasses are submerged aquatic angiosperms that colonise well-illuminated soft-bottom habitats. Their distribution encompasses all of the world’s latitudinal climates (i.e. tropical, temperate & polar) (Green & Short, 2003, Orth et al., 2006). As ecosystems they form the foundations for coastal productivity in protected to moderately exposed marine waters (Short & Wyllie-Echeverria, 1996). Much of the carbon flux from seagrass ecosystems can also be attributed to the assemblages of epiphytic algae that colonise seagrass leaves (Silberstein et al., 1986). As such, seagrass habitats provide ecosystem services and promote the maintenance of biodiversity by supporting trophic food-webs and important commercial fish species (Heck et al., 2008, Jackson *et al.*, 2001, Sogard, 1992, Waycott et al., 2009). Their role in providing nutrient cycling, carbon sequestration and habitat stabilisation that limits turbidity and sedimentation all help to maintain optimal functioning of coastal ecosystems (Duarte et al., 2005, Orth et al., 2006, Russell et al., 2013, Touchette & Burkholder, 2000). Seagrasses share one critical trait with humans; a natural propensity to inhabit protected and sandy coastlines. Concomitant with human population growth, almost a third of globally recorded seagrass meadows have been lost during the last 130 years (Waycott et al., 2009). In many instances seagrass meadows continue to decline (Waycott et al., 2009), which occurs despite the abatement of primary threats, e.g. reduction of nutrient inputs to coastal environments (Nayar et al., 2012). Of principal importance is that once certain seagrass habitats are lost, their slow natural recruitment and meadow expansion rates mean that regeneration can take decades to centuries to occur, if ever (Bryars & Neverauskas, 2004, Irving, 2013, Kirkman & Kuo, 1990), which is particularly common in many southern temperate species.

Many established and emerging anthropogenic factors threaten to compound the decline of seagrass meadows over the coming decades (Duarte, 2002, Orth et al., 2006, Short & Wyllie-Echeverria, 1996). Seagrasses have a host of environmental requirements that control their physiological performance and growth (Bulthuis, 1987, Short & Neckles, 1999, Touchette & Burkholder, 2000). Light for photosynthesis is critical for seagrasses, which due to their evolution from terrestrial plants, have a high energy demand to support below ground biomass (Dennison, 1987, Lee et al., 2007). Therefore, primary threats are often associated with increased light attenuation in marine habitats resulting from changes in terrestrial land use. Such activities create run-off, causing increases in turbidity and sedimentation that can shade and smother plants (Collier et al., 2012, Ralph et al., 2007). On a more localised scale, physical disturbances, such as dredging (Lavery et al., 2009), coastal development (Short & Wyllie-Echeverria, 1996), mining (Irving, 2013) and benthic anchorages (Walker et al., 1989) have all contributed to seagrass decline. Such direct physical mechanisms can also combine with ecological change to promote seagrass loss. Where terrestrial inputs increase coastal nutrient levels these subsidies can fuel blooms of free-living and epiphytic algae, which shade or colonise seagrass, in-turn competing for a number of finite resources (e.g. light, CO_2 & nutrients) (Borowitzka et al., 2006, Bryars et al., 2011, Bulthuis & Woelkerling, 1983, Sand-Jensen, 1977). Human harvesting of large predatory organisms (e.g. fish) can also modify top-down control in seagrass systems, enabling expansions of lower-order consumers to over-graze upon seagrass (Eklöf et al., 2008). These locally driven processes (i.e. over-harvesting and nutrient subsidies) commonly combine along urbanised coastlines to modify top-down (i.e. grazing) and bottom-up (i.e. nutrients) control (Hughes et al., 2013, Tewfik et al., 2005, Vitousek et al., 1997), which have long been of great fascination for ecologists in understanding habitat dynamics (Menge, 2000, Terborgh et al., 2001).

1.2 CHANGING ABIOTIC CONDITIONS OF GLOBAL ORIGIN

On a global-scale imminent changes to seagrasses are centred on increasing levels of dissolved CO_2 in the ocean that result from rising atmospheric CO_2 (Koch et al., 2013, Short & Neckles, 1999). While the negative environmental effects associated with increasing atmospheric CO_2 have caused global concern for many organisms (Orr et al., 2005, Thomas et al., 2004, Thuiller et al., 2005), there is also evidence that many photosynthetic organisms will derive physiological benefits from a CO_2-enriched world (Beer & Koch, 1996, Curtis & Wang, 1998, Wand et al., 1999). When angiosperms first entered the aquatic realm nearly 90 million years ago atmospheric carbon dioxide (CO_2) levels were much greater (approximately 3 - 7 times) than today (Beardall et al., 1998, Beer & Koch, 1996, Berner & Kothavala, 2001). In fact, higher CO_2 is hypothesised to have played a role in facilitating the transition of flowering plants into the aquatic realm, because of the greater availability of dissolved CO_2 to support aquatic photosynthesis (Beardall et al., 1998, Beer & Koch, 1996).

In present times, increasing levels of atmospheric CO_2 are anticipated to benefit the physiology and growth of seagrasses by altering the concentrations of different dissolved inorganic carbon (C_i) forms used for photosynthesis (Björk et al., 1997, Koch et al., 2013). While many seagrass species use both dissolved CO_2 and bi-carbonate (HCO_3^-) for photosynthesis (Millhouse & Strother, 1986), photosynthesis might be limited under contemporary C_i concentrations due to the inefficiency with which seagrasses utilise HCO_3^- and the extremely low concentrations of dissolved CO_2 relative to HCO_3^- (Beer, 1989, Björk et al., 1997, Lee et al., 2007, Touchette & Burkholder, 2000). This is compounded by the slow diffusion rate of CO_2 in water (10,000 times slower than in air) that can create significant boundary layer CO_2 depletion around leaves (Prins & Elzenga, 1989). Seagrass responses to changing CO_2 will be species-specific, owing to existing adaptations to utilise HCO_3^- for photosynthesis (Björk et al., 1997, Koch et al., 2013). Although there are few controlled long-term manipulations that examine growth, the available evidence to date predicts elevated CO_2 will increase seagrass biomass or productivity, provided other factors (e.g. light, nutrients and habitat availability) do not become limiting (Alexandre et al., 2012, Campbell & Fourqurean, 2013a, Hall-Spencer et al., 2008, Jiang et al., 2010, Palacios & Zimmerman, 2007). Although, there remains few accounts of how CO_2 might indirectly modify ecological outcomes in seagrass habitats if such changes also impact the physiology and growth of competing primary producers or herbivores that graze seagrass.

1.3 CHANGING BIOTIC INTERACTIONS OF GLOBAL ORIGIN

Understanding changes to biotic interactions from CO_2 is needed given the importance of top-down grazing effects (e.g. keystone echinoderms) and bottom-up resource effects (e.g. algal vs. seagrass growth) in modifying habitats (Eklöf et al., 2008, Thomsen et al., 2012). Studies of forecasted CO_2 across terrestrial and marine systems indicate such shifts in resources might benefit those plants which are strong competitors, meanwhile inhibiting others via interactive processes such as shading (Falkenberg et al., 2012, Reekie & Bazzaz, 1989). Seagrass-epiphyte systems are known to display such ecology-mediated responses whereby coastal nutrient enrichment, which theoretically could benefit both seagrass and epiphytes by releasing them from nutrient limitation (Kelaher et al., 2013), typically causes disproportionate increases in epiphyte proliferation that results in seagrass overgrowth and decline (Bryars et al., 2011, Orth et al., 2006, Silberstein et al., 1986, Walker & McComb, 1992). While physiology predicts the direct benefits of CO_2 enrichment for seagrass could exceed those for algae (Beer & Koch, 1996), purely physiological studies might have temporal constraints that do not reconcile changing ecological interactions, such as those between seagrass and their algal epiphytes. Importantly, any increase in epiphyte cover due to CO_2 enrichment has the potential to accelerate global seagrass loss (Short & Neckles, 1999).

The effects of global change will not be limited to interactions between primary producers, but also interactions across different trophic levels. Concurrent warming and acidification of global oceans will affect the physiology of organisms that graze upon primary producers (Calosi et al., 2013, Kordas et al., 2011). For example, warmer waters are predicted to increase the metabolic demands of grazers and thus promote greater consumption of primary producers (Hillebrand et al., 2009, O'Connor, 2009). In contrast, CO_2-induced acidification appears to have variable effects on grazers, particularly those such as echinoderms that produce calcium carbonate shells (Dupont et al., 2010). Calcifiers could be subject to CO_2 induced dissolution or a reduction in synthesis of shell material (Courtney *et al.*, 2013, Orr et al., 2005, Ries et al., 2009), therefore it has been suggested acidification will disrupt biological processes such that feeding ability and opportunities may be reduced (e.g. exo-skeletal structural damage, increased predator avoidance and damage to feeding parts) (Bibby et al., 2007, Marchant et al., 2010, Wood et al., 2008), but in other instances metabolic demands associated with physiological maintenance under acidified conditions might elevate energetic requirements and thus grazing (Gooding et al., 2009, Li et al., 2013). These direct effects on grazing herbivores will not act independently, but may also be modified if global changes create resources shifts in food quality (Falkenberg et al., 2013b). Such direct changes on herbivore physiology and indirect influences on food sources will have important implications for top-down processes that shape habitats.

1.4 SOUTHERN AUSTRALIAN SEAGRASSES

In southern Australia the primary sub-tidal habitat-forming seagrasses in Gulf St Vincent (Fig. 1.1) come from two genera, *Amphibolis and Posidonia*, although it should be mentioned for completeness that there are two other genera also commonly found (i.e. *Halophila* & *Zostera*), but these are more prevalent in estuarine, shallow, deep or disturbed habitats (Bryars et al., 2008). Therefore, *Amphibolis and Posidonia* are largely the two climax genera which can form dense long-lived sub-tidal seagrass meadows, with *Amphibolis* sometimes acting as an earlier coloniser that paves the way for *Posidonia* (Bryars et al., 2008, Shepherd & Sprigg, 1976). The genus *Amphibolis* which is endemic to southern Australia has two species, *Amphibolis antarctica* and *Amphibolis griffithii*, the latter of which is relatively rare in the semi-protected waters of Gulf St Vincent (Shepherd & Sprigg, 1976). In comparison, *Posidonia* has three species that are common to Gulf St Vincent, which constitute the *australis* complex. These two genera of seagrasses are often found in contiguous or interspersed mixed meadows (Bryars & Rowling, 2009), particularly *P. sinuosa* and *A. antarctica* (Cambridge & Kuo, 1979). In addition to some differences in distribution, the structural and morphological differences between these two genera are significant. *Amphibolis* spp. have an upright woody vertical shoot, with a number of short shoots and leaf heads on each stem, whereas the above ground biomass of *Posidonia* spp. consists entirely of strap like leaves (Fig. 1.2). Thus, the leaf meristems of *Amphibolis* spp. are elevated in the seagrass canopy, whereas those of *Posidonia* spp. are basal and protected in the sediment. For a more in depth descriptions of these two genera see Marbà and Walker (1999).

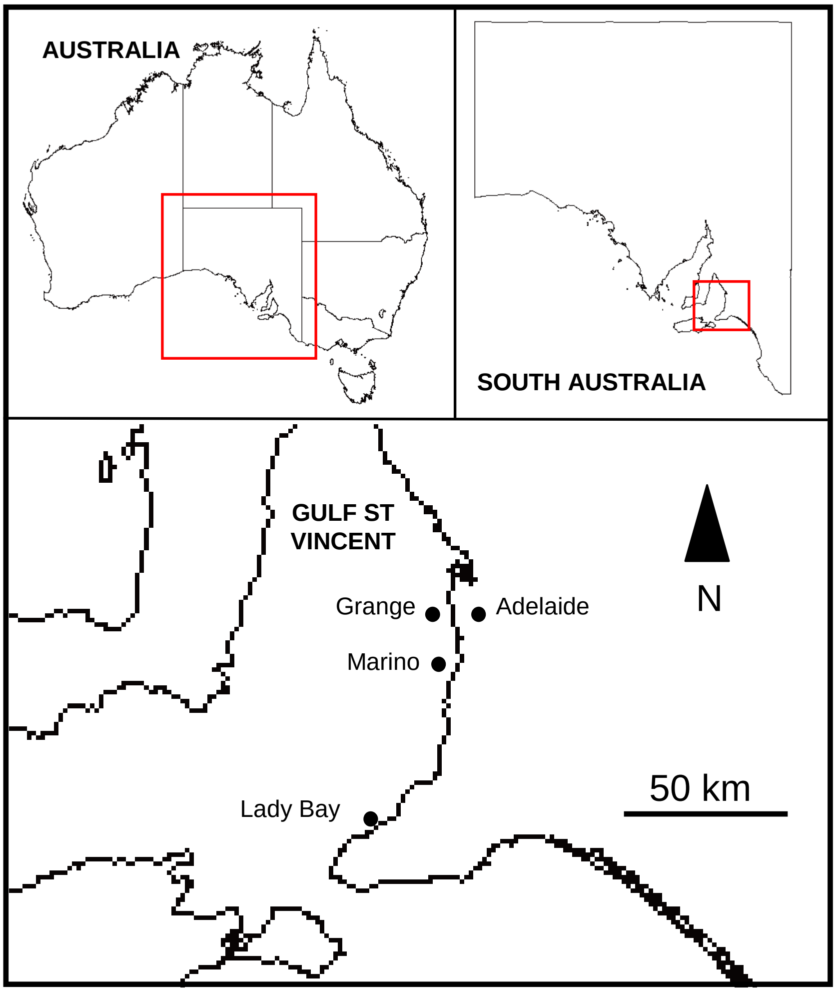


Figure 1.1 Gulf St Vincent, South Australia, including the primary metropolitan centre of Adelaide (approx. pop. of 1.2 million) and the three sites where seagrasses were collected or studied *in situ*, including Grange, Marino & Lady Bay.

Figure 1.2 The differing morphology of the two primary sub-tidal habitat-forming genera of seagrass from southern Australia. Above: An *Amphibolis antarctica* meadow. Below: A *Posidonia* sp. meadow. Photo credits: Owen Burnell. Illustrations: Tracey Saxby, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

1.5 CHANGING BIOTIC INTERACTIONS OF LOCAL ORIGIN

In seagrass habitats limited direct grazing upon leaves has traditionally led to the application of a bottom-up paradigm to describe the structuring and maintenance processes for these systems (Valentine & Duffy, 2006). Similarly, in many regions of southern Australia top-down grazing control of habitats is generally considered to be of relatively minor influence, including kelp-forests and seagrasses (Bulleri et al., 2012, Edgar & Shaw, 1995, Fowler-Walker & Connell, 2002). For seagrasses there are few accounts of significant fish grazing (Edgar & Shaw, 1995, Wressnig & Booth, 2008), some isolated cases of macro-invertebrate grazing (Cambridge et al., 1986, Kendrick et al., 2002, Langdon et al., 2011, Larkum & West, 1990) and certainly no large mammalian or reptilian herbivores (e.g. dugongs and turtles) that are of greater consequence in tropical and sub-tropical regions (Thayer et al., 1984, Valentine & Heck, 1991). Top-down herbivore driven events are more commonly documented worldwide for grazing on sub-tidal rocky reefs, where increased sea-urchins densities can cause phase-shifts from highly productive macro-algal forests to relatively unproductive barrens (Chapman & Johnson, 1990, Estes & Palmisan.Jf, 1974, Ling et al., 2009, Pearse, 2006). Once established these barrens can inhibit the recovery of macro-algae as urchins consume algal re-colonisers (Leinaas & Christie, 1996) and other physical feedbacks, such as sedimentation limit recruitment success (Valentine & Johnson, 2005). However, evidence for top-down grazing influences in seagrass meadows is accumulating worldwide with documented cases increasing over recent decades (Eklöf et al., 2008, Orth et al., 2006, Valentine & Duffy, 2006). An emerging local threat in a southern Australian seagrass meadow suggests grazing of seagrasses by the urchin *Amblypneustes* *pallidus* can denude habitats to a point of impairment where a temporary or permanent phase-shift from a healthy seagrass canopy to bare sand and dead shoots can occur (Fig. 1.3). Further to this, morphological traits such as those evident in the genus *Amphibolis* (i.e. elevated meristems) are known to mediate the vulnerability of other similar seagrass species to lasting top-down grazing effects (Alcoverro & Mariani, 2002). Such top-down grazer effects are defined as overgrazing where herbivory exceeds growth or in cases where plant fitness is significantly reduced (Eklöf et al., 2008). Importantly, such occurrences of overgrazing worldwide are particularly common in disturbed regions where changes in nutrients or overfishing modify seagrass-epiphyte-urchin interactions (Eklöf et al., 2008, Ruíz et al., 2009, Walker & McComb, 1992).

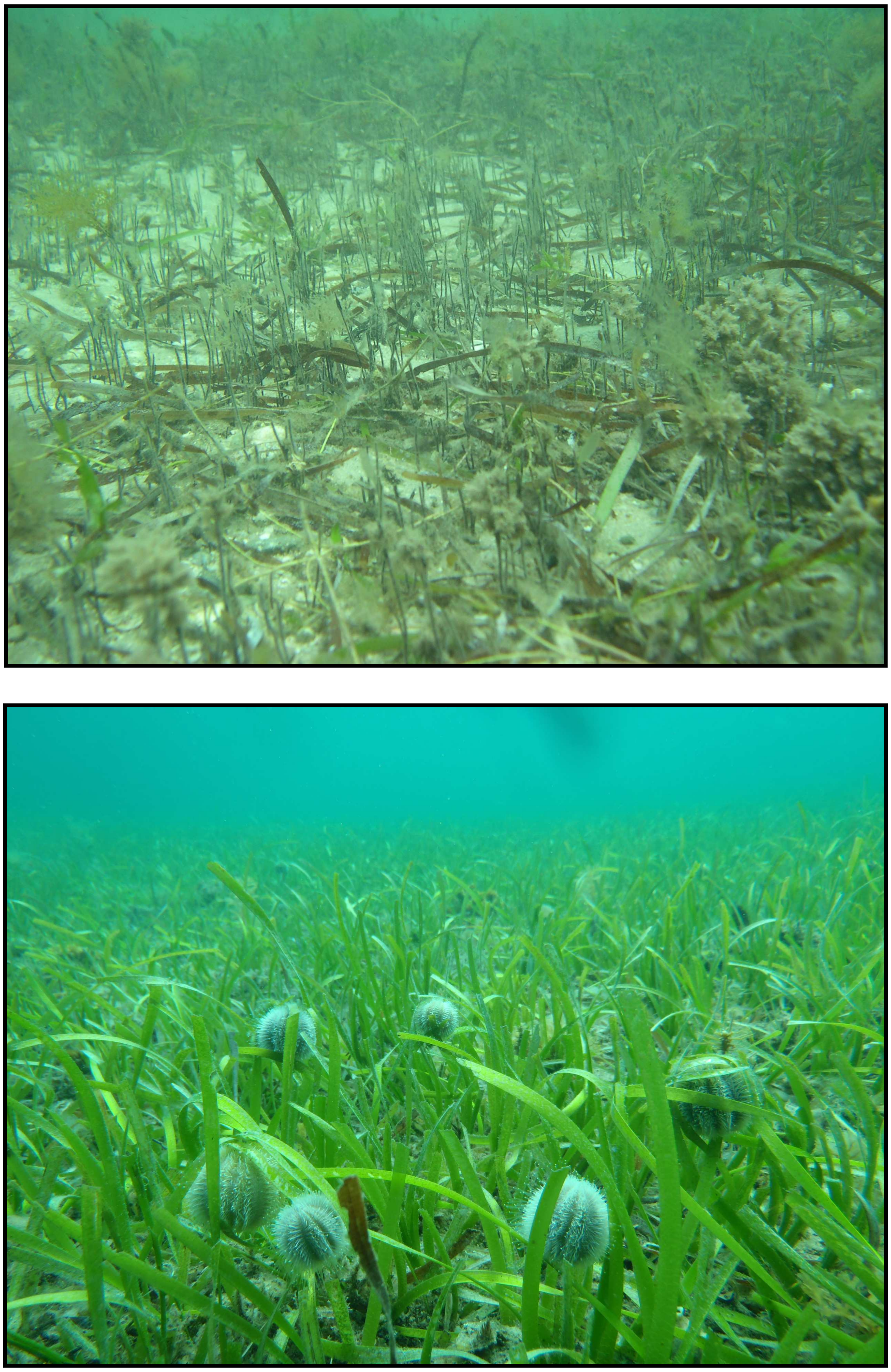


Figure 1.3 Contrasting damage to different seagrasses from sea-urchin grazing, Above: An *Amphibolis antarctica* meadow that has been heavily grazed by urchins; only dead shoots and detritus remain. Below: A *Posidonia* sp. meadow with aggregations of short-spined urchins *Amblypneustes pallidus*. Photo credits, Above: Andrew Irving; Below: Owen Burnell

Anthropogenic nutrient enrichment has been responsible for widespread declines in seagrass meadows (Walker & McComb, 1992, Waycott et al., 2009). While such declines commonly result from blooms of free-living and epiphytic algae that shade and smother seagrass (Bryars et al., 2011, Short & Wyllie-Echeverria, 1996, Walker & McComb, 1992), observations in eutrophic meadows suggest urchin grazing can be more prolific, such that it appears the combination of these changes often culminates in overgrazing (Eklöf et al., 2008, Kirkman & Young, 1981, Ruíz et al., 2001, Ruíz et al., 2009, Walker & McComb, 1992). However, grazing has traditionally received relatively less recognition as a direct driver of habitat loss; more often renowned for its indirect benefits as herbivores remove potentially damaging epiphytes (Hughes et al., 2004, McGlathery, 1995, Valentine & Duffy, 2006) or graze older seagrass leaves incidentally as these are often heavily covered with epiphytes (Wressnig & Booth, 2007). Such selective consumption might occur as urchins can obtain a high proportion of their nutritional requirements from epiphytes and epibiota growing on seagrass leaves, particularly in eutrophic meadows (McGlathery, 1995, Prado et al., 2010, Ruíz et al., 2009, Tewfik et al., 2005), therefore, in many instances epiphytes might reduce or redirect grazing impacts away from seagrass leaves (McGlathery, 1995, Tewfik et al., 2005, van Montfrans et al., 1984, Yamamuro, 1999). In some instances it appears that urchins only switch diets to seagrass leaves once more nutritious algal sources are exhausted (McGlathery, 1995). Nonetheless, evidence for the direct grazing on seagrasses continues to accumulate, suggesting the ingestion of these plants can contribute significantly to herbivore nutrition (Valentine & Duffy, 2006, Valentine & Heck, 1999). Thus, eutrophication might modify the structural and chemical composition of seagrass tissue, which indirectly modifies herbivore grazing (de los Santos et al., 2012, Vergés et al., 2011, Vergés et al., 2007b). The complexity of such interactions (seagrass-nutrient-epiphyte-urchin) in natural meadows means there is still much to learn about what drives changes in *per capita* and population grazing effects. A better understanding of how nutrient enrichment interacts with herbivory might shed light on the reasons for urchin overgrazing events.

1.6 THE STUDY OF MULTIPLE STRESSORS

Understanding how local- and global-scale changes combine will enable better forecasts of habitat loss or maintenance (Russell et al., 2009). Importantly, interactions between changing conditions may have a synergistic, antagonistic or ameliorative nature, whereby predictions based on independent or additive studies could misrepresent net impacts (Darling et al., 2010, Folt et al., 1999, Guinotte & Fabry, 2008, Przeslawski et al., 2005, Reynaud et al., 2003). Non-linear effects and interactions between stressors makes forecasting such responses inherently difficult, but research of this nature is important given most studies in marine systems report such outcomes (Crain et al., 2008). For example, in sub-tidal algae communities, CO_2 induced acidification is anticipated to deliver benefits to algal growth, but opposing negative effects from warming might act concurrently ameliorating any benefit, the complexity of which is even greater in systems with significant competitive interactions and trophic forces (Harley et al., 2012). Such forecasted opposing effects will also occur for abiotic changes in seagrass meadows, further complicated by the effect of changing biotic interactions, such as overgrowth by algal epiphytes and grazing by keystone echinoderms, which can both inhibit seagrass meadows.

1.7 SCOPE AND OUTLINE OF THE THESIS

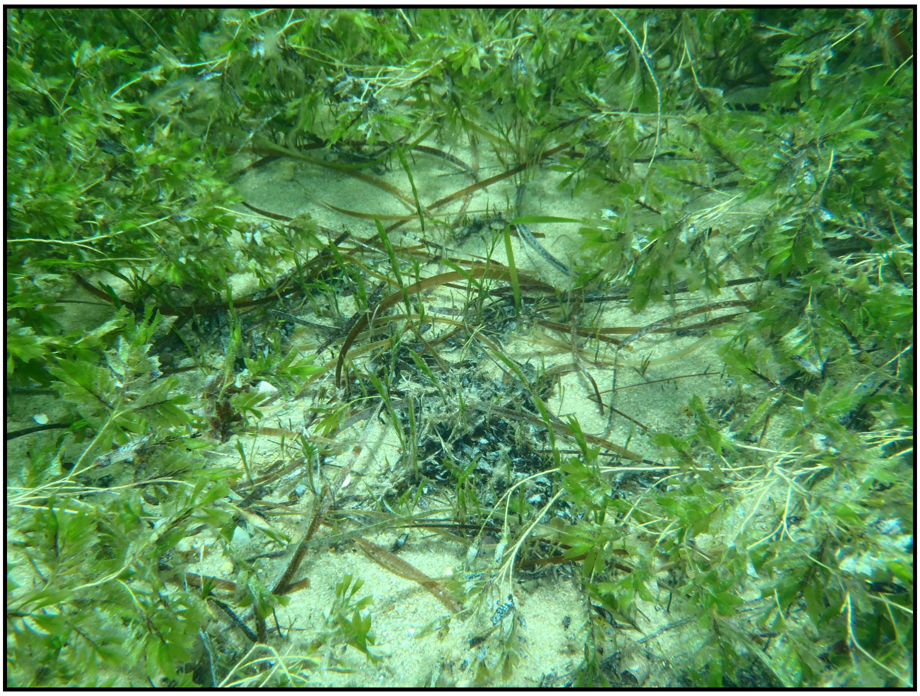
This thesis summarises my doctoral research regarding *Seagrass ecology and physiology in a locally and globally changing environment*, focussing upon abiotic conditions and biotic interactions that can modify seagrass habitats. The thesis focus predominantly on the seagrass species *Amphibolis antarctica*, which is endemic to southern Australia and has experienced great declines over the preceding century (Bryars et al., 2011, Walker & McComb, 1992). Firstly, I examine how the grazing of a sea-urchin poses a contemporary threat in local seagrasses. Next, I consider the carbon physiology of seagrasses with reference to its implications for the future productivity and growth of seagrass in response to increasing CO_2. I then examine how a critical biotic interaction in seagrass meadows (i.e. seagrass and their algal epiphytes) could be modified by CO_2 and light, which are two of the most commonly limiting abiotic conditions for seagrasses. Finally, I use seagrass-urchin interactions as a model system to identify how local- and global-scale changes (i.e. nutrients, warming and CO_2) could modify grazing loss. The specific aims were:

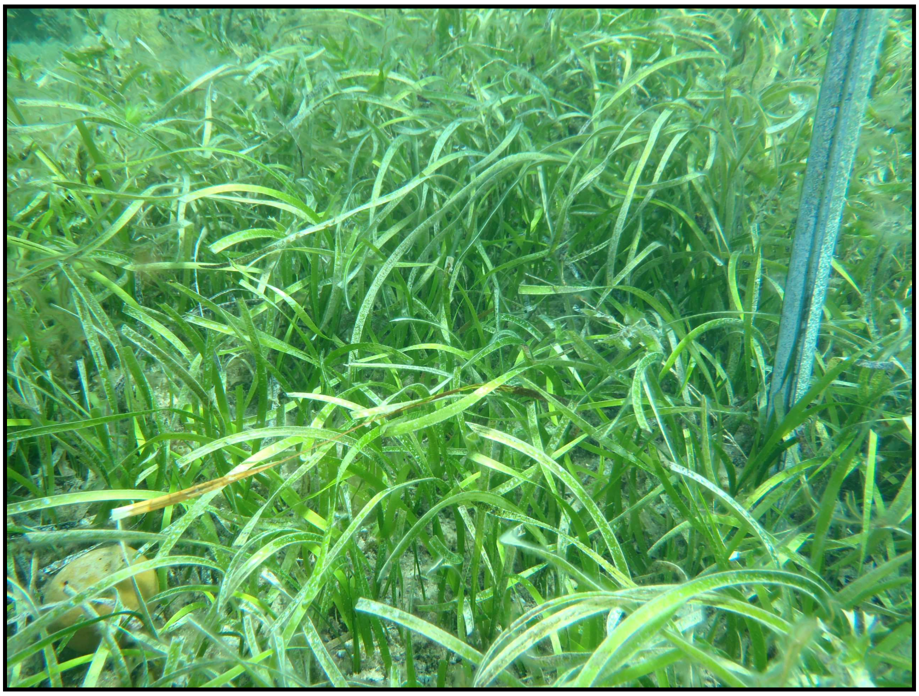
1. To quantify the grazing impact of the sea-urchin *Amblypneustes pallidus* on two different seagrass species from the most common habitat-forming sub-tidal genera in southern Australia (i.e. *Amphibolis* and *Posidonia*), based on their loss and recovery rate.
2. To test if the negative effects of nutrient enrichment on seagrasses (i.e. epiphytic overgrowth) could be weakened by sea-urchin grazing. Thus an antagonistic interaction might emerge where greater food quantity or quality

(i.e. bottom-up effects) could reduce direct grazing impacts (i.e. top-down effects) on seagrass habitats.

3. To quantify the usage of energetically costly bicarbonate for photosynthesis by seagrass from the genera *Amphibolis* and *Posidonia*, and test implications for photosynthetic efficiency and growth under forecasted future CO_2.
4. To test if epiphyte growth could modify the predicted direct benefits of forecasted CO_2 for seagrass under conditions where epiphyte growth is favoured (i.e. high light).
5. To test how local- and global-scale change to abiotic conditions (i.e. nutrients, warming and CO_2) could combine to modify sea-urchin grazing in future seagrass meadows.

While each of these research aims is addressed in a different thesis chapter and forms an independent body of work, the logical sequence begins with locally significant biotic interactions in seagrass meadows (i.e. urchin population expansions), transitioning to globally derived changes which are forecast primarily as a result of greater atmospheric CO_2 (i.e. warming and CO_2). Collectively, this thesis builds upon our understanding of the many abiotic and biotic factors that can influence seagrass habitat abundance, both now and in the future.





Asymmetric patterns of recovery

Above: A cleared plot of *Amphibolis antarctica* shows little recovery after eight weeks. Below: A cleared plot of *Posidonia sinuosa* is almost indistinguishable eight weeks after clearance.

Photo credits: Owen Burnell

CHAPTER TWO

ASYMMETRIC PATTERNS OF RECOVERY IN TWO HABITAT FORMING SEAGRASS SPECIES FOLLOWING SIMULATED OVERGRAZING BY URCHINS

Owen W. Burnell, Sean D. Connell, Andrew D. Irving and Bayden D. Russell

School of Earth and Environmental Sciences,
The University of Adelaide, Adelaide, SA 5005 Australia

Journal of Experimental Marine Biology and Ecology 2013, 448 pp 114-120

STATEMENT OF AUTHORSHIP

In this paper I executed the laboratory work and analysed the data. I wrote the manuscript and undertook the field experiment, assisted by Sean D. Connell, Andrew D. Irving & Bayden D. Russell. All authors contributed to the experimental design. Bayden D. Russell, Sean D. Connell & I provided funding for the project.

Signatures of co-authors:

Professor Sean D. Connell

Dr Andrew D. Irving

Dr Bayden D. Russell

PREAMBLE

Chapter 2 was motivated by Andrew D. Irving, who observed the often inconspicuous sea-urchin *Amblypneustes pallidus* in large aggregations and inflicting significant damage to the seagrass *Amphibolis antarctica*. The chapter is a reproduction of a co-authored manuscript published in the *Journal of Experimental Marine Biology and Ecology*, and therefore is written in plural throughout and uses the journal formatting. The paper is primarily written for a global audience with an interest in grazing ecology and how this can interact with species-specific morphology and recovery. The manuscript can be cited as:

Burnell OW, Connell SD, Irving AD, Russell BD (2013) Asymmetric patterns of recovery in two habitat forming seagrass species following simulated overgrazing by urchins. *Journal of Experimental Marine Biology and Ecology*, 488: 114-120.

CHAPTER TWO

ASYMMETRIC PATTERNS OF RECOVERY IN TWO HABITAT FORMING SEAGRASS SPECIES FOLLOWING SIMULATED OVERGRAZING BY URCHINS

2.1 ABSTRACT

The persistence of seagrass meadows reflects variation in factors that influence their productivity and consumption. Sea-urchins (*Amblypneustes pallidus*) can over-graze seagrass (*Amphibolis antarctica*) to create sparse meadows in South Australia, but this effect is not observed in adjacent *Posidonia sinuosa* meadows despite greater densities of inhabiting urchins. To test the effect of urchin grazing on seagrass biomass, we elevated the density of urchins in meadows of *A. antarctica* and *P. sinuosa* and quantified seagrass decline. Urchins removed similar amounts of biomass from both seagrass species, but the loss of leaf meristems was 11-times greater in *A. antarctica* than *P. sinuosa*. In a second experiment to assess the recovery of seagrass, we simulated urchin grazing by clipping seagrass to mimic impacts measured in the first experiment, as well as completely removing all above ground biomass in one treatment. Following simulated grazing, *P. sinuosa* showed a rapid trajectory toward recovery, while *A. antarctica* meadows continued to decline relative to control treatments. While both *A. antarctica* and *P. sinuosa* were susceptible to heavy grazing loss, consumption of the exposed meristems of *A. antarctica* appears to reduce its

capacity to recover, which may increase its vulnerability to long-term habitat phase-shifts and associated cascading ecosystem changes.

2.2 INTRODUCTION

Species that create widespread habitats provide the foundations for entire ecological systems (Barbier, et al., 2011), but tend to be susceptible to disturbances that increase their consumption relative to productivity (Connell, et al., 2011; Steneck, et al., 2002). This disruption to the balance between productivity and consumption is perhaps most renown along sub-tidal rocky coasts where sea-urchins can overgraze kelp forests to create ‘barrens’ (Chapman and Johnson, 1990; Ling, et al., 2009; Pearse, 2006). Overgrazing can occur where the rate of herbivory exceeds the rate of plant productivity for sustained periods, resulting in diminished ecosystem functions (Eklöf, et al., 2008). In seagrass meadows, overgrazing by urchins can cause phase-shifts to bare habitats (Eklöf, et al., 2008; Rose, et al., 1999), which can impact coastal productivity, biodiversity, food-webs, sediment stabilisation, turbidity, nutrient cycling, and carbon sequestration (Duarte, 2002; Orth, et al., 2006). Such impacts may be persistent given that many species can take decades to centuries to recover from disturbance (Bryars and Neverauskas, 2004; Kirkman and Kuo, 1990; Irving, 2013).

On southern Australian coasts, the seagrasses *Amphibolis* spp. and *Posidonia* spp. can form extensive co-occurring habitats on moderately wave-exposed shores (Bryars and Rowling, 2009; Shepherd and Womersley, 1981). Widespread historical losses (Walker and McComb, 1992), limited re-colonisation (Bryars and

Neverauskas, 2004; Kendrick, et al., 2002; Irving, 2013), and slow meadow expansion rates (Marbà and Walker, 1999) have demonstrated the susceptibility of these seagrasses to natural and anthropogenic impacts. Among natural effects, overgrazing has been observed but appears to disproportionately impact *Amphibolis antarctica* (author's pers. obs.), possibly because it is a species that produces a canopy of accessible meristematic leaf clusters while *Posidonia* spp. meristems are protected below ground (Alcoverro and Mariani, 2002; Marbà and Walker, 1999; Short and Duarte, 2001).

The purpose of this study was to determine how sea-urchin grazing causes the loss and affects the recovery of the seagrasses *A. antarctica* and *Posidonia sinuosa*. In southern Australia, the short-spined sea-urchin *Amblypnuestes pallidus* can over-graze *A. antarctica* with little to no recovery for at least 3 years (author's pers. obs.). However, grazing effects on *P. sinuosa*, which often co-exist in mixed or adjacent meadows with *A. antarctica*, appear less pronounced despite urchins often occurring in greater densities in *P. sinuosa*. We experimentally tested the hypothesis that a fixed density of *A. pallidus* would disproportionately impact the biomass of *A. antarctica* relative to *P. sinuosa*. In a second experiment we tested the hypothesis that *P. sinuosa* would show greater recovery than *A. antarctica* following simulated grazing damage.

2.3 MATERIALS & METHODS

2.3.1 Study region

All experiments were done at Lady Bay (35°28.036 S, 138°17.198 E), approximately 70 km South-West of the city of Adelaide, South Australia. Seagrass meadows in this region are largely intact and unaffected by anthropogenic impacts more common to the Adelaide metropolitan coastline (e.g. eutrophication). Meadows comprise a mosaic of *P. sinuosa*, *Posidonia angustifolia* and *A. antarctica*, interspersed by small rocky outcroppings and sand patches. The short-spined sea-urchin *A. pallidus* is a small (i.e. 2 - 4 cm) and often inconspicuous seagrass grazer that is normally sparsely distributed but occasionally greatly exceeds background densities (author's pers. obs.). All sampling was done between depths of 4 - 5.5 m.

2.3.2 Field sampling

Approximately three months after a sea-urchin overgrazing event in *A. antarctica* meadows during February 2010, seagrasses were sampled within affected and unaffected meadows. Meadows were defined as 'affected' where urchins had been observed actively grazing seagrass and there was clear evidence of defoliation and dead shoots. Meadows were defined as 'unaffected' where urchins had not been observed grazing seagrass and there was limited evidence of defoliation or dead shoots. Five 0.0625 m^2 quadrats of *A. antarctica* were harvested at the substratum, transported on ice under dark conditions and frozen for later analysis of above ground biomass and shoot density.

As no conspicuous effects of grazing were observed in *P. sinuosa* meadows, we tested for a relationship between urchin and *P. sinuosa* biomass, on three different dates, from December 2011 to March 2012. At four sites, each separated by a minimum of 1 km, urchin abundance was counted in five 10 × 1 m transects. Twenty urchins were then haphazardly collected and weighed while wet to estimate total biomass m^{-2}. Five 0.0625 m^2 quadrats of *P. sinuosa* were then harvested at the substratum to quantify above ground biomass. We undertook the same sampling procedure in nearby *A. antarctica* meadows, however, urchin numbers were so low (zero at 66% of sites) that no relationship could be established, therefore only average urchin densities are presented.

2.3.3 Urchin grazing (Experiment 1)

Manipulative experiments testing the effect of *A. pallidus* grazing on seagrass biomass were done within nearby mono-specific meadows of *A. antarctica* and *P. sinuosa*, where the naturally occurring urchin populations observed during the experimental period (March to August 2011) were zero. For each seagrass species, fifteen experimental plots were randomly interspersed over 500 m^2 to separate plots by at least four meters. Five plots were designated as treatments of elevated grazing by adding two urchins within a wire mesh cage anchored by fence droppers (mesh size 0.02 × 0.02 m, cage size 0.4 × 0.4 × 0.4 m) to create urchin densities within the natural range observed at Lady Bay (12.5 m^{-2}; average wet urchin biomass = 29.45 ± 0.40 g). Urchins were collected from nearby beds of *Posidonia* spp. and *A. antarctica*, with weekly replacement of dead individuals if necessary. A procedural control was established in another five plots of each species using a partial cage to test for caging artefacts on seagrass. The remaining five plots were un-manipulated and served as controls. Cages were cleaned of algal growth weekly. One grazing replicate from each species of seagrass was lost due to storm damage during June. These treatments were not replaced.

Destructive sampling of seagrass was done at 82 and 169 days. Small 0.01 m^2 samples of seagrass (i.e. 6 % of experimental plots) were harvested after 82 days and their location marked to prohibit re-sampling. Larger 0.0625 m^2 samples were taken after 169 days. Above ground biomass was harvested at the substratum, transported on ice under dark conditions and frozen for later measurement of above ground leaf biomass, meristem survival and grazing damage. Below ground biomass was only sampled at the conclusion of the experiment (i.e. 169 days) by excavating roots and rhizomes in 0.0625 m^2 quadrats to a depth of 20 cm.

Seagrass biomass was measured by removing any fleshy epiphytes with a razor blade, rinsing in MilliQ water to remove salts and sediment, and then drying at 60°C for 48 h. For *A. antarctica,* vertical rhizomes and leaves were separated prior to drying to determine above ground leaf biomass in grams of dry weight per square metre (gdw m^{-2}). Leaf meristems were counted in *A. antarctica* if the apical meristem was intact on each leaf cluster, determined by the survival of the youngest leaf in the cluster. Leaf meristems of *P. sinuosa* were counted if sheaths contained at least one living leaf. In the final samples all *A. antarctica* shoots were classified as either alive (i.e. containing living meristems) or dead. As urchins can graze *A. antarctica* directly at the shoot, this was the best way to estimate rates of grazing damage. For *P. sinuosa* 20 individual leaves were selected and the leaf tips identified as grazed if possessing an apex consistent with urchin grazing scar.

2.3.4 Grazing experiment statistical analyses

Repeated measures ANOVA was used to compare the effects of grazing and seagrass species on leaf biomass for both the halfway (82 day) and final (169 day) sampling dates of the grazing experiment using SPSS. Urchin grazing, seagrass species (between subject factors) and time (within subject factor) were treated as fixed. Halfway and final above ground leaf biomass were fourth-root transformed to satisfy the assumption of homogeneity of variances and normality, which were analysed using Levene's test and Shapiro-Wilk's test, respectively. Sphericity could not be evaluated as there were only two levels of the repeated measure time.

Two-factor ANOVA was used to test for the effects of urchin grazing and seagrass species on below ground biomass, meristem survival and grazing damage using PERMANOVA+ for Primer v6. Urchin grazing and seagrass species were treated as fixed factors in the orthogonal design. Cochran's *C*-test was used to test for homogeneity of variance prior to analysis. Below ground biomass and meristem survival violated the assumption of homogeneity of variance, therefore data was log-transformed prior to analysis. Grazing damage was ArcSin (%) transformed prior to analysis. Pairwise tests were used where significant interactions terms were detected.

2.3.5 Recovery from simulated grazing (Experiment 2)

To quantify seagrass recovery from overgrazing, urchin grazing was simulated in both *A. antarctica* and *P. sinuosa* using scissors to clip above ground biomass. The recovery experiment was completed in different mono-specific beds of *A. antarctica* and *P. sinuosa* adjacent to where the grazing experiment was undertaken. Three treatments were established: control (no biomass removed), partial removal (removal equal to the urchin effects sampled in the grazing experiment; see section 2.6) and complete removal (100 % removal of above ground biomass). Five replicate plots 0.4 × 0.4m (0.16 m^2) for each treatment were established in both seagrass species, randomly interspersed over a 500 m^2 area for each species and marked using star droppers. The experiment commenced seven weeks after the grazing experiment and continued for 109 days from October 2011 to January 2012, after which above ground biomass was harvested by destructively sampling within 0.0625 m^2 quadrats before processing seagrass, as described above.

2.3.6 Partial removal treatment

Due to the different morphologies of *Amphibolis* spp. and *Posidonia* spp. unique removal techniques were utilised to accurately replicate grazing loss for each species. While *Amphibolis* spp. have woody vertical rhizomes, with a number of short shoots and leaf heads on each stem, the above ground biomass of *Posidonia* spp. consists entirely of strap like leaves. For a detailed summary of the morphology of these species see Marbà and Walker (1999). Because urchins can remove either entire or partial sections of vertical rhizomes and leaves, clipping of both entire and partial vertical rhizomes (*A. antarctica*) and leaves (*P. sinuosa*) was required to accurately replicate grazing loss. Firstly, clipping of entire vertical rhizomes (*A. antarctica*) and leaves (*P. sinuosa*) at the substrate was undertaken to match loss recorded between control and urchin grazing treatments in Experiment 1. Secondly, clipping of individual leaf heads (*A. antarctica*) and partial leaves (*P. sinuosa*) was undertaken to account for the removal of partial vertical rhizomes and leaves from Experiment 1. For each experimental plot (0.4 × 0.4m) in *A. antarctica*, 90 entire vertical rhizomes (560 m^{-2}) at an average 0.19 gdw vertical $rhizome^{-1}$ and 132 individual leaf heads (825 m^{-2}) at an average 0.07 gdw leaf $head^{-1}$ were removed for a total removal of 26.28 gdw $plot^{-1}$ (164.2 g m^{-2}). For each experimental plot (0.4 × 0.4m) in *P. sinuosa* 197 entire leaves (1232 m^{-2}) at an average of 0.06 gdw $leaf^{-1}$ and 435 partial leaves (2720 m^{-2}) at an average of 0.03 gdw partial $leaf^{-1}$ were removed for a total removal of 24.77 gdw $plot^{-1}$ (154.8 g m^{-2}). Average weights of vertical rhizomes and leaf heads (*A. antarctica*) and leaves (*P. sinuosa*) were calculated from Experiment 1.

2.3.7 Recovery from simulated grazing statistical analyses

Two-factor ANOVA was used to test for the effects of simulated grazing and seagrass species on seagrass recovery using PERMANOVA+ for Primer v6. Species and simulated grazing were treated as fixed factors. Partial removal and complete removal treatments were compared independently of one another against controls in two separate analyses. This approach was taken as the partial removal treatment tested urchin effects from Experiment 1 (i.e. different pre-recovery biomass for each species) whereas the complete removal tested simulated grazing of all above ground biomass (i.e. identical pre-recovery biomass for each species). Cochran's *C*-was used to test for homogeneity of variance prior to analysis of variance. Pairwise tests were used where significant interaction terms were detected.

2.4 RESULTS

2.4.1 Field sampling

Sampling of *A. antarctica* meadows following observations of elevated *A. pallidus* densities suggested that urchins may have been responsible for the loss of > 83 % of above ground biomass (133.2 *v.* 21.7 gdw m^{-2}) and > 96 % of living shoot density (360.6 *v.* 12.8 shoots m^{-2}) in un-affected *v.* affected meadows. While *P. sinuosa* meadows appeared un-impacted by urchin grazing during our initial observations, we later sampled a highly variable negative relationship between urchin and *P. sinuosa* biomass, with regression coefficient values ranging between $0.11 \geq r^2 \geq 0.90$ over three sampling dates. The maximum density of urchins recorded in *P. sinuosa* across the three sampling dates and sites was 8.76 ± 0.50 m^{-2}, with mean densities of 1.03 ± 0.78 m^{-2}, although densities as high as 34.6 ± 3.88 m^{-2} were recorded two months prior to the first sampling date. During the same period in nearby *A. antarctica* meadows, urchin densities did not exceed 0.70 ± 0.11 m^{-2}, with mean densities of 0.12 ± 0.11 m^{-2}.

2.4.2 Grazing experiment (Experiment 1)

Urchin grazing reduced the above ground leaf biomass of *A. antarctica* and *P. sinuosa*. After 169 days there was no difference in the remaining leaf biomass between the two species (Fig. 2.1A,B Table 2.1), while, the rate of decline appeared faster in *P. sinuosa* than in *A. antarctica*; this effect was not significant in the repeated measures ANOVA (Table 2.1, Time × Species × Grazing interaction). The procedural controls had no effect on the leaf biomass of seagrass at either halfway or final sampling point (Fig. 2.1A, B, Table 2.1).

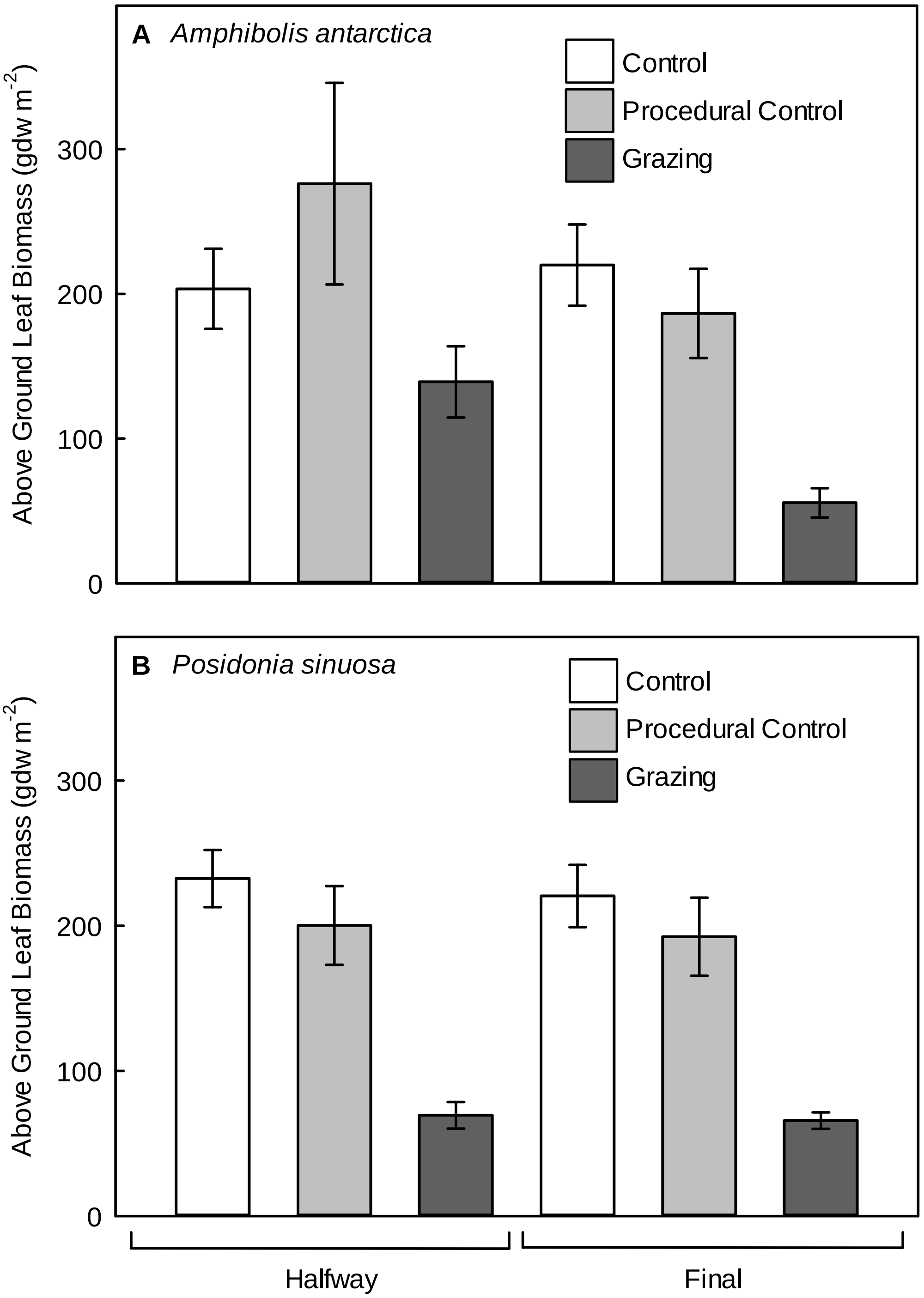


Figure 2.1 The effect of sea-urchins *Amblypneustes pallidus* (control, procedural control *v.* grazing) on the halfway (82 days) and final (169 days) above ground leaf biomass (A. *Amphibolis antarctica*; B. *Posidonia sinuosa*) of seagrasses (gdw m^{-2}). Bars indicate the mean and standard error.

Table 2.1 Repeated measures analysis of variance comparing the effect of urchin grazing (control, procedural control *v.* grazing), seagrass species (*Amphibolis antarctica v. Posidonia sinuosa*) and time (halfway *v.* final) on the above ground leaf biomass of seagrasses. Significant effects indicated in bold where p<0.05.

Source	**df**	**MS**	***F***	**p**
Leaf Biomass				
Between Subjects				
Species	1	0.064	0.528	0.475
Grazing	**2**	**4.779**	**39.536**	**0.000**
Species × Grazing	2	0.087	0.716	0.499
Residual	24	0.121		
Within subjects				
Time	**1**	**0.423**	**4.626**	**0.042**
Time × Species	1	0.253	2.772	0.109
Time × Grazing	2	0.157	1.714	0.202
Time × Species × Grazing	2	0.181	1.986	0.159
Residual	24	0.091		

Pairwise comparison: Leaf Biomass

Urchin grazing: control = procedural control > grazing

Grazing damage, quantified by the percentage of dead shoots (*A. antarctica*) and leaves with grazing scars (*P. sinuosa*), increased where urchins were present, however, there was no difference between the two species (Fig. 2.2A,B , Table 2.2). It is worth noting that data for both *A. antarctica* and *P. sinuosa* may underestimate the frequency of grazing damage; given that in *A. antarctica* all leaf heads had to be removed for a shoot to be classified as dead and in *P. sinuosa* some consumed leaves may have been totally removed leaving no evidence of grazing.

Urchin presence significantly reduced meristem survival in *A. antarctica*, but had no impact in *P. sinuosa*. While *A. antarctica* had a greater abundance of leaf meristems than *P. sinuosa* where urchins were absent, this effect was reversed where urchin densities were elevated, highlighting the significant loss of *A. antarctica* leaf heads and associated meristems due to grazing (Fig. 2.2C,D, Table 2.2: Grazing × Species interaction).

Below ground biomass was greater in *P. sinuosa* than in *A. antarctica*, but the grazing treatment had no impact on below ground biomass of either species (Fig. 2.2E,F, Table 2.2).

2.4.3 Seagrass recovery from simulated grazing (Experiment 2)

Recovery of biomass in *P. sinuosa* following simulated grazing exceeded that for *A. antarctica* (56 and 62 % *v.* 7 and 25 % of controls in the complete and partial removal treatments, respectively) (Fig. 2.3, Tables 2.3 & 2.4). Recovery of *P. sinuosa* was greater where more seagrass was removed (a direct relationship between removal and recovery), whereas in *A. antarctica* recovery declined as removal increased (an inverse relationship between removal and recovery) (Table 2.3).

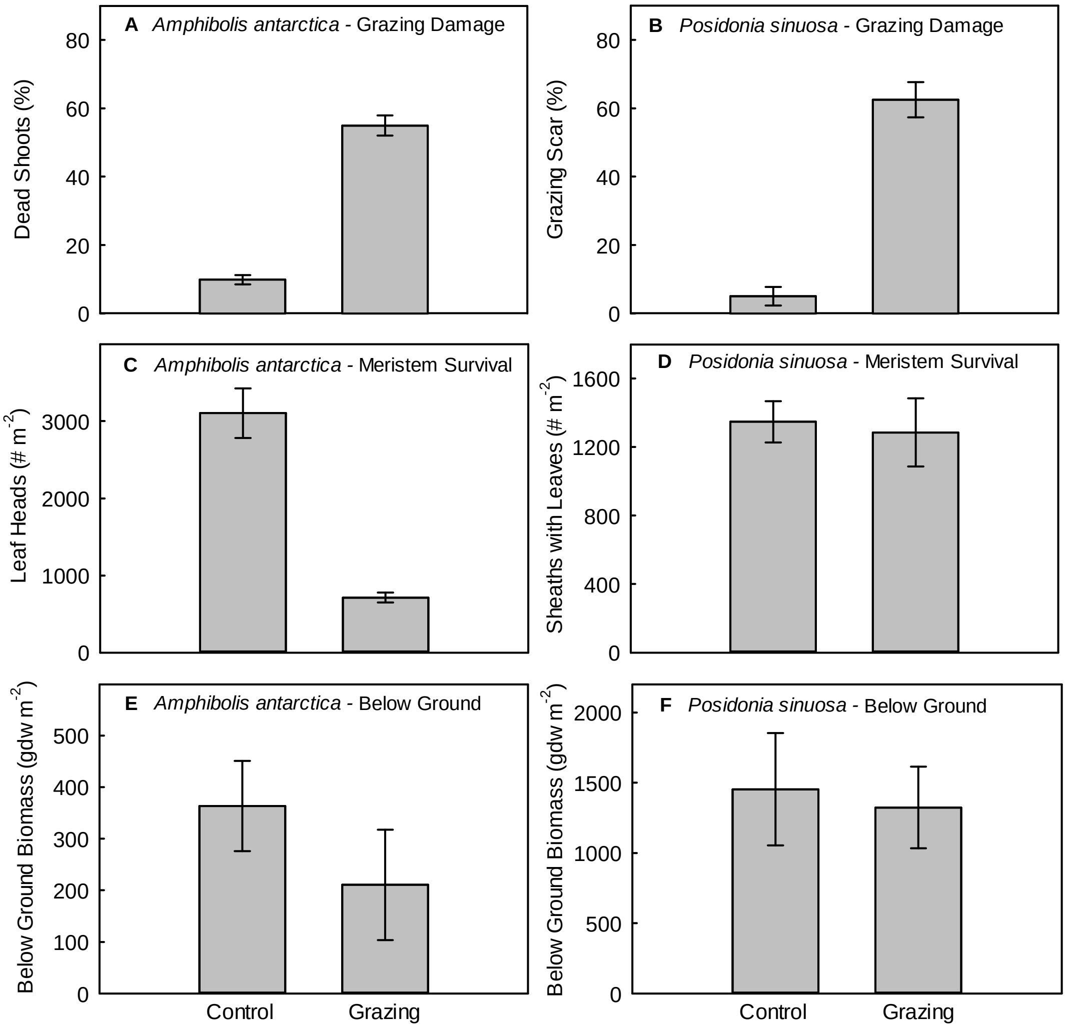


Figure 2.2 The effect of sea-urchins *Amblypneustes pallidus* (control *v.* grazing), on grazing damage (A. *Amphibolis antarctica*, percentage dead shoots; B. *Posidonia sinuosa*, percentage of leaves with grazing scar), leaf meristem survival (C. *Amphibolis antarctica*, # leaf heads; D. *Posidonia sinuosa*, # sheaths with leaves) and final (169 days) below ground biomass (E. *Amphibolis antarctica*, gdw m^{-2}; F. *Posidonia sinuosa*, gdw m^{-2}). Bars indicate the mean and standard error.

Table 2.2 Two factor analysis or variance comparing the effect of urchin grazing (control *v.* grazing) and seagrass species (*Amphibolis antarctica v. Posidonia sinuosa*) on grazing damage, leaf meristem survival and below ground biomass of seagrasses. Significant effects indicated in bold where p<0.05.

Source	df	MS	*F*	p
Grazing Damage				
Grazing	**1**	**1.557**	**194.610**	**0.001**
Species	1	0.003	0.388	0.526
Grazing × Species	1	0.027	3.380	0.092
Residual	16	0.008		
Leaf Meristem Survival				
Grazing	1	0.543	61.834	0.001
Species	1	0.016	1.794	0.213
Grazing × Species	**1**	**0.463**	**52.778**	**0.001**
Residual	16	0.009		
Below Ground Biomass				
Grazing	1	0.072	0.715	0.407
Species	**1**	**2.623**	**26.086**	**0.001**
Grazing × Species	1	0.135	1.344	0.296
Residual	16	0.101		

Pairwise comparisons: Leaf Meristem Survival

Urchin grazing: *A. antarctica*, control > grazing; *P. sinuosa*, control = grazing;

Seagrass species: control, *A. antarctica* > *P. sinuosa*; grazing, *A. antarctica* < *P. sinuosa*.

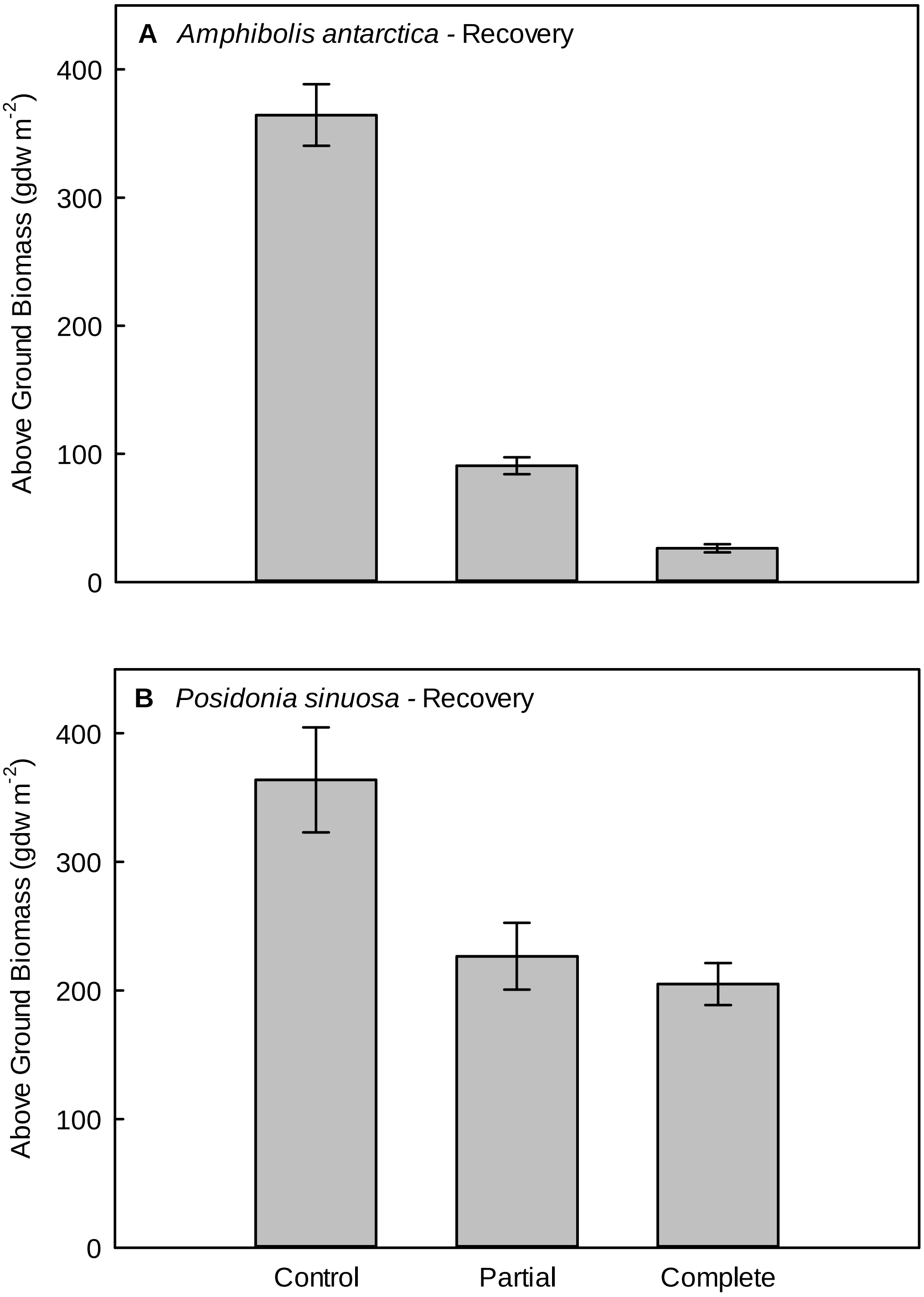


Figure 2.3 The effect of simulated urchin grazing (control, partial removal *v.* complete removal) on the above ground leaf biomass (A. *Amphibolis antarctica*; B. *Posidonia sinuosa*) of seagrass (gdw m^{-2}) after 109 days. Bars indicate the mean and standard error.

Table 2.3 Final seagrass dry weight from Experiment 1 (Grazing Experiment) and Experiment 2 (Simulated Grazing Experiment) used to calculate biomass recovery during the Simulated Grazing Experiment. Recovery was calculated by subtracting leaf biomass at the conclusion of the Grazing Experiment from leaf biomass at the conclusion of the Simulated Grazing Experiment, given that the biomass removed in the partial removal treatment of the Simulated Grazing Experiment mimicked that removed by urchins.

Treatment	**Urchin Grazing (Exp 1) (gdw m^{-2})**	**Simulated Grazing (Exp 2) (gdw m^{-2})**	**Calculated Recovery (Exp 2) (gdw m^{-2})**
Amphibolis antarctica			
Control	219.84 ± 28.11	364.32 ± 23.99	144.48
Urchin Grazing/Partial Removal	55.64 ± 10.12	90.75 ± 6.64	35.11
Complete Removal	n/a	26.24 ± 3.17	26.24
Posidonia sinuosa			
Control	220.58 ± 21.47	363.78 ± 40.79	143.20
Urchin Grazing/Partial Removal	65.76 ± 5.72	226.62 ± 25.92	160.86
Complete Removal	n/a	204.96 ± 16.28	204.96

Table 2.4 Analysis of variance comparing the effect of simulated grazing (control *v.* partial removal and control *v.* complete removal) and species (*Amphibolis antarctica v. Posidonia sinuosa*) on above ground leaf biomass following 109 days of recovery. Significant effects indicated in bold where p<0.05.

Source	df	MS	*F*	p
Leaf Biomass (Partial Removal)				
Grazing	1	210860	57.08	0.001
Species	1	22892	6.20	0.032
Grazing × Species	**1**	**23262**	**6.30**	**0.024**
Residual	16	3694		
Leaf Biomass (Complete Removal)				
Grazing	1	308630	98.20	0.001
Species	1	39683	12.63	0.006
Grazing × Species	**1**	**40169**	**12.78**	**0.003**
Residual	16	3143		

Pairwise comparisons: Partial Removal

Grazing: *A. Antarctica*, control > partial removal, *P. sinuosa*, control > partial removal; **Species:** control, *A. antarctica* = *P. sinuosa*, partial removal, *A. Antarctica* < *P. sinuosa*;

Pairwise comparisons: Complete Removal

Grazing: *A. Antarctica*, control > complete removal, *P. sinuosa*, control > complete removal; **Species:** control, *A. antarctica* = *P. sinuosa*, complete removal, *A. antarctica* < *P. sinuosa*.

2.5 DISCUSSION

Grazing by sea-urchins has often been considered important for shaping the composition and extent of habitats among sub-tidal rock and coral reefs. While seagrass habitats were long believed to be predominantly bottom-up controlled, recent literature demonstrates that top-down forces can also play an important role in regulating seagrass ecosystems (Eklöf, et al., 2008; Valentine and Duffy, 2006). Here, we identify that sea-urchins living at elevated though naturally occurring densities can switch habitat from dense to sparse seagrass meadows through overgrazing, highlighting the susceptibility of seagrasses to top-down grazing under certain conditions. While net grazing effects were consistent across both species of seagrass, rates of recovery following simulated grazing were far greater in *P. sinuosa* than in *A. antarctica.* Such asymmetric patterns in seagrass recovery from overgrazing suggest that the duration of phase shifts from seagrass to bare habitats caused by overgrazing, and any cascading ecosystem effects, can be dependent on the identity of species which is lost.

The substantial loss of *A. antarctica* during a natural overgrazing event, compared to the apparent stability of *Posidonia* spp. biomass in adjacent meadows, suggested that urchin grazing effects may be species-specific. Therefore, the similar decline in seagrass biomass for both *A. antarctica* and *P. sinuosa* during the grazing experiment was initially surprising. The strong positive trajectory toward the recovery of *P. sinuosa* following simulated grazing, however, was in stark contrast to *A. antarctica*, which showed minimal recovery. An elevated capacity for regrowth is a successful strategy used by many terrestrial plants exposed to high grazing pressure (Strauss and Agrawal, 1999; van der Meijden, et al., 1988), and may better prepare some species for herbivore driven disturbances. Given variability in urchin densities, periods with reduced urchin presence are common (Pearse and Hines, 1987; Rose, et al., 1999). Therefore, the combination of per capita grazing intensity, urchin return or recruitment interval and rates of seagrass recovery from grazing may all be crucial to long-term seagrass habitat persistence (Alcoverro and Mariani, 2002; Burnell et al., 2013b).

While only one period of natural overgrazing was observed in *A. antarctica* during intermittent visits to the study area over three years, recovery of seagrasses at the grazed sites appeared negligible during this time. Following natural overgrazing of *A. antarctica*, urchins were only present within adjacent *Posidonia* spp. suggesting the inability of *A. antarctica* to recover from grazing and hence provide a stable habitat may have resulted in urchin migration, starvation or predation. While grazing impacts appear persistent within *A. antarctica* meadows over the short-term, longer term studies would be required to accurately gauge if the frequency of these events exceed seagrass recovery from meadow expansion or recruitment.

Morphology and growth strategy can be important for seagrass regeneration and proliferation (Tomlinson, 1974). While both *A. antarctica* and *P. sinuosa* grow by continually replacing old leaves (Short and Duarte, 2001), the leaf meristems of *Amphibolis* spp. are elevated in the canopy, whereas those of *Posidonia* spp. are below-ground (Marbà and Walker, 1999). The exposure of leaf meristems on vertical shoots above the sediment can result in strong grazing impacts (Alcoverro and Mariani, 2002). Since seagrass regeneration is reliant on active meristems

(Tomlinson, 1974), seagrasses that have lost raised apical meristems can only regenerate by creating new shoots from existing rhizomes (Alcoverro and Mariani, 2002; Rose, et al., 1999). Consequently, when *A. antarctica* is overgrazed until the majority of shoots are completely defoliated, its recovery becomes reliant on new shoot growth from below ground rhizome extension, whereas basal meristems of *Posidonia* spp. may continue to produce new leaves in a largely uninterrupted way. This species-dependent capacity for re-growth based on meristem location appears a likely explanation for the patterns of recovery we observed and is analogous to findings for the recovery of other seagrasses exposed to significant meristem damage (i.e. *Syringodium filiforme, Zostera marina & Thalassodendron ciliatum*) (Alcoverro and Mariani, 2002; 2005; Rivers and Short, 2007; Rose, et al., 1999). In fact, grazing pressure may even play a significant role in determining seagrass species distribution (Vonk, et al., 2008). The tropical seagrass *T. ciliatum*, which has a similar morphology to *A. antarctica* and a high vulnerability to urchin grazing (Alcoverro and Mariani, 2002; 2005), is dominant in zones protected from fishing where urchin populations remain low (McClanahan, et al., 1994), but lower in unprotected zones where urchin abundance is greater (Alcoverro and Mariani, 2004). Such patterns suggest that morphology may interact with grazing pressure to shape the distribution of some seagrass species.

Recovery of *P. sinuosa* biomass during the simulated grazing experiment was greater where more seagrass was removed, whereas in *A. antarctica* biomass recovery declined as removal increased. Seagrass growth in reponse to changing grazing intensity is rarely linear (Cebrián, et al., 1998; Eklöf, et al., 2008) and can be highly species-specific (Alcoverro and Mariani, 2005; Cebrián, et al., 1998). The greater loss of leaf meristems in *A. antarctica* where more seagrass was removed is likely to have limited the availability of active meristems that could produce new growth. In comparison, simulated grazing in *P. sinuosa* potentially induced an overcompensatory growth response, where recovery rate increased as a function of removal. Alternatively, natural senescence and shedding of older leaves by *P. sinuosa* in the control and partial removal treatment could explain the lower recovery, in comparison to the complete removal treatment where no older leaves remained.

While it appears that meristem damage from grazing may modify the vulnerability and post-grazing recovery of *A. antarctica*, inherent biological differences between the two species should not be discounted and could also have contributed to the species-specific differences we found (Strauss and Agrawal, 1999). The greater below ground resources we recorded for *P. sinuosa*, which is consistently evident when compared with *A. antarctica* (Cambridge, 1999), could have aided rapid recovery from defoliation as resources can be translocated from roots and rhizomes to help leaf growth and recovery (Cebrián, et al., 1998; Strauss and Agrawal, 1999). Regardless of the mechanism to recovery, however, it appears evident that *P. sinuosa* has a greater tolerance to urchin grazing than *A. antarctica*.

It is important to note that simulated grazing is rarely an exact substitute for actual grazing (Eklöf, et al., 2009; Ibarra-Obando, et al., 2004; Strauss and Agrawal, 1999), suggesting the recovery we recorded should be interpreted cautiously. It was necessary to use new experimental plots for the recovery experiment as we destructively sampled seagrass to quantify urchin effects during the grazing experiment. Therefore, any deterioration of below ground resources that occurred during the grazing experiment was not replicated in the simulated grazing experiment. While *A. antarctica* showed a greater loss of below ground biomass than *P. sinuosa* during the grazing experiment, albeit non-significantly, replicating this effect in the simulated grazing experiment would only have exacerbated the species differences we observed, as fewer resources would have been available to *A. antarctica* for recovery. Simulated grazing can also underestimate potential benefits of herbivory. For example, urchin faeces can increase seagrass productivity by enhancing the rate of nutrient cycling, effectively fertilising any remaining plants (Koike, et al., 1987; Vonk, et al., 2008). The absence of this effect could have potentially underestimated seagrass recovery during the simulated grazing experiment, although this effect should be consistent across both species and is therefore unlikely to alter the inter-specific differences seen here. In addition, the rapid re-growth of *P. sinuosa* suggests that plants at the study site were unlikely to be nutrient-limited.

We considered it important to quantify the rate of biomass decline in both species despite the possibility that sub-sampling halfway through the grazing experiment may have impacted adjacent seagrasses due to the clonal nature of ramets. Importantly, *Amphibolis* and *Posidonia* rhizomes form a complex matrix where many individual plants overlap, rather than being a single clonal individual (Bryars, et al., 2011), making the removal of small amounts of above ground biomass unlikely to destroy entire plants. While sub-sampling itself does not appear to send seagrass meadows into significant decline (Bryars, et al., 2011; Valentine and Heck, 1991; Vonk, et al., 2008), it is plausible that biomass removal could have had a larger effect where seagrasses were already under stress from urchin grazing, possibly exacerbating grazing impacts. Yet we anticipate that our results are not an overestimation of grazing impacts for two reasons. First, only a small amount of above-ground biomass was removed (6% of plots) and belowground biomass was undamaged so there is no reason to assume that translocation of belowground resources was unduly interrupted. Second, while we removed 6% of the areal cover in all treatments, seagrass cover and biomass were already reduced in grazing treatments and by sampling in a standard area more biomass was actually removed in the control treatments. Nonetheless, non-destructive estimates of grazing impact, such as shoot density and canopy height could be used in future studies, though this can be inherently difficult due to species-specific morphology and seasonal changes in biomass composition.

2.6 CONCLUSION

The current rate of decline in seagrass meadows places them among the most threatened ecosystems worldwide (Waycott, et al., 2009). While some species of seagrass have evolved traits to tolerate high grazing intensity, such as rapid regeneration and basal leaf meristems (Valentine and Duffy, 2006), others such as *A. antarctica* appear vulnerable to long lasting impacts from sudden increases in grazing pressure due to a reduced capacity for recovery post-disturbance. Habitats created by such species appear particularly vulnerable to long-term, top-down induced phase-shifts. The cascading ecosystem effects of wholesale seagrass habitat loss threaten coastal productivity and biodiversity, through a reduction in sediment stabilisation, nutrient cycling and support of trophic food webs (Duarte, 2002; Orth, et al., 2006). Understanding the causes of sudden urchin population increases, and under what conditions seagrasses may be more or less susceptible to overgrazing, may help us predict and prepare for the effects of seagrass loss.

Nutrient enrichment modifies epiphyte cover

Above: A leaf head of *Amphibolis antarctica* from an ambient nutrient treatment.
Below: A leaf head of *Amphibolis antarctica* from an enriched nutrient treatment.

Photo credits: Kingsley Griffin

CHAPTER THREE

NUTRIENT ENRICHMENT MODERATES TOP-DOWN CONTROL OF SEAGRASS BY SEA-URCHIN GRAZING

Owen W. Burnell, Sean D. Connell, Andrew D. Irving and Bayden D. Russell

School of Earth and Environmental Sciences,
The University of Adelaide, Adelaide, SA 5005 Australia

STATEMENT OF AUTHORSHIP

In this paper I executed the laboratory work and analysed the data. I wrote the manuscript and undertook the field experiment, assisted by Sean D. Connell, Andrew D. Irving & Bayden D. Russell. All authors contributed to the experimental design. Sean D. Connell, Bayden D. Russell & I provided funding for the project.

Signatures of co-authors:

Professor Sean D. Connell

Dr Andrew D. Irving

Dr Bayden D. Russell

PREAMBLE

Chapter 3 was motivated by the significant role nutrient enrichment has played in the decline of seagrass meadows, and the general observation that overgrazing is common in areas influenced by nutrient enrichment. Thus Lady Bay, a relatively oligotrophic seagrass meadow with high abundances of herbivores, provided an excellent natural laboratory to test the effects of nutrient enrichment on grazing. The chapter is a co-authored draft manuscript and therefore is written in plural throughout. The paper is written for a global audience with an interest in how nutrient enrichment can modify the role of grazers.

CHAPTER THREE

NUTRIENT ENRICHMENT MODERATES TOP-DOWN CONTROL OF SEAGRASS BY SEA-URCHIN GRAZING

3.1 ABSTRACT

On urbanised coastlines worldwide over-harvesting of large predatory organisms combined with land-derived nutrient subsidies can affect top-down (i.e. herbivory by grazers) and bottom-up (i.e. resource availability) processes that shape marine communities. In seagrass meadows top-down sea-urchin effects often appear synergistic with bottom-up nutrient effects, such that they culminate in greater grazing intensity and habitat loss. However, bottom-up nutrient effects can result in contrasting outcomes for producers, whereby habitat-forming seagrasses commonly undergo loss from opportunistic epiphytic overgrowth, but nutrient enrichment can also change the quality and quantity of food for herbivores, which might modify individual grazing rates on seagrass. The sea-urchin *Amblypneustes pallidus* can over-graze seagrasses to contribute to habitat loss in oligotrophic waters. By elevating urchin densities and nutrients within meadows of the seagrass *Amphibolis antarctica*, we tested whether the *per capita* effect of grazing would be decreased under nutrient enrichment, and if such declines related to changes in seagrass structure and chemistry from nutrient enrichment (i.e. nitrogen content, epiphyte cover, grazing deterrents & leaf strength). Under

oligotrophic conditions, urchins removed 46 – 73 % of total seagrass biomass, whereas nutrient enrichment reduced urchin driven biomass loss to 10 – 51 %. Reductions in *per capita* grazing loss related to greater nitrogen content of seagrass leaves and thus probably better quality of food for urchins. This finding supports the notion that in circumstances where nutrient enrichment culminates in urchin overgrazing this would likely be associated with population effects (e.g. mass recruitment of urchins to seagrass meadows), rather than increases to *per capita* grazing.

3.2 INTRODUCTION

The role of top-down and bottom-up processes can be fundamental for understanding causes of habitat loss across many biomes (Eklöf et al., 2008, Estes et al., 1998, Power, 1992, Rosemond et al., 1993). In marine habitats, grazing by herbivores (top-down) and anthropogenic nutrient enrichment (bottom-up) are key drivers of ecosystem dynamics (Burkepile & Hay, 2006, McGlathery, 1995, Smith et al., 2010). On urbanised coastlines worldwide, over-harvesting of large predatory organisms combined with land-derived nutrient subsidies have modified the effects of these top-down and bottom-up processes for habitat maintenance (Tewfik et al., 2005, Vitousek et al., 1997). While over-harvesting often results in population growth of grazing herbivores released from predatory control (Estes & Palmisan.Jf, 1974, Estes et al., 2011), nutrient subsidies modify ecosystems by changing resource availability and growth rates of primary producers (Bulleri et al., 2012, Scheffer et al., 2001). Understanding how human activities can modify top-down and bottom-up processes might help explain mechanisms behind accelerating habitat losses frequently observed on urbanised coasts, particularly as ~75 % of studies in marine systems report antagonistic or synergistic effects between multiple stressors (Crain et al., 2008).

Nutrient subsidies can have disparate effects on primary producers that range from increases in productivity and growth to widespread biogenic habitat loss (Anderson & Polis, 1999, Anton et al., 2011, Bryars et al., 2011, Kelaher et al., 2013). The negative effects of nutrient enrichment on seagrasses are well established, particularly their ability to induce epiphytic overgrowth that inhibits seagrass photosynthesis and growth (Bryars et al., 2011, Sand-Jensen, 1977, Short & Wyllie-Echeverria, 1996, Walker & McComb, 1992). Under such circumstances, grazing is often considered beneficial to seagrasses as herbivores can target and remove these epiphytes (Hughes et al., 2004, Valentine & Duffy, 2006). Thus, nutrient subsidies might divert grazing effort away from seagrass leaves, as alternative foods are of greater quality or quantity (Anton et al., 2011, McGlathery, 1995, Tewfik et al., 2005).

There is also evidence, however, for direct negative effects of grazing on seagrass habitats (Burnell et al., 2013a, Eklöf et al., 2008, Rose et al., 1999, Valentine & Duffy, 2006, Valentine & Heck, 1991). While the ingestion of seagrass itself is often considered incidental to obtaining the more nutritious epiphytes or epibiota that colonise leaves (Eklöf et al., 2008, Tewfik et al., 2005), there is evidence that absorption of seagrass tissue can deliver nutritional benefits (Valentine & Duffy, 2006) and induce compensatory feeding behaviour (Valentine & Heck, 2001). Overgrazing events by sea-urchins on seagrasses appear disproportionately common in meadows affected by nutrient enrichment (Eklöf et al., 2008, Kirkman

& Young, 1981, Ruíz et al., 2009, Walker & McComb, 1992), generating interest in understanding if changes in *per capita* consumption of leaves might contribute to such declines. Several laboratory manipulations suggest the *per capita* grazing effect of urchins can decline where nutrient content is greater (Burnell et al., 2013b, Lares & McClintock, 1991, Valentine & Heck, 2001), but nutritional value can also have negligible (Lawrence et al., 1989) or even positive (Beddingfield & McClintock, 1998) effects on consumption. Principally those attributes of seagrass leaves that can modify grazing and could vary with nutrient enrichment, include the nitrogen content (Anton et al., 2011, de los Santos et al., 2012, Valentine & Heck, 2001), phenolic content (i.e. grazing deterrents) (Vergés et al., 2007a, Vergés *et al.*, 2007b) and the physical toughness or strength of leaves (de los Santos et al., 2012, La Nafie et al., 2012, Vergés et al., 2007b).

We replicated changes to top-down and bottom-up process common on urbanised coasts, by elevating both the density of urchins and the concentration of nutrients in an oligotrophic seagrass meadow, to test whether the *per capita* grazing effect of urchins on seagrass would change, and if the structural and chemical properties of seagrass were associated with such changes to grazing. We hypothesised that the negative effects of nutrient enrichment on seagrasses (i.e. epiphytic overgrowth) would be weakened on balance by lower *per capita* grazing upon seagrass leaves by urchins. Thus an antagonistic interaction would emerge where greater food quantity or quality could reduce direct grazing impacts on seagrass habitats. Such evidence would also support theory that overgrazing of seagrass commonly observed in eutrophic waters would likely be a population response (e.g. mass recruitment of grazers) rather than a consequence of increasing *per capita* grazing.

3.3 MATERIALS AND METHODS

3.3.1 Study site

The study was completed within a meadow of seagrass *Amphibolis antarctica,* of approximately 4 m depth at Lady Bay, South Australia (35°28.036 S, 138°17.198 E), during the Austral Autumn-Winter (Mar-2011 to Aug-2011). For a description of seagrass meadows at this site and the population dynamics of the short-spined sea-urchin *Amblypneustes pallidus* in the region, see Burnell et al., (2013a).

3.3.2 Experimental design

Manipulative experiments tested the relative and combined effects of urchin grazing and nutrient enrichment on seagrass in a 3 × 2 crossed experimental design. Thirty-five replicate plots were randomly interspersed over 500 m^2 of seagrass meadow to separate plots by approximately four meters, where the naturally occurring urchin populations observed during the experimental period were zero. Ten plots were designated as treatments of moderate grazing (hereafter MG) and ten of high grazing (hereafter HG), by adding one (6.25 individuals m^{-2}) or two urchins (12.5 individuals m^{-2}), respectively, within a wire mesh cage (cage size 0.4 × 0.4 × 0.4 m, mesh size 0.02 × 0.02 m) (average urchin mass = 29.45 ± 0.40 g $urchin^{-1}$). Ten plots remained un-manipulated and were designated as plots of no urchin grazing (hereafter Absent Grazing or AG). A procedural control was established in another five plots using a partial cage to test for caging artefacts on seagrass. Procedural controls were found to have no detectable effects on any of the response variables measured. Urchin densities replicated high natural densities that have been observed in seagrass meadows nearby the study site (Burnell et al., 2013a). Urchins were collected from nearby beds of seagrass with weekly replacement of any dead individuals in experimental plots. Urchin densities were used in cross combination with two levels of nutrient concentration, ambient (hereafter AN) and enriched (hereafter EN). Within each of the urchin levels five plots were designated as EN, while the other five plots were designated as AN. Treatments were maintained for 169 days and cages were scrubbed free of epiphytes weekly.

Nutrients were supplied to EN plots by adding 1 kg of Osmocote Plus® 12-14 month controlled release fertiliser (N:P:K – 17:4.3:8.2 - Scotts, Sydney, Australia). Fertiliser pellets were evenly separated into four nylon bags with 1 mm mesh (i.e. 250g bag^{-1}) and anchored with stainless steel pegs at the substrate level along the four edges of each EN plot to provide an even distribution of nutrients. Bags were replaced every 8 – 10 weeks with ones containing new fertiliser (i.e. three separate additions of 1 kg throughout the experiment). Water samples were taken from all plots five centimetres inside the plot boundary at several times after the initial deployment of nutrients (i.e. ~24 hours, ~3 weeks, ~6 weeks and ~10 weeks after deployment) to monitor treatments (see ‘Results’). Water was sampled using sterile 25 ml syringes, filtered at 0.45 µm and frozen for later analysis. Analysis was undertaken using a Lachat Quickchem 8500 Flow Injection Analyser (Hach, Colorado, USA) for nitrate and nitrate (NO_x), Phosphate (PO_4) and ammonia (NH_3).

3.3.3 Experimental sampling

Destructive sampling of seagrass was undertaken twice, approximately halfway through the experiment (i.e. 82 days) and at the end of the experiment (i.e. 169 days). The first samples were small; 0.10 m^2 or 6 % of experimental plots and their location was marked to prohibit re-sampling. These initial samples were used to quantify changes in tissue biochemistry and epiphyte cover, without unduly impacting upon the health of the remaining seagrass. The second samples were larger; 0.25 m^2 or 39 % of experimental plots, and thus detected biomass effects as the bigger quadrat size controlled for natural variation in stem density. During biomass sampling seagrass above ground biomass was harvested at the substrate, transported under dark conditions on ice to the laboratory and frozen for later analysis. Measurements taken from these samples were meristem number, leaf biomass, living stem density, epiphyte cover, biochemistry of leaf tissue (i.e. carbon and nitrogen content), secondary metabolites (i.e. % leaf phenolics) and leaf strength (i.e. relative force required to pierce leaves with a blunt probe). Phenolics and leaf strength were only measured on the final samples.

3.3.4 Seagrass parameters

The number of living meristems was recorded by counting those leaf heads where the youngest leaf, which is the position of new growth for *A. antarctica*, was intact. Shoot density or living stems was recorded by counting the number of stems that supported at least one living meristem. Where all above ground meristems had been lost stems were classified as dead. Leaf biomass was determined by separating leaves from stems before rinsing in MilliQ water and drying for 48 h at 60°C. Samples were then acid washed in 5 % HCl to remove any calcified epiphytes, re-dried and weighed to determine final dry weight of seagrass. For a detailed summary of the anatomy and morphology of *A. antarctica* see Marbà and Walker (1999).

3.3.5 Leaf growth rates

Seagrass growth was quantified by recording new leaf initiation. Trimming of the corner on the second youngest leaf was implemented and the stem marked at the base with flagging tape to assist re-location. This occurred for three leaves in each plot twice throughout the experimental period, firstly during May for two weeks and the secondly in June for four weeks. Due to significant loss of marked leaves, all samples were pooled for statistical analysis, nonetheless even after pooling no significant differences were detected between experimental treatments (Table 3.S2).

3.3.6 Epiphyte cover

Cover of calcified algal epiphytes on seagrass leaves was quantified over a 3 × 5 mm grid on the 2nd, 5th & 7th leaf on three separate leaf heads from each plot. Each mm^2 within the rectangular grid was scored as either being dominated by epiphyte cover or bare, which was then used to calculate percentage cover. The leaf epiphyte assemblage was almost exclusively encrusting coralline algae, which is typical for seagrass in this area (James et al., 2009), particularly during winter months (O. W. Burnell pers. obs.). As such, no quantification of other epiphyte groups on seagrass leaves was necessary or indeed possible.

3.3.7 Biochemical and physical properties of seagrass

Biochemistry of seagrass tissue was quantified to determine if the treatments modified the nutritional value of leaves. Seagrass tissue from each experimental plot was ground with a mortar and pestle and analysed for % nitrogen and % carbon (hereafter % N and % C, respectively) on an Isotope Ratio Mass Spectrometer (Hydra 2020 ANCA-GSL Version 4.0, Sercon, Cheshire, UK).

Phenolic content of seagrass leaves was determined using Folin-Ciocalteau methodology adapted from Bolser et al., (1998). Seagrass leaves were dried and ground (as described above). A 5 mg subsample was extracted in 1 ml of 50 % MeOH under dark refrigerated conditions for 24 h. Following centrifuging, 100 µl of the supernatant was added to 3.9 ml of MilliQ water and mixed, 0.25 ml of Folin-Ciocalteau reagent was then added followed by 0.75 ml of sodium carbonate solution after a 2-min delay. Samples were then refrigerated for two hours for colour development, before absorbencies were recorded on a single beam photospectrometer at 760 nm. A standard gallic acid equivalence curve was generated from serial dilutions, and used to read the concentration of total phenolics.

Leaf strength was measured using a custom designed leaf penetrometer. Two wooden blocks fastened to one another held a seagrass leaf in position. A blunt 1 mm probe with an attached weight vessel was suspended over the seagrass by resting the tip on the centre of the blade. Weight was slowly added to measure the force required for the probe to puncture the seagrass leaf. Force was calculated in micro-newtons (mN) of force by summing the mass of the probe, container and added weight required to puncture the leaf, and multiplying by the force of gravity, similar to the methodology implemented by Pennings and Paul (1992).

3.3.8 Statistical analyses

Univariate two-factor ANOVA was used to compare the effect of urchin grazing and nutrient enrichment on seagrass parameters (i.e. meristem number, leaf biomass & living stem density), leaf growth, biochemistry (C:N ratio, % N & % C), total phenolics, leaf strength and water chemistry using the program PERMANOVA+ for Primer v6. Univariate three-factor ANOVA was used to compare the compare the effect of urchin grazing, nutrient enrichment and leaf position on epiphyte cover of seagrass. All factors used in the analyses were treated as fixed and orthogonal. Averages from each plot were used as replicates, except for leaf growth where samples recovered were pooled, due to loss of all samples from many plots. Posthoc pairwise comparisons were used where significant terms were detected. Procedural controls were compared against control plots to determine any artefacts of caging, of which no effect was detected in any response variable. Partitioning of variance for all ANOVAs was undertaken to estimate the magnitude of effects (ω^2) using equations from Vaughan and Corballis (1969). Magnitude of effects provide an estimate of the variation explained by each individual or combination of experimental factors.

3.4 RESULTS

3.4.1 Seagrass habitat

Sea-urchin grazing had a strong negative effect on all three seagrass habitat parameters (Fig. 3.1, Table 3.1). Variance partitioning indicated urchin grazing explained a large proportion of variation in meristem number ($\omega^2 = 0.59$), leaf biomass ($\omega^2 = 0.46$) and living stem density ($\omega^2 = 0.38$), which is an intuitive sequence of destruction given grazers would firstly consume individual leaf heads/meristems, in turn impacting leaf biomass and eventually stem mortality (i.e. loss of all living above ground meristems). However, an interaction with nutrient enrichment moderated urchin effects on seagrass meristem number and leaf biomass (i.e. $\omega^2 = 0.08$ and $\omega^2 = 0.07$, respectively) (Fig. 3.1, Table 3.1). This interaction occurred as urchin elevations were responsible for a biomass decrease of 46 – 73 % at ambient nutrients (AN), but only 10 – 51 % at enriched nutrients (EN), for moderate grazing (MG) and high grazing (HG) treatments, respectively. Nutrients also independently decreased meristem number in the absent grazing (AG) treatment, but had no effect where urchins were present. These opposing effects of nutrients in the absence verses presence of urchins resulted in a negligible effect of nutrients once variance was partitioned (i.e. $\omega^2 < 0.01$ in all three seagrass habitat parameters), suggesting stronger top-down control over seagrass habitat under the tested conditions (Fig. 3.1, Table 3.1). No effects of grazing or nutrient enrichment on seagrass biomass were detected between treatments at the halfway sampling point (Table 3.S1), which is likely to be due to the small sampling unit and natural variability in biomass inflating the sampling error around the mean. Neither nutrient enrichment nor urchin grazing affected the rate of leaf initiation during the experiment (Table 3.S2).

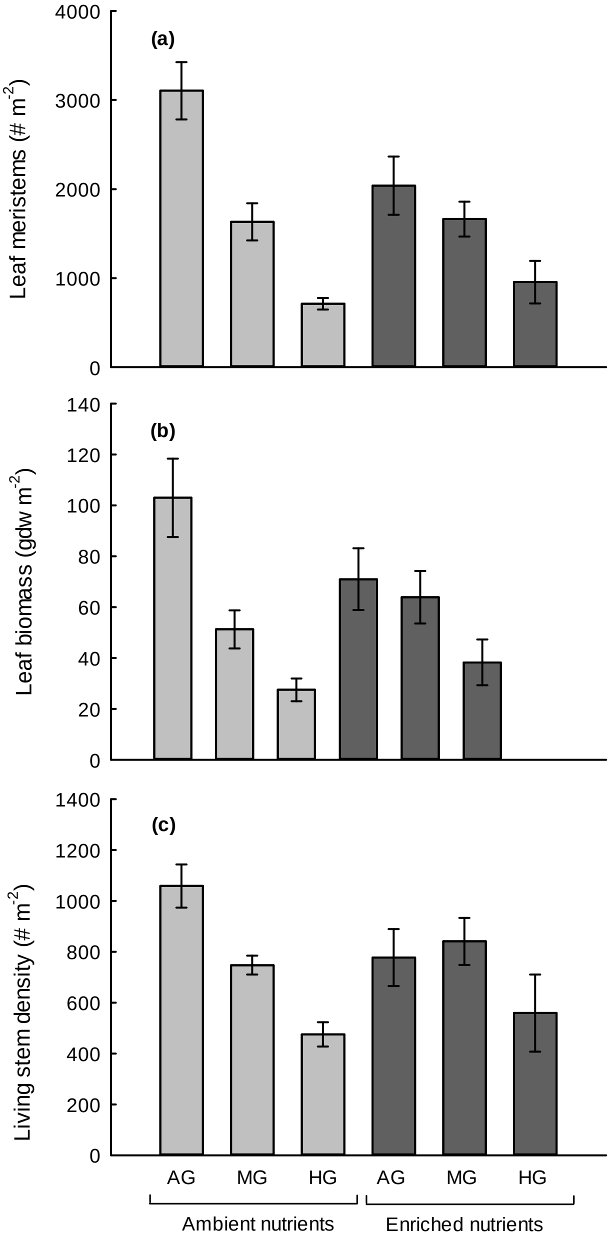


Figure 3.1 a) Leaf meristems, b) leaf biomass and c) living stem density for *Amphibolis antarctica* exposed to sea-urchin grazing *Amblypneustes pallidus* (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched) for 169 days. Bars represent mean ± SE (*n* = 5). AG = absent grazing, MG = moderate grazing, HG = high grazing.

Table 3.1 ANOVA and variance partitioning for leaf meristems, leaf biomass and living stem density of seagrass exposed to sea-urchin grazing (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched) for 169 days. G = grazing, N = Nutrients.

Source	df	MS	F	P	ω^2	Pairwise
Leaf Meristems						
G	2	75.5×10^5	29.18	0.001	0.59	[a] at AN, AG > MG > HG, at EN, AG = MG > HG. At AG, AN > EN, at MG, AN = EN, at HG, AN= EN
N	1	52.0×10^4	2.01	0.182	0.01	
G × N	**2**	$\mathbf{12.4 \times 10^5}$	**4.77**	**0.015**[a]	0.08	
Residual	24	25.9×10^4			0.32	
Leaf Biomass						
G	2	73.4×10^2	15.08	0.001	0.46	[b] at AN, AG > MG > HG, at EN, AG = MG, AG > HG, MG = HG. At AG, AN = EN, at MG, AN = EN, at HG, AN = EN
N	1	6.22×10^1	0.13	0.71	-0.01	
G × N	**2**	$\mathbf{15.9 \times 10^2}$	**3.26**	**0.042**[b]	0.07	
Residual	24	4.87×10^2			0.49	
Living Stem Density						
G	**2**	$\mathbf{42.0 \times 10^4}$	**11.40**	**0.001**[c]	0.38	[c] Living Stem Density pairwise for G, AG = MG > HG
N	1	90.1×10^2	0.24	0.642	-0.01	
G × N	2	11.4×10^4	3.10	0.065	0.08	
Residual	24	36.9×10^3			0.56	

3.4.2 Epiphyte cover

Nutrient enrichment and urchin presence both modified epiphyte cover upon leaves. Epiphyte cover was greater at EN than AN for all leaf positions sampled, but younger leaves had lower total cover than older leaves (i.e. 2nd < 5th = 7th) (Fig. 3.2, Table 3.2). The presence of urchins also reduced epiphyte cover on older leaves (i.e. on 5th and 7th leaves, AG > MG = HG) (Fig. 3.2, Table 3.2). Variance partitioning indicated nutrients and leaf position had by far the greatest influence on epiphyte cover (i.e. $\omega^2 = 0.40$ each), while the effect of urchins ($\omega^2 = 0.07$) and the interaction between nutrients and leaf position ($\omega^2 = 0.11$) were smaller (Fig. 3.2, Table 3.2).

3.4.3 Biochemical and physical properties of seagrass

Nitrogen in seagrass leaves increased at EN, which coincided with a reduction in their C:N ratio (Fig. 3.3, Table 3.3). The % C did not differ among any of the experimental treatments. Urchin presence also had an effect on tissue chemistry, whereby % N decreased in the presence of urchins at both the halfway and final sampling date (Fig. 3.3, Table 3.3). This effect was only evident in EN treatments at halfway, but by the final sampling period grazing consistently reduced % N (and increased C:N) across all treatments. A large decline in % C was observed between the two sampling dates, almost certainly due to seasonal over-wintering effects. The percentage of secondary metabolites, measured as total phenolics, declined in seagrass leaves at EN, but did not respond to grazing manipulations (Fig. 3.3, Table 3.3). Penetrometer measurements revealed no differences in the breaking force required to pierce leaves amongst any of the experimental treatments (Table 3.S3).

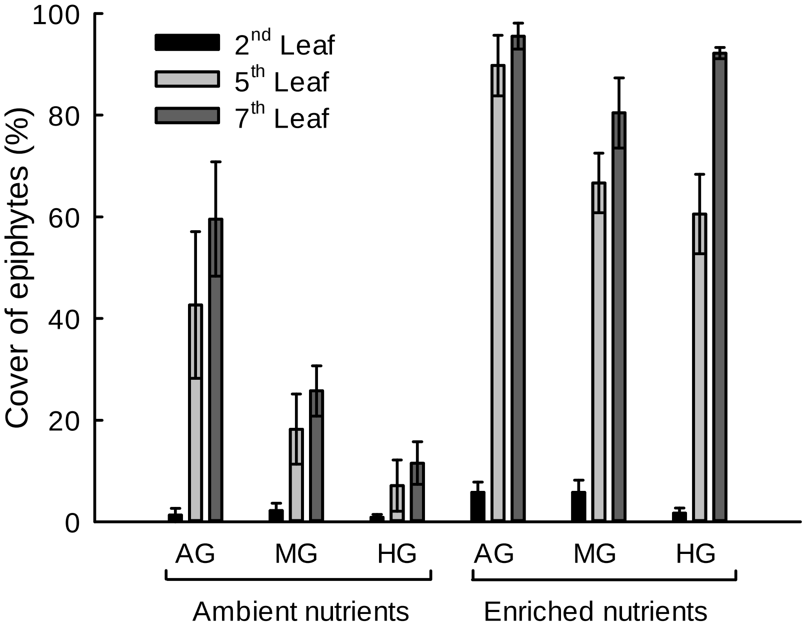


Figure 3.2 Epiphyte percentage cover at different leaf positions of seagrass exposed to sea-urchin grazing (absent *v.* moderate *v.* high) and nutrients (ambient *v.* high) for 169 days. Bars represent mean ± SE (*n* = 5). AG = absent grazing, MG = moderate grazing, HG = high grazing.

Table 3.2 ANOVA and variance partitioning for the percentage cover of coralline epiphytes on different leaf positions (2nd *v.* 5th *v.* 7th) of seagrass exposed to sea-urchin grazing (absent *v.* moderate *v.* high), nutrients (ambient *v.* enriched) for 169 days. G = grazing, N = nutrients, L = leaf position.

Source	**df**	**MS**	**F**	**P**	**ω^2**	**Pairwise**
G	2	4893	15.83	0.001	0.07	^{a} on 2nd L, AG = MG = HG, on 5th L, AG > MG = HG, on 7th L, AG > MG = HG. At AG, 2nd < 5th = 7th, at MG 2nd < 5th = 7th, at HG, 2nd < 5th = 7th.
N	1	24705	79.89	0.001	0.40	
L	2	24891	80.49	0.001	0.40	
G × N	2	89.3	0.29	0.755	0.00	
G × L	**4**	**1050**	**3.40**	**0.012^{a}**	0.02	
N × L	**2**	**5225**	**16.90**	**0.001^{b}**	0.11	^{b} on 2nd L, AN < EN, on 5th L, AN < EN, on 7th L, AN < EN. At AN, 2nd < 5th = 7th, at EN, 2nd < 5th = 7th.
G × N × L	4	206.5	0.67	0.605	0.00	
Residual	72	309.3			0.00	

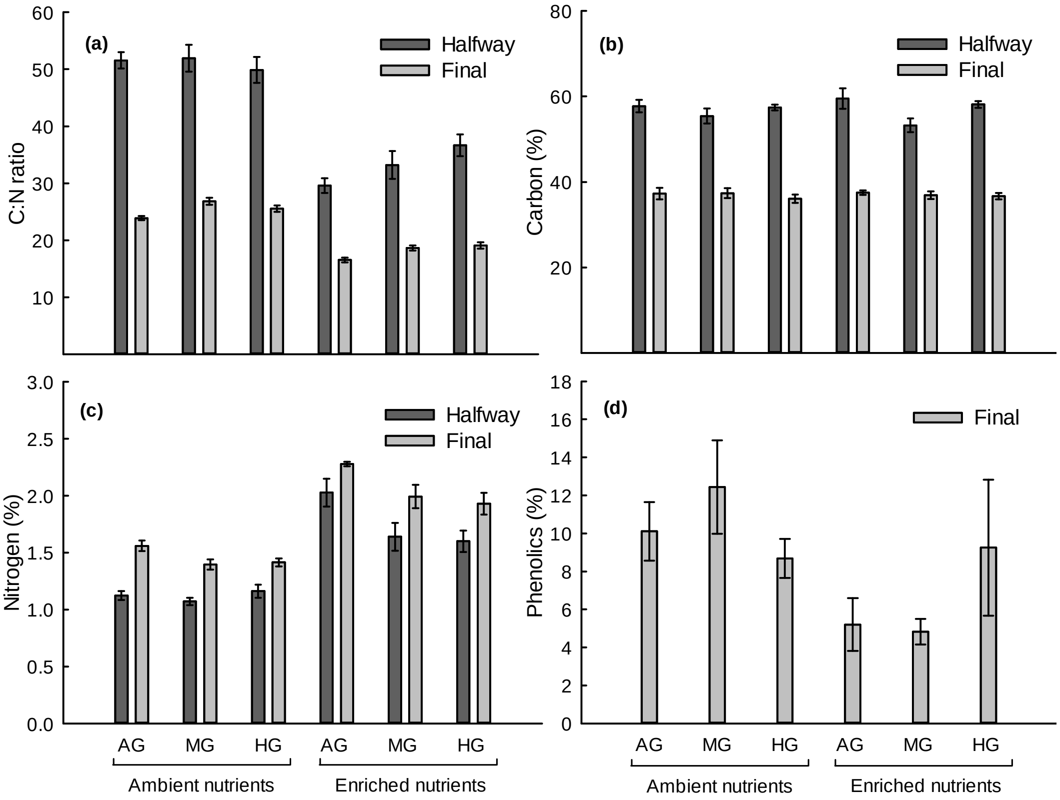


Figure 3.3 a) Carbon to nitrogen (ratio), b) nitrogen (%), c) carbon (%) and d) phenolics (%) of dry weight of seagrass exposed to sea-urchin grazing (absent *v*. moderate *v*. high) and nutrients (ambient *v*. enriched) for 82 (halfway) and 169 (final) days. Bars represent mean ± SE (*n* = 5). AG = absent grazing, MG = moderate grazing, HG = high grazing.

Table 3.3 Significance values from ANOVA and variance partitioning for C:N ratio, nitrogen (%) and phenolics (%) for dry weight of seagrass exposed to sea-urchin grazing (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched) for 82 (halfway) and 169 (final) days. No significant differences in Carbon (%), therefore analyses not presented. G = grazing, N = nutrients.

	C:N halfway		**C:N final**		**% N halfway**		**% N final**		**% Phenolics**	
	p	ω^2	p	ω^2	p	ω^2	p	ω^2	p	ω^2
G	0.364	0.00	**0.001**[b]	0.07	0.031	0.05	**0.004**[d]	0.09	0.753	-0.04
N	**0.001**[a]	0.79	**0.001**[b]	0.86	0.001	0.67	**0.001**[d]	0.73	**0.007**[e]	0.18
G × N	0.097	0.02	0.137	0.04	**0.032**[c]	0.05	0.315	0.00	0.085	0.10
Res	n/a	0.19	n/a	0.06	n/a	0.23	n/a	0.18	n/a	0.76

[a] C:N halfway pairwise for N, AN > EN;

[b] C:N final pairwise for G, AG < MG = HG, for N, AN > EN;

[c] % N halfway pairwise for G × N, at AN, AG = MG = HG, at EN, AG > MG = HG. At AG, AN = EN, at MG, AN = EN, at HG, AN = EN;

[d] % N final pairwise for G, AG > MG = HG, for N, AN < EN;

[e] % Phenolics final pairwise for N, AN> EN.

3.4.4 Water column nutrients

Average NO_x (nitrate + nitrite) (~4.5 fold), PO_4 (~1.3 fold) and NH_3 (~1.5 fold) concentrations were greater at EN compared to AN. Average values were only statistically different for NO_x ($F_{1,18} = 24.04$, $p = 0.002$) and PO_4 ($F_{1,18} = 10.07$, $p = 0.005$). Nutrient levels varied between grazing treatments on certain individual days, but grazing had no statistical effect on average values over the four sampling dates (Fig. 3.4, Table 3.S4). The effect of EN treatments diminished as a function of time from deployment, evident from the initial spike ~24 hours after new fertiliser was introduced, which subsequently decreased over time.

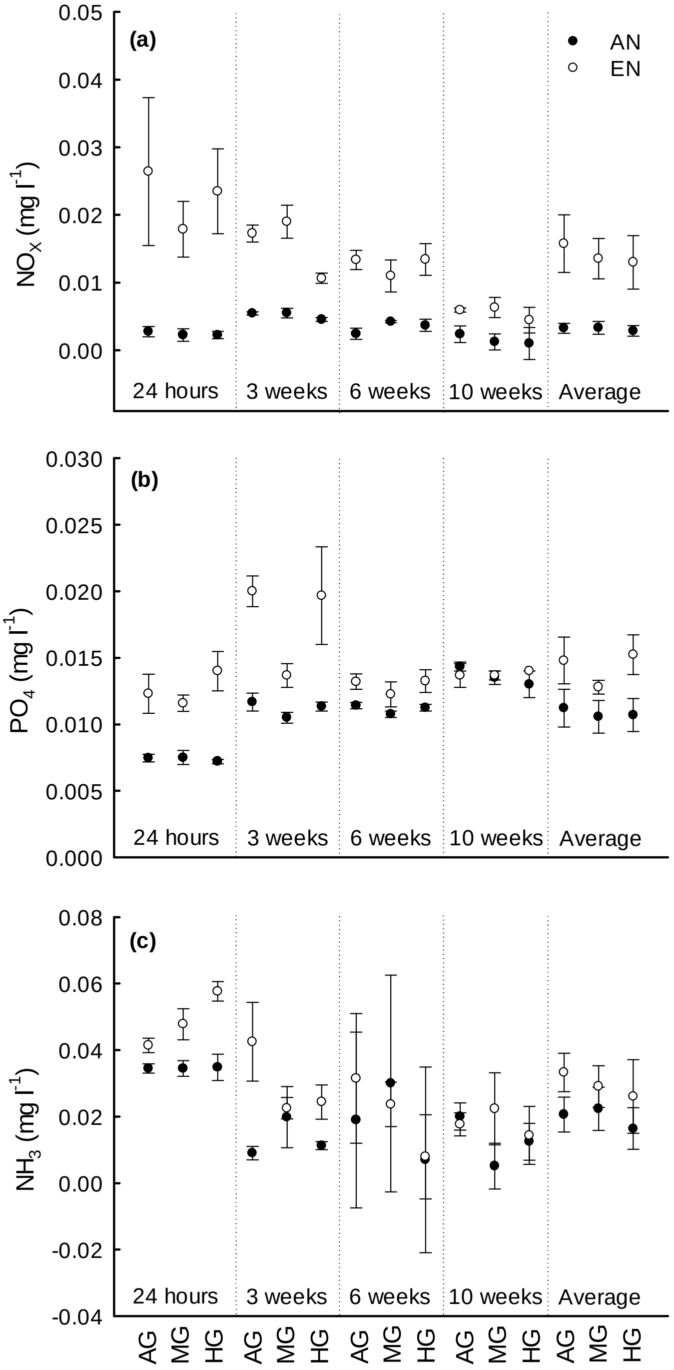


Figure 3.4 Water column nutrients of a) NO_x, b) PO_4 and c) NH_3 within seagrass plots exposed to sea-urchin grazing *Amblypneustes pallidus* (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched). Ambient nutrients (closed circles) & enriched nutrients (open circles). Data points represent mean ± SE (*n* = 5, besides average where *n* = 4). AG = absent grazing, MG = moderate grazing, HG = high grazing.

3.5 DISCUSSION

Strong top-down grazing effects were moderated when nutrients were enriched, which may have been a compensatory reduction in grazing that was driven by greater nitrogen content in seagrass tissue. Due to the importance of nitrogen in organism growth and natural shortages that commonly result in limitation for many organisms, herbivores can exhibit compensatory feeding in response to its availability (Mattson, 1980). While variable responses in urchin grazing to nutritional content have been observed (Beddingfield & McClintock, 1998, Lares & McClintock, 1991, Lawrence et al., 1989, Valentine & Heck, 2001), the moderations in grazing we found supports the notion that nutrient-induced overgrazing events would be driven by population-level phenomena (e.g. mass recruitment), rather than increasing *per capita* grazing rates. Indeed this would support observations of increased urchin activity in eutrophic meadows (Cambridge et al., 1986, Ruíz et al., 2001, Ruíz et al., 2009, Tewfik et al., 2005) and their greater reproductive potential when supplied with more nutritious food (Beddingfield & McClintock, 1998).

The moderation of seagrass lost to grazers where nutrients were enriched suggests urchins might have become increasingly satiated from the greater nutritional content of seagrass. Changing biochemistry of many primary producers in seagrass ecosystems from nutrient enrichment is known to increase food quality and palatability to consumers (Anton et al., 2011, Cebrián et al., 2009). In many instances it appears epiphytes and epifauna living upon seagrass are actually the primary source of nutrition for urchins (Beddingfield & McClintock, 1998, Ruíz et al., 2009, Tewfik et al., 2005). In fact, a facultative switch and trophic plasticity in feeding has been shown for some urchins, where detritus, epiphytes or epifauna are consumed preferentially, but when nutrient availability limits the growth of these preferred food sources, they switch their feeding to seagrass leaves (McGlathery, 1995, Prado et al., 2010).

In this study, however, it seems unlikely that epiphytes on seagrass would have offered a nutritious subsidy. The epiphyte assemblage in this study was comprised almost solely of encrusting coralline algae, which is typical for seagrass in this area (James et al., 2009), particularly during the winter (O. W. Burnell pers. obs.). Further to this, in oligotrophic regions epiphytes might actually have lower nutritional value than the seagrasses they colonise (Burkholder et al., 2013). The encrusting nature of epiphytes upon seagrass meant their consumption appeared inextricably linked to one another, except when urchins grazed on younger leaves where epiphytes were almost absent. Therefore, greater overall ingestion under nutrient enrichment might have contributed to satiation. However, calcified algae generally have a very low nutritional content (Foster et al., 1999, Littler & Littler, 1980, Neighbors & Horn, 1991, Niell, 1976) and may actually deter grazing (Pennings & Paul, 1992, Pennings & Svedberg, 1993, Schupp & Paul, 1994), although this effect appears minimal for urchins, due to their almost neutral gut pH, which is well equipped to dissolve $CaCO_3$ (Pennings & Svedberg, 1993). Regardless of the mechanism (i.e. greater food quality, quantity or even a deterrent), it does appear the net outcome of nutrient enrichment is a moderation of *per capita* grazing on seagrass blades. Further investigation of isotope analysis in urchins and their food sources (i.e. algae and epiphytes) could offer an effective tool for assessing nutritional pathways and potential mechanisms behind grazing effects in *A. antarctica* meadows (e.g. Tewfik et al., 2005).

The dampening effect of nutrients on grazing should not be interpreted to suggest eutrophication is beneficial to seagrass. We found nutrients decreased the number of meristems, and while it had marginally non-significant effects on leaf biomass, other longer-term studies highlight the detrimental effects of nutrients on this species of seagrass (Bryars et al., 2011). While food quantity and quality can be one important ecosystem service provided by seagrasses, the loss of biogenic habitat is by and large detrimental (Anton et al., 2011). Importantly, urchin populations are generally a transient and often localised threat (Burnell et al., 2013a, Cambridge et al., 1986), when compared to the chronic and widespread effects of nutrient enrichment (Bryars et al., 2011, Silberstein et al., 1986, Walker & McComb, 1992). While grazing effects were reduced by nutrient enrichment, the combination of these two factors did not modify overall seagrass loss, thus high grazing is unlikely to safeguard meadows from the detrimental effects of nutrient enrichment.

While we did not detect any changes in leaf initiation rates, a concurrent study aimed exclusively at the effect of nutrients on leaf production found decreases in initiation of ~40 % and almost double the leaf shedding rate at only half the nutrient additions we tested (Falkenberg et al., unpublished). Changes of this nature would explain the nutrient induced decline in seagrass we recorded. Leaf production is inherently difficult to measure for *A. antarctica* compared with other seagrasses, as elongation of each leaf is negligible soon after initiation. Therefore production is measured in discreet bundles (i.e. new leaves initiated or shed), which average one leaf every ~14 days (Marbà & Walker, 1999). We recorded slightly longer average initiation rates than previous findings (i.e. ~15 days leaf^{-1} in May and ~25 days leaf^{-1} in June), probably due to seasonally low water temperatures during May (~16.6°C) and June (~14.5°C). Due to this low initiation rate many of our measurements were either 0 or 1 leaf meristem^{-1}, making it very difficult to detect meaningful variation. This was compounded by the high proportion of marked leaves that were not recovered presumably due to the effects of nutrient enrichment or grazing.

The lower epiphyte cover we found on the older seagrass leaves as a result of the grazing treatments was unexpected. Therefore, while our observations suggest urchins do not directly or intentionally consume encrusting calcified epiphytes, we do not discount the possibility entirely. Other potential mechanisms behind this decline in epiphytes are increased abrasion or a reduction in recruitment propugules where grazing reduced the seagrass biomass. Water movement is greatly reduced in *Amphibolis* meadows where leaf material is more abundant (van Keulen & Borowitzka, 2002), and while epiphytes decline under increased flow, calcified assemblages are generally most resistant to abrasion (Lavery et al., 2007). Alternatively, a subtle undetected increase in leaf initiation rate in response to grazing could have impacted the age and therefore the time for epiphyte recruiment at each respective leaf position.

Interestingly, grazing treatments reduced the % N in seagrass tissue, an effect that was generally stronger at enriched nutrients. This may have occurred if urchins preferentially consumed young tissue (i.e. meristems), as % N content in *A. antarctica* can decrease from >4 % in the youngest leaf to ~1 % of dry mass in the older leaves (Pedersen et al., 1997). Such a pattern of grazing would have resulted in older tissue being disproportionately represented in the biochemical analyses, which pooled all above ground leaf tissue from each replicate treatment. A preference for younger tissue appears likely given the magnitude of effects calculations found grazing explained 59 % of variation in meristem number, in comparison to only 46 % for total leaf biomass. Alternatively, grazing could have modified seagrass health and the ability of plants to incorporate available nitrogen, or a subtle undetected increase in growth could have diluted nitrogen content.

Phenolics and leaf strength did not appear to modify grazing between treatments, nor did it appear grazing stimulated changes in these leaf properties. The total phenolic content recorded in *A. antarctica* (~8.5 % of DW) was relatively high compared to reports for other seagrasses (Agostini et al., 1998, Darnell & Heck Jr, 2013, Grignon-Dubois et al., 2012, Vergeer & Develi, 1997, Vergés et al., 2011, Vergés et al., 2007b), however, in some instances similarly high levels have been found (McMillan, 1984, Steele & Valentine, 2012, Vergeer et al., 1995, Vergés et al., 2007b). We suggest these high levels could be due to accumulation from a combination of low seasonal temperatures and slow growth (Coley, 1988, Dumay et al., 2004, Vergeer et al., 1995, Vergés et al., 2008). Interestingly, we found no effect of nutrients on leaf strength, whereas a highly significant positive relationship between the strength of *A. antarctica* leaves and their biochemistry has previously been reported (de los Santos et al., 2012), although disparate methodologies might explain these differences as we did not remove epiphytes prior to strength trials unlike the aforementioned study, because we considered their structural presence might be an important determinant of leaf strength and thus palatability.

Evidence for the disparate effects of nutrient enrichment on seagrass meadows continues to accumulate, with consequences varying from seemingly positive to highly negative (Anton et al., 2011, Bryars et al., 2011, Kelaher et al., 2013). Responses are dependent upon the many environmental conditions that make each seagrass meadow distinctive (e.g. exposure, depth, temperature, season, nutrient status, grazing & connectivity) (Anton et al., 2011, Kelaher et al., 2013). From an ecosystem services perspective, nutrient enrichment consistently increases the quality of food provided to first-order consumers, but simultaneously the effects of enrichment can progress to be largely negative as habitats are lost (Anton et al., 2011). Thus, an initially positive effect when examining typically short-term *per capita* effects (i.e. lower grazing by first-order consumers due to greater food quality or quantity), could transition to be negative over longer temporal scales if nutrient enrichment inhibits seagrass or indirectly increases populations of herbivores. Importantly, herbivores often grow faster, accumulate greater biomass and, as a population, graze a larger proportion of primary production where the nutritional quality of food is greater (Cebrián, 1999, Cebrián et al., 2009). This is consistent with the observation of increased urchin abundance in meadows exposed to nutrient subsidies (Cambridge et al., 1986, Eklöf et al., 2008, Ruíz et al., 2001, Ruíz et al., 2009, Tewfik et al., 2005) and increases in urchin reproductive condition when fed high quality diets (Beddingfield & McClintock, 1998); although see (Livore & Connell, 2012a). Therefore in many eutrophic events long-term population responses in urchin abundance may temper any benefits to seagrass from moderations in *per capita* grazing.

In summary, nutrient enrichment moderated grazing impacts on seagrass leaves, but top-down grazer effects still had an overwhelming influence on seagrass habitats. Thus, in a region where top-down control is considered relatively weak (Bulleri et al., 2012, Livore & Connell, 2012b), we confirm high densities of urchins can exceed bottom-up effects (i.e. nutrient enrichment) over relatively short time scales that likely correspond to the transient nature of urchin population flux. The provision of greater food quality (nutrients) or potentially quantity (epiphytes) appeared to be the moderating factor on *per capita* grazing, supporting the notion that nutrient-induced overgrazing would likely be associated with population effects (e.g. mass recruitment of urchins to seagrass meadows) rather than changing *per capita* grazing rates.

3.6 SUPPLEMENTARY DATA

Table 3.S1 Leaf biomass (gdw m^{-2}) for seagrass exposed to sea-urchin grazing (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched) for 82 days (n = 5).

	Leaf biomass (gdw m^{-2})	
Absent Grazing + Ambient Nutrients	94.27	± 12.83
Moderate Grazing + Ambient Nutrients	91.95	± 19.78
High Grazing + Ambient Nutrients	69.83	± 12.35
Absent Grazing + Enriched Nutrients	103.86	± 36.75
Moderate Grazing + Enriched Nutrients	87.69	± 12.91
High Grazing + Enriched Nutrients	109.20	± 32.21

(ANOVA: Grazing: $F_{2,24}$ = 0.130, p = 0.896, Nutrients: $F_{1,24}$ = 0.736, p = 0.392, Grazing × Nutrients $F_{2,24}$ = 0.549, p = 0.562)

Table 3.S2 Leaf initiation per day for seagrass exposed to sea-urchin grazing (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched) (*n* = 5-14).

	1st period (May)	**2nd period (June)**
Absent Grazing + Ambient Nutrients	0.077 ± 0.000	0.036 ± 0.012
Moderate Grazing + Ambient Nutrients	ND[a] ND[a]	0.041 ± 0.009
High Grazing + Ambient Nutrients	0.038 ± 0.022	0.040 ± 0.007
Absent Grazing + Enriched Nutrients	0.077 ± 0.028	0.033 ± 0.011
Moderate Grazing + Enriched Nutrients	ND[a] ND[a]	0.025 ± 0.008
High Grazing + Enriched Nutrients	0.077 ± 0.020	0.060 ± 0.010

[a] ND = No data taken

(1st: Grazing: $F_{1,20}$ = 1.085, p = 0.305, Nutrients: $F_{1,20}$ = 1.085, p = 0.297, Grazing × Nutrients $F_{1,20}$ = 1.085, p = 0.308)

(2nd: Grazing: $F_{2,52}$ = 1.276, p = 0.303, Nutrients: $F_{1,52}$ = 0.001, p = 0.988, Grazing × Nutrients $F_{2,52}$ = 1.462, p = 0.249)

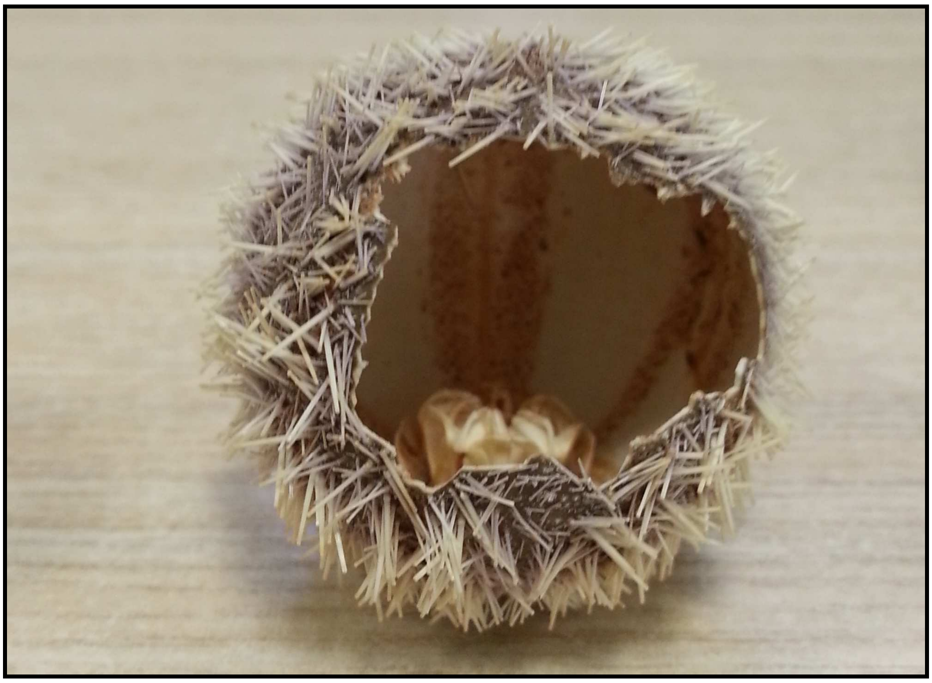
Table 3.S3 Breaking force (mN) for seagrass exposed to sea-urchin grazing (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched) for 169 days (*n* = 5).

	Breaking force (mN)
Absent Grazing + Ambient Nutrients	1538.13 ± 117.78
Moderate Grazing + Ambient Nutrients	1787.01 ± 084.76
High Grazing + Ambient Nutrients	1730.25 ± 148.02
Absent Grazing + Enriched Nutrients	1758.04 ± 178.49
Moderate Grazing + Enriched Nutrients	1620.23 ± 121.91
High Grazing + Enriched Nutrients	1767.82 ± 040.67

(Grazing: $F_{2,24} = 0.320$, p = 0.751, Nutrients: $F_{1,24} = 2.832$, p = 0.117, Grazing × Nutrients $F_{2,24} = 0.578$, p = 0.580)

Table 3.S4 ANOVA for the effect of sea-urchin grazing (absent *v.* moderate *v.* high) and nutrients (ambient *v.* enriched) on the average water column nutrients of NO_x, PO_4 & NH_3 within experimental plots over four dates following nutrient deployment.

Source	df	MS	F	P	Pairwise
NO_x					
Grazing (G)	2	5.15×10^{-6}	0.17	0.850	[a] NO_x pairwise for average N, AN < EN
Nutrients (N)	**1**	**7.20×10^{-4}**	**24.04**	**0.002**[a]	
G × N	2	3.54×10^{-6}	0.12	0.893	
Residual	18	3.00×10^{-5}			
PO_4					
Grazing	2	4.56×10^{-6}	0.64	0.551	[b] PO_4 pairwise for average N, AN < EN
Nutrients	**1**	**7.13×10^{-5}**	**10.07**	**0.005**[b]	
G × N	2	2.67×10^{-6}	0.38	0.689	
Residual	18	7.08×10^{-6}			
NH_3					
Grazing	2	7.22×10^{-5}	0.36	0.720	
Nutrients	1	5.62×10^{-4}	2.79	0.107	
G × N	2	1.76×10^{-5}	0.09	0.919	
Residual	18	2.02×10^{-4}			



Predation of urchins

Above: A test of the urchin *Amblypneustes pallidus* subject to fish or crustacean predation. Below: A test of the urchin *Amblypneustes pallidus* subject to mollusc predation.

Photo credits: Owen Burnell

CHAPTER FOUR

CONTEMPORARY RELIANCE ON BICARBONATE ACQUISITION PREDICTS INCREASED GROWTH OF SEAGRASS *AMPHIBOLIS ANTARCTICA* IN A HIGH CO_2 WORLD

Owen W. Burnell, Sean D. Connell, Andrew D. Irving, Jennifer R. Watling and Bayden D. Russell

School of Earth and Environmental Sciences,

The University of Adelaide, Adelaide, SA 5005 Australia

STATEMENT OF AUTHORSHIP

In this paper I collected the field samples, executed the laboratory experiment and analysed the data. I wrote the manuscript, assisted by Sean D. Connell, Andrew D. Irving, Jennifer R. Watling & Bayden D. Russell. All authors contributed to the experimental design. Sean D. Connell and Bayden D. Russell provided funding for the project.

Signatures of co-authors:

Professor Sean D. Connell

Dr Andrew D. Irving

A/Prof Jennifer R. Watling

Dr Bayden D. Russell

PREAMBLE

Chapter 4 was motivated by the significant role CO_2 plays in the photosynthesis and growth of seagrass. While the preceding chapters focus on the local grazing ecology of seagrass, this chapter considers the consequences of a globally changing resource (i.e. CO_2) that could deliver significant benefits to seagrass. The chapter is a reproduction of a co-authored manuscript currently in review at *Conservation Physiology,* and therefore is written in plural throughout and uses the journal formatting. The paper is written for a global audience with an interest in how CO_2 could modify seagrass photosynthesis and growth.

Burnell, O.W., Connell, S.D., Irving, A.D., Watling, J.R. & Russell, B.D. (2014) Contemporary reliance on bicarbonate acquisition predicts increased growth of seagrass *amphibolis antarctica* in a high CO2 world.
Conservation Physiology, under review

NOTE:
This publication is included on pages 104-131 in the print copy of the thesis held in the University of Adelaide Library.

Filamentous epiphytes on seagrass

Amphibolis antarctica with heavy filamentous epiphyte growth during the Austral summer.

Photo credits: Owen Burnell

CHAPTER FIVE

SEAGRASS RESPONSE TO CO_2 CONTINGENT ON EPIPHYTIC ALGAE: INDIRECT EFFECTS CAN OVERWHELM DIRECT EFFECTS

Owen W. Burnell, Bayden D. Russell, Andrew D. Irving and Sean D. Connell

School of Earth and Environmental Sciences,
The University of Adelaide, Adelaide, SA 5005 Australia

STATEMENT OF AUTHORSHIP

In this paper I collected the field samples, executed the laboratory experiment and analysed the data. I wrote the manuscript, assisted by Bayden D. Russell, Andrew D. Irving & Sean D. Connell. All authors contributed to the experimental design. Bayden D. Russell and Sean D. Connell provided funding for the project.

Signatures of co-authors:

Dr Bayden D. Russell

Dr Andrew D. Irving

Professor Sean D. Connell

PREAMBLE

Chapter 5 was motivated by the significant role epiphytes play in seagrass ecology. While there is a vast amount of literature available on the independent physiological response of seagrass to short-term CO_2 modifications, few studies focus on future longer-term ecological interactions. The chapter is a reproduction of a co-authored manuscript currently in review at *Oecologia*, and therefore is written in plural throughout and uses the journal formatting. The paper is written for a global audience with an interest in how CO_2 could modify seagrass and epiphyte growth and the critical role of light in mediating this response.

CHAPTER FIVE

SEAGRASS RESPONSE TO CO_2 CONTINGENT ON EPIPHYTIC ALGAE: INDIRECT EFFECTS CAN OVERWHELM DIRECT EFFECTS

5.1 ABSTRACT

Increased availability of dissolved CO_2 in the ocean can enhance the productivity and growth of marine plants such as seagrasses and algae, but realised benefits may be contingent on additional conditions (e.g. light) that modify biotic interactions between these plant groups. The combined effects of future CO_2 and differing light were tested on the growth of seagrass and their algal epiphytes by maintaining juvenile seagrasses *Amphibolis antarctica*, under three different CO_2 concentrations representing ambient, moderate future and high future forecasts (i.e. 390, 650 *v.* 900 $\mu l\ l^{-1}$) and two light levels representing low and high PAR (i.e. 43 *v.* 167 $\mu mol\ m^{-2}\ s^{-1}$). Above and below ground biomass, leaf growth, epiphyte cover, tissue chemistry and photosynthetic parameters of seagrasses were measured. At low light, there was a neutral to positive effect of elevating CO_2 on seagrass biomass and growth; at high light this effect of CO_2 switched toward negative as growth and biomass decreased at the highest CO_2 level. These opposing responses to CO_2 appeared closely linked to the overgrowth of seagrass by filamentous algal epiphytes when high light and CO_2 were combined. Importantly, all seagrass plants maintained positive leaf growth throughout the

experiment, indicating growth was inhibited by some experimental conditions, but not arrested entirely. Therefore, while greater light and CO_2 provided direct physiological benefits for seagrasses, when combined such benefits were likely negated by overgrowth of epiphytic algae. This result demonstrates how indirect ecological effects from epiphytes can modify independent physiological predictions for seagrass associated with global change.

5.2 INTRODUCTION

Trees, grasses, aquatic plants, and other primary producers are widely anticipated to derive physiological benefits from a CO_2-enriched atmosphere (Curtis and Wang 1998; Koch et al. 2013; Wand et al. 1999). Thus, despite the substantive concerns regarding the harmful effects of CO_2 and climate change on many organisms (Orr et al. 2005; Thomas et al. 2004; Thuiller et al. 2005), CO_2-enriched oceans might benefit marine autotrophs, such as seagrasses and algae (Beer and Koch 1996; Palacios and Zimmerman 2007), which can presently be carbon limited or reliant on energetically-costly pathways for photosynthesis (i.e. use of bicarbonate rather than dissolved CO_2) (Beardall et al. 1998; Koch et al. 2013; Raven 1991). While much focus has surrounded these direct physiological responses of marine plants to global changes in CO_2, biotic interactions can indirectly modify responses (Connell et al. 2011), especially among taxa that compete for resources (Connell et al. 2013; DeLucia et al. 1999).

Interactions between seagrasses and the epiphytes that colonise them are often concerning in locations that suffer seagrass loss as a consequence of changing environmental resources (Bryars et al. 2011; Thomsen et al. 2012). Declines in seagrass abundance and distribution are commonly associated with excessive epiphyte proliferation, often due to increases in nutrient availability (Mabrouk et al. 2013; Orth and Moore 1983; Walker and McComb 1992). While seagrasses facilitate algal epiphytes by providing a substrate for their recruitment and growth, epiphytes can have detrimental effects on their benefactors by competing and acting as a physical barrier for light, dissolved inorganic carbon (C_i) and nutrients (Borowitzka et al. 2006; Nayar et al. 2009; Nayar et al. 2010; Sand-Jensen 1977). Due to the high light requirements of seagrasses for photosynthesis, they are particularly vulnerable to environmental changes that affect its attenuation (Duarte 1991; Orth et al. 2006; Short and Wyllie-Echeverria 1996), but growth can similarly be inhibited by secondary limitations, such as CO_2 or nutrients (Alexandre et al. 2012; Touchette and Burkholder 2000).

Under contemporary CO_2 conditions seagrasses appear to experience greater carbon limitation and reliance on energetically-costly pathways for photosynthesis (i.e. use of bicarbonate rather than dissolved CO_2) than many algae, which suggests seagrass may benefit more from future CO_2 enrichment (Beer and Koch 1996; Koch et al. 2013). However, realised responses could depend on local-scale biotic interactions between seagrasses and their algal epiphytes (e.g. competition for resources). In terrestrial ecosystems the importance of combining ecology and physiology to understand the role of CO_2 in shaping communities has long been emphasised (Reekie and Bazzaz 1989; Williams et al. 1986). For example, changes in CO_2 can modify the community structure of terrestrial plants by favouring those species with greater canopy height that dominate light acquisition (Reekie and Bazzaz 1989). Seagrass systems are known to display similar environmentally mediated responses to different abiotic conditions. For example, coastal nutrient enrichment, which can benefit both seagrass and epiphytes by releasing them from nutrient limitation (Kelaher et al. 2013), typically causes disproportionate increases in epiphyte growth that results in seagrass decline (Bryars et al. 2011; Orth et al. 2006; Walker and McComb 1992). Importantly, any increase in epiphyte cover due to CO_2 enrichment has the potential to accelerate global seagrass loss (Short and Neckles 1999), with almost one-third of historical abundance already lost during the past ~ 130 years (Waycott et al. 2009).

Evidence from kelp-dominated systems indicates that CO_2 enrichment can promote the growth and recruitment of filamentous algae (Connell and Russell 2010; Falkenberg et al. 2013; Hepburn et al. 2011; Russell et al. 2011), which as epiphytes can colonise and damage seagrass. Limited testing of seagrass-algal interactions to moderate changes in CO_2 suggests that seagrass biomass could increase relative to free-living and epiphytic macro-algae; however, under additional conditions that favour algal growth (i.e. warming) positive effects on seagrass might be ameliorated (Eklöf et al. 2012). Similarly, light which varies concomitantly with many different environmental factors (e.g. depth, eutrophication, coastal sedimentation or season), can favour the proliferation of different algal epiphyte groups that grow on seagrass (Borowitzka et al. 2006; Collings et al. 2006). In particular, filamentous algae are known to grow and occupy available space more rapidly under shallow well-illuminated conditions (Russell et al. 2011).

The seagrass *Amphibolis antarctica* has undergone significant loss in southern Australia likely as a result of epiphyte overgrowth (Bryars et al. 2011; Bryars et al. 2008; Walker and McComb 1992) and its recovery following disturbance appears to be particularly slow (Bryars and Neverauskas 2004; Burnell et al. 2013a). Physiology alone might predict a positive response of this seagrass to increasing CO_2 (Beer and Koch 1996; Koch et al. 2013), however, concomitant positive epiphytic responses could modify any realised benefits on seagrass. We tested how the availability of CO_2 and light can affect the growth of *A. antarctica* and its algal epiphytes, by rearing juvenile seagrass for three months under various combinations of CO_2 concentrations and Photosynthetically Active Radiation (PAR). We tested the hypothesis that the benefits of CO_2 enrichment on seagrass growth would be reduced at high light intensities potentially due to excessive growth of filamentous epiphytes on seagrass leaves. If supported, this would demonstrate how indirect ecological effects can modify independent physiological predictions associated with global change.

5.3 MATERIALS AND METHODS

5.3.1 Collection site and biology

Juvenile seagrasses *Amphibolis antarctica* and any epiphytes growing on these plants were collected by hand from snorkelers at a depth of 3-4m near Marino Rocks in Gulf St Vincent, South Australia (35° 02.806′S, 138° 30.350′E). Plants were then transported in aerated aquaria to The University of Adelaide and kept in a glasshouse for one week to acclimatise to ambient conditions prior to the commencement of the experimental trials. The seagrass genus *Amphibolis* produces viviparous seedlings which typically become detached from adult plants during winter, and recruit to rough surfaces (e.g. seagrass rhizome banks) using a grappling apparatus; see Ducker et al. (1977). These juveniles can be transplanted into a laboratory environment before developing any root or rhizome network, therefore, avoiding common problems of laboratory trails such as root exposure and associated necrosis. Using juvenile seagrasses also ensured individual plants did not have differential nutritional support via translocation of resources from the large network of integrated clones in mature meadows. The distinctive morphology of *Amphibolis* includes leaf clusters upon short shoots, a number of which extend from each vertical rhizome. In mature plants this vertical rhizome becomes a perennial stem which can accumulate many epiphytes. Partly to avoid epiphyte accumulation on leaves, older leaves are continually shed, while new leaves are continually produced at the centre or meristem of each leaf cluster, approximately one every 14 days (Marbà and Walker 1999). For a detailed summary of the morphology of juvenile and mature *A. antarctica* plants; see Ducker et al. (1977) and Marbà and Walker (1999), respectively.

5.3.2 Experimental design

The effects of CO_2 and light were tested by planting seagrasses (average height = 8.56 ± 0.10 cm plant^{-1}, average fresh weight = 1.11 ± 0.07 g plant^{-1}) in sediment from the collection within transparent 2 l cylindrical microcosms (*n* = 36 microcosms, height = 32 cm, diameter = 10 cm, water depth = 25 cm, sediment depth = 5 cm). Seagrasses were introduced to the experimental trials with epiphyte communities present from the collection site. These communities were dominated by calcified and filamentous epiphytes living on the older leaves of each plant. Temperatures were maintained at 20°C using heater/chiller units

(TECO, Ravenna, Italy) attached to a recirculating water bath. This temperature was chosen as an average seawater temperature at the collection site over the experimental period. Due to the dynamic nature of the light climate in the near shore environment this temperature is relevant for plants across a wide array of light intensities. Salinity and water quality were maintained by replacing 70% of the microcosm volume with new seawater twice weekly. While the salinity and alkalinity levels reported may appear abnormally high (Table 1), low rainfall and high evaporation in southern Australia results in many bays and estuaries, including Gulf St Vincent, with naturally high salinity and alkalinity, and seagrass species such as *A. antarctica* show great tolerance and prosperity under such conditions (Walker 1985).

Three CO_2 levels (ambient CO_2, moderate CO_2 & high CO_2, hereafter ACO_2, MCO_2 & HCO_2) and two light levels (low light & high light, hereafter LL & HL) were used in a fully orthogonal design, creating a total of six treatment combinations. Seagrasses were maintained in microcosms for 12 weeks, with six replicate microcosms for each treatment and four juvenile seagrasses planted within each microcosm. Light treatments were fixed in two different blocks, while CO_2 treatments were randomly allocated to microcosms within each of these blocks. This approach ensured all microcosms could be included within the same water bath that maintained a constant temperature of 20°C.

5.3.3 CO_2

Carbon dioxide levels were chosen to cover a range of possible levels forecasted prior to the year 2100 (Meehl et al. 2007) representing current atmospheric CO_2 (~390 µl l^{-1}), moderate future (~650 µl l^{-1}) and high future (~900 µl l^{-1}) scenarios. To control CO_2 the experimental microcosms were constantly aerated (0.8 l min^{-1}) with either ambient or CO_2-enriched air from Pegas gas mass flow controllers (Columbus Instruments, CO, USA). Temperature, salinity and pH were measured daily and total alkalinity was measured weekly to calculate carbonate chemistry outcomes from the microcosms (Table 1).

5.3.4 Light

Levels of light were chosen to represent a range of natural irradiances that occur in seagrass meadows within Gulf St Vincent, South Australia (Bryars et al. 2011; Irving 2009). Environmental conditions in natural meadows, such as depth, eutrophication, wind direction and season deliver changes in attenuation, such that light intensities vary on both a spatial and temporal scale. Seagrasses were exposed to natural Photosynthetically Active Radiation (PAR) in the glasshouse by sheltering them from full surface irradiance using 50% black shade cloth over the entire experimental glass house (HL treatment) and additional 70% black shade cloth over half of the microcosms (LL treatment). Light was recorded in lux using Hobo® waterproof light and temperature pendant data loggers (Onset®, MA, USA), and converted to give an approximation of PAR using the constant (1 lux = 54 µmol m^{-2} s^{-1}) for sunlight as recommended by Thimijan and Heins (1983). Approximately 75% of PAR was attenuated by the additional shade cloth over LL treatments, when compared with HL treatments, such that average daily PAR across a 12-hr photoperiod for HL = 166.9 ± 9.5 µmol m^{-2} s^{-1}, compared with LL = 42.5 ± 2.7 µmol m^{-2} s^{-1}. These treatments resembled levels below and above ambient light previously recorded in a nearby seagrass meadow of 3.5m depth (i.e. 70 µmol m^{-2} s^{-1}; see Irving (2009). Peak daily irradiances occurred at ~1:00 pm for HL = 541.6 ± 45.7, compared with LL = 111.3 ± 11.7 (see Electronic Supplementary Material).

5.3.5 Biomass and growth parameters

Filamentous algal epiphytes that were dominated by Phaeophyta (i.e. brown algal species) were sampled from both shed leaves and living plants. The final four leaves shed in each experimental microcosm were used to determine average epiphyte biomass per shed leaf. Filamentous epiphytes were removed by lightly scraping shed leaves using a razor blade before washing filamentous epiphytes in 5% HCl to remove any sediment trapped amongst the algae. After 12 weeks whole individual seagrasses were scraped in the same fashion. All filamentous algae were dried and weighed to measure dry weight. To measure biomass seagrass leaves and shoots (i.e. above ground biomass) were separated from roots and rhizomes (i.e. below ground biomass) and dried for 48 hours at 60 °C. Above ground components of seagrass were then acid washed in 5% HCl to remove any calcified algal epiphytes and re-dried for 48 hours at 60 °C to measure dry weight as recommended in Kendrick and Lavery (2001). Calcified epiphyte biomass that consisted entirely of encrusting species was quantified by subtracting the final seagrass dried weight after acid washing from initial dried weight prior to acid washing for both shed leaves and living plants. Seagrass tissue from living plants was then ground with a mortar and pestle and analysed for carbon and nitrogen (%

N and % C) on an Isotope Ratio Mass Spectrometer (Hydra 2020 ANCA-GSL Version 4.0, Sercon).

Seagrass growth during the experiment was measured by recording new leaf initiation, quantified by clipping the corner of the second youngest leaf on each plant at the commencement of the treatment period. This method is less obtrusive than sheath marking or using leaf ties, which can impact meristem growth and plant buoyancy (O. W. Burnell pers. obs.). Because *Amphibolis* spp. grow by continually initiating new leaves at the centre (or meristem) of each leaf cluster it is possible to track growth by counting the leaves produced above the clipped leaf. The total number of leaves on each leaf cluster was also recorded before planting and again after 12 weeks to calculate total leaf balance and shedding (i.e. Leaf Balance = (Final # Leaves – Initial # Leaves)/Days, Leaf Shedding = (New Leaf Initiation – Leaf Balance)/Days).

5.3.6 PAM Fluorometry

A diving PAM fluorometer (Walz, Germany) was used to record Rapid Light Curves (RLCs) and Maximum Quantum Yield (QY_{max}) for seagrasses acclimated to experimental conditions after 12 weeks. The PAM generated a RLC by delivering nine successive irradiances (0, 18, 37, 62, 92, 125, 186, 256 & 400 μmol m^{-2} s^{-1}) of 10 seconds each followed by a saturating pulse of light, to record Effective Quantum Yield (Φ_{PSII}) at each successive light level. All RLC measurements were taken between 11:00 am – 12:30 pm. To calculate absolute Electron Transport Rate (ETR) the light absorbance of the sample leaf was taken by placing the quantum sensor directly behind the leaf and measuring the percentage of light that was absorbed. ETR was calculated from the equation ETR = Φ_{PSII} × PAR × leaf absorbance × 0.5. Actual leaf absorbance was measured in favour of using the traditional ETR factor of 0.84. The traditional ETR factor is more relevant for terrestrial plants, particularly as reflection from the leaf surface is negligible during underwater measurements (Beer et al. 2001). QY_{max} was determined from pre-dawn fluorescence measurements.

5.3.7 Statistical analyses

Prior to all analyses homogeneity of variances was tested using Levene's test, however, normality was not tested as the permutation based analysis used makes no explicit assumptions regarding normality. Only ETR_{max} was found to violate the assumption of homogeneity of variance, therefore, data were log transformed prior to analysis. Univariate two-factor ANOVA was then used to compare the effect of CO_2 and light treatments on seagrass (i.e. biomass, leaf growth, ETR_{max}, QY_{max} & tissue C/N) and epiphytes (i.e. filamentous and calcified biomass) using the program PERMANOVA+ for Primer v6. CO_2 and light were treated as fixed and orthogonal. As epiphytes can increase with substrate availability, seagrass leaf biomass was used as a covariate for both filamentous and calcified epiphyte biomass in the ANOVAs. Averages from each microcosm were used as replicates. Permutation based unplanned post-hoc pairwise comparisons were used where significant terms were detected. Linear regression was used to compare the effect of total epiphyte biomass and total seagrass biomass on % nitrogen in plant tissues.

5.4 RESULTS

5.4.1 Seagrass biomass

A contrasting pattern emerged for both above ground and below ground biomass, whereby CO_2 had a neutral or positive effect on seagrass at low light (LL), but a neutral or negative effect at high light (HL). This pattern involved a complex interaction between the two experimental factors of light and CO_2 (Table 2a). Under LL there was no difference in above ground biomass between the three different concentrations of CO_2, whereas at HL seagrass biomass was lower under high CO_2 (HCO_2) compared with ambient CO_2 (ACO_2) and moderate CO_2 (MCO_2) plants. When the effect of light was considered on above ground biomass, HL resulted in greater biomass at ACO_2, but this effect was not present at either MCO_2 or HCO_2 (Fig. 1a). For below ground biomass under LL both MCO_2 and HCO_2 had greater biomass than ACO_2, whereas under HL biomass at HCO_2 was lower than MCO_2. When the effect of contrasting light conditions was considered on below ground biomass, HL resulted in greater biomass at ACO_2, but this effect was not present at either MCO_2 or HCO_2 (Fig. 1b, Table 2b).

5.4.2 Leaf growth

Leaf growth parameters displayed a similar pattern to biomass. While at LL CO_2 had no significant effect on new leaf initiation, at HL new leaf initiation decreased for seagrass under HCO_2 when compared with MCO_2 treatments. Similarly, the effect of HL progressed from positive at ACO_2 to neutral or negative at HCO_2. For example, at both ACO_2 and MCO_2 HL promoted greater new leaf initiation, but this effect was absent at HCO_2 (Fig. 2, Table 2c). Similarly, the positive effect of light on leaf balance at MCO_2, switched to become negative at HCO_2 (Fig. 2,

Table 2d). This change in leaf balance was a combination of both lower new leaf initiation and greater leaf shedding at HL and HCO_2. While the pairwise comparisons for the interaction between CO_2 and light varied between biomass and leaf growth, consistent patterns within the data show the disparate effect of HCO_2 on seagrass at LL compared with HL.

Table 5.1 Sea water chemistry in the experimental microcosm. Measurements of pH, temperature, salinity and total alkalinity were used to calculate average carbonate chemistry values for the experimental period. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. A_T = total alkalinity; TCO_2 = total CO_2; pCO_2 = CO_2 partial pressure; HCO_3^- = bicarbonate; CO_3^{2-} = carbonate; Pa = pascals; kgsw = kilogram of seawater

	pH		**A_T (µmol kgsw^{-1})**		**Salinity (g kgsw^{-1})**		**Temp (° C)**		**TCO_2 (µmol kgsw^{-1})**	**pCO_2 (Pa)**	**HCO_3^- (µmol kgsw^{-1})**	**CO_3^{2-} (µmol kgsw^{-1})**	**CO_2 (µmol kgsw^{-1})**
ACO_2 & LL	8.12	± 0.002	2700.88	± 26.90	40.30	± 0.06	19.84	± 0.01	2308.73	38.067	2011.41	285.47	11.84
ACO_2 & HL	8.16	± 0.002	2664.34	± 15.35	40.39	± 0.07	19.86	± 0.01	2250.79	34.075	1942.12	298.08	10.59
MCO_2 & LL	7.94	± 0.004	2669.33	± 11.63	39.95	± 0.07	19.86	± 0.01	2400.38	62.050	2176.99	204.06	19.33
MCO_2 & HL	7.96	± 0.002	2688.77	± 27.36	40.16	± 0.04	19.87	± 0.01	2406.84	59.646	2175.58	212.80	18.56
HCO_2 & LL	7.82	± 0.003	2696.77	± 34.73	39.96	± 0.05	19.87	± 0.01	2497.59	88.369	2308.82	161.24	27.52
HCO_2 & HL	7.83	± 0.002	2729.44	± 16.38	40.26	± 0.06	19.85	± 0.02	2518.55	85.614	2322.71	169.21	26.63

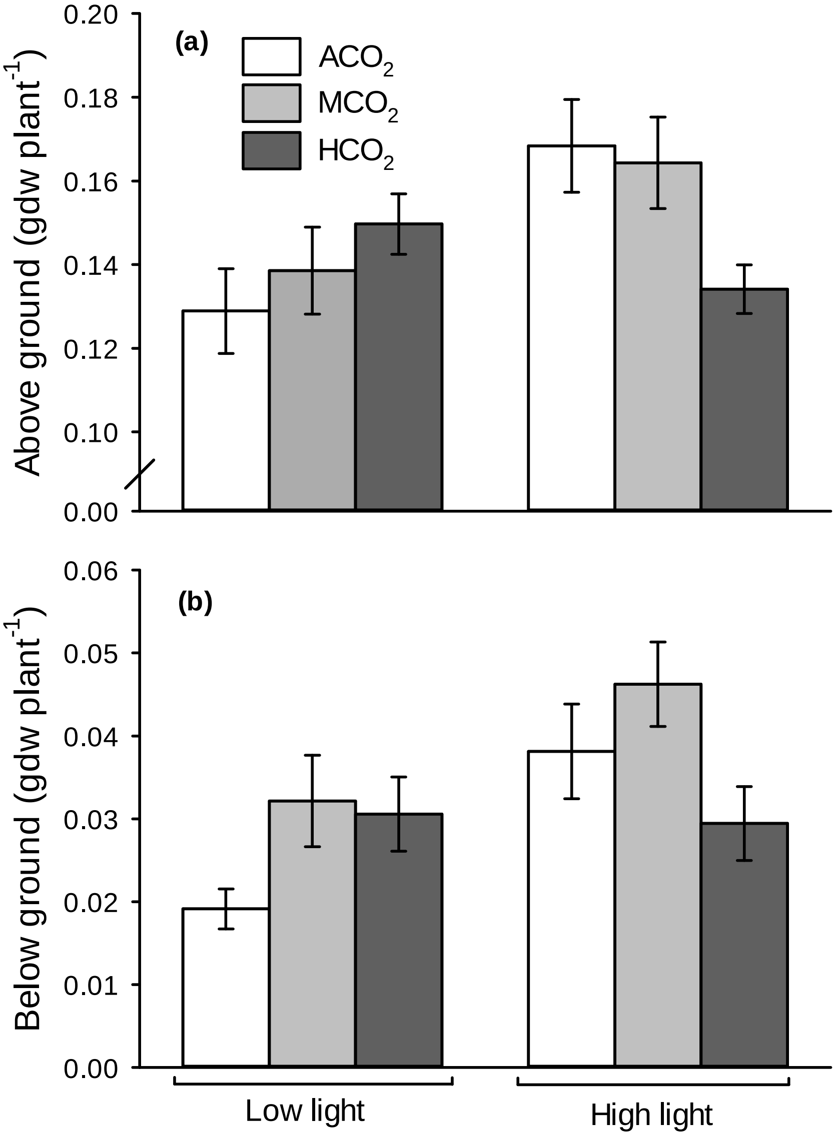


Figure 5.1 a) Above ground and b) below ground biomass of juvenile seagrass *Amphibolis antarctica* plants grown at different CO_2 and light levels for 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. Bars represent Mean ± SE (*n* = 6)

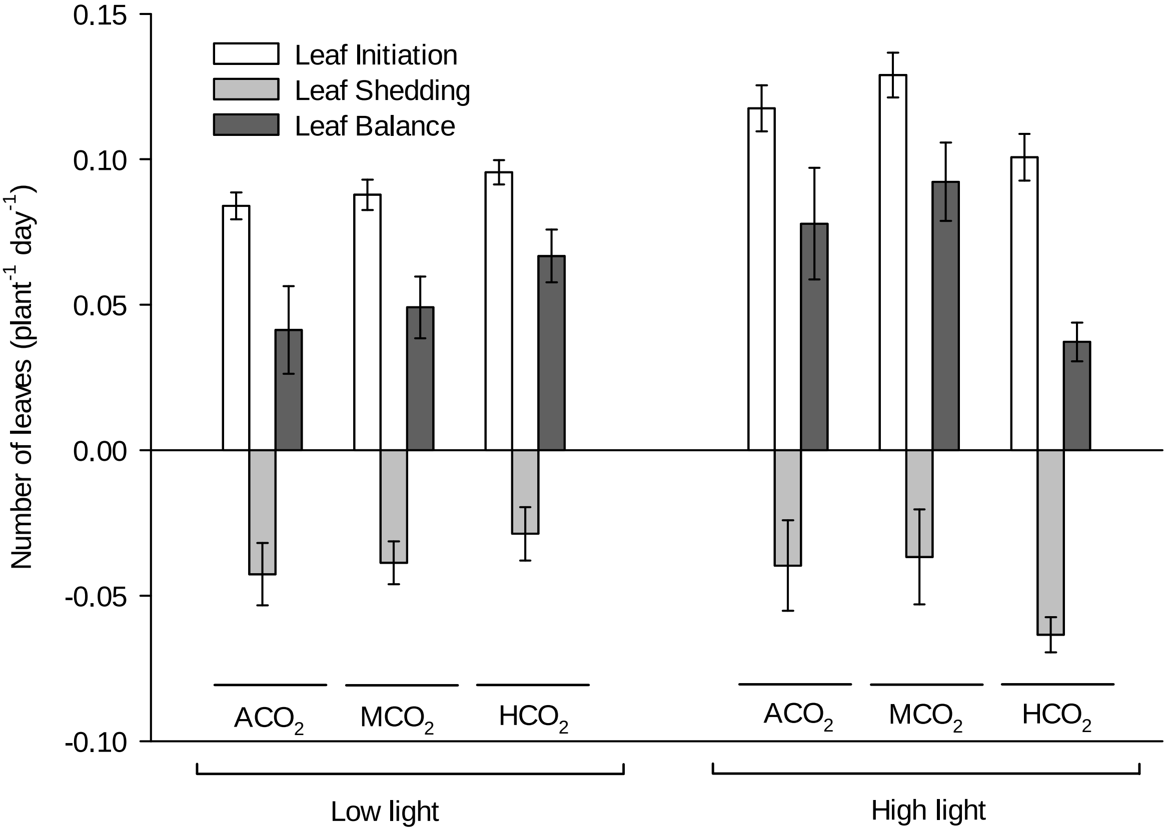


Figure 5.2 Leaf initiation, shedding and net balance for juvenile seagrass grown at different CO_2 and light levels for 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. Bars represent Mean ± SE (*n* = 6)

Table 5.2 ANOVA comparing the effects of CO_2 and Light on the a) above ground, b) below ground biomass, c) leaf initiation and d) leaf balance of juvenile seagrass *Amphibolis antarctica* after 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. Significant effects from ANOVA are highlighted in bold

Source	df	MS	*F*	p	pairwise
a) Above Ground					
CO_2	2	2.88×10^{-4}	0.5351	0.58	[a] at LL, $ACO_2 = MCO_2 = HCO_2$; at HL, $ACO_2 = MCO_2 > HCO_2$; at ACO_2, LL < HL; at MCO_2, LL = HL; at HCO_2, LL = HL.
Light	1	2.46×10^{-3}	4.5769	0.035	
CO_2 × Light	**2**	**2.46×10^{-3}**	**4.5713**	**0.022**[a]	
Residual	30	5.39×10^{-4}			
b) Below Ground					
CO_2	2	4.84×10^{-4}	3.8464	0.033	**[b]** at LL, $ACO_2 < MCO_2 = HCO_2$; at HL, $ACO_2 = MCO_2$, $ACO_2 = HCO_2$, $MCO_2 > HCO_2$; at ACO_2, LL < HL; at MCO_2, LL = HL; at HCO_2, LL = HL.
Light	1	8.34×10^{-4}	6.6277	0.018	
CO_2 × Light	**2**	**5.00×10^{-4}**	**3.9741**	**0.027**[b]	
Residual	30	1.26×10^{-4}			
c) Leaf Initiation					
CO_2	2	3.40×10^{-4}	1.34	0.31	**[c]** at LL, $ACO_2 = MCO_2 = HCO_2$; at HL, $ACO_2 = MCO_2$, $ACO_2 = HCO_2$, $MCO_2 > HCO_2$; at ACO_2, LL < HL; at MCO_2, LL < HL; at HCO_2, LL = HL.
Light	1	6.38×10^{-3}	25.146	0.001	
CO_2 × Light	**2**	**1.08×10^{-3}**	**4.2697**	**0.022**[c]	
Residual	30	2.54×10^{-4}			
d) Leaf Balance					
CO_2	2	1.06×10^{-3}	1.0429	0.36	**[d]** at LL, $ACO_2 = MCO_2 = HCO_2$; at HL, $ACO_2 = MCO_2$, $ACO_2 = HCO_2$, $MCO_2 > HCO_2$; at ACO_2, LL = HL; at MCO_2, LL < HL; at HCO_2, LL > HL.
Light	1	2.51×10^{-3}	2.4711	0.13	
CO_2 × Light	**2**	**4.86×10^{-3}**	**4.7802**	**0.013**[d]	
Residual	30	1.02×10^{-3}			

5.4.3 Seagrass epiphytes

The biomass of filamentous epiphytes was greater at HL. CO_2 also increased filamentous epiphyte biomass when elevated at HL. While these effects were not significant when whole plants were sampled at the conclusion of the experiment, they were clearly evident on the older leaves continually being shed by seagrasses (Figs. 3a,b). Filamentous epiphytes accumulated dispropotionately on the older leaves at HL, thus sampling singular shed leaves was the most accurate way to measure this pattern non-destructively. Under the combination of HCO_2 and HL the shed leaves had accumulated greater biomass of filamentous epiphytes than leaves under combined ACO_2 and HL conditions, while no differences were observed between CO_2 treatments under LL. Light had a strong positive effect on the growth of filamentous epiphytes, as biomass was greater in all HL treatments (Table 3a). The opposite was found for calcified epiphytes, whereby CO_2 had no effect on shed leaves and decreased biomass on living seagrass (Fig. S2a,b). The biomass of both algal epiphyte groups generally had a positive relationship with leaf biomass, which was incorporated as a covariate in all ANOVA for epiphyte biomass. This association was particularly strong for calcified epiphytes (r^2 = 0.75 and 0.82, on shed and living leaves, respectively), but less so for filamentous epiphytes (r^2 = 0.34 and 0.09, on shed and living leaves, respectively), and thus was only a signifcant covariate in three of the four analyses (Tables 3a,b, S1a,b).

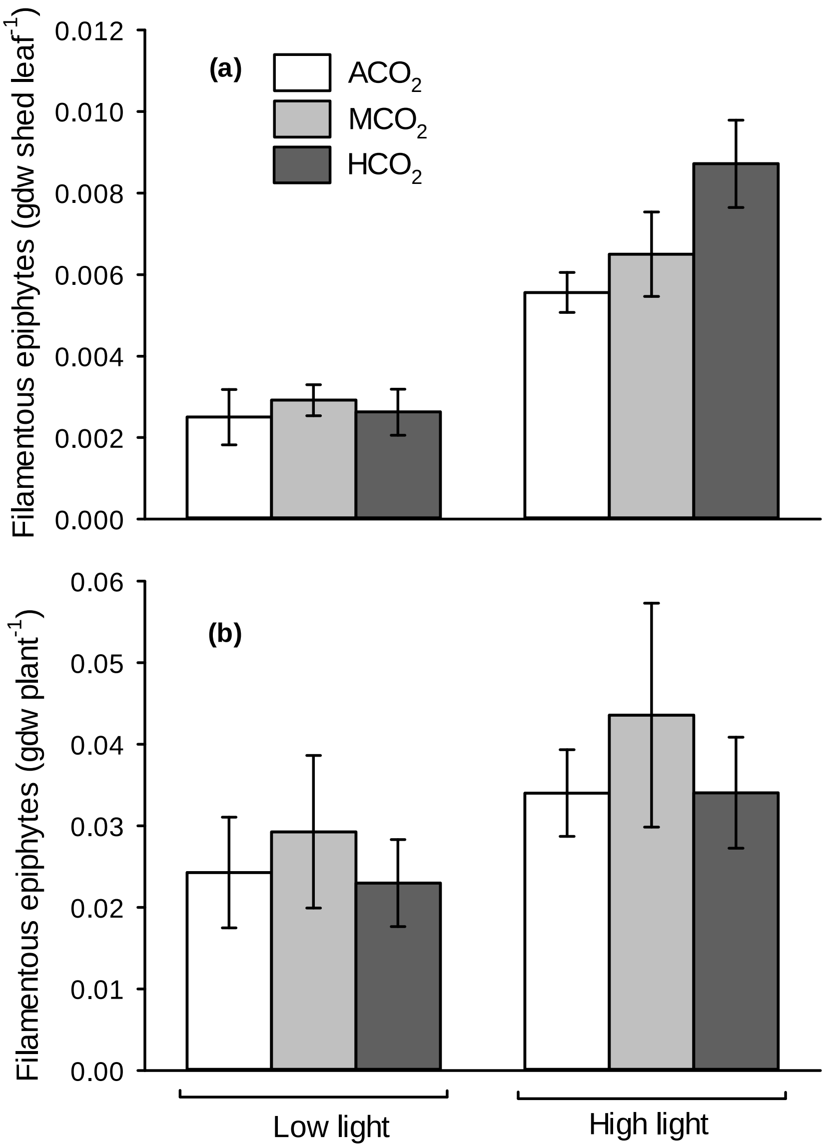


Figure 5.3 a) Filamentous epiphytes on a) shed leaves and b) living plants of juvenile seagrass grown at different CO_2 and light levels for 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. Bars represent Mean ± SE (*n* = 6)

Table 5.3 ANOVA comparing the effects of CO_2 and Light on the biomass of filamentous epiphytic algae on juvenile seagrass *Amphibolis antarctica* after 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. Significant effects from ANOVA are highlighted in bold

Source	df	MS	*F*	*P*	pairwise
a) Shed leaves					
Covariate	**1**	**1.64×10^{-5}**	**6.046**	**0.022**	[a] at LL, $ACO_2 = MCO_2 = HCO_2$; at HL, $ACO_2 = MCO_2$, $MCO_2 = HCO_2$, $ACO_2 < HCO_2$; at ACO_2, LL < HL; at MCO_2, LL < HL; at HCO_2, LL < HL.
CO_2	2	7.77×10^{-6}	2.860	0.062	
Light	1	8.85×10^{-5}	32.56	0.001	
CO_2 × Light	**2**	**1.08×10^{-5}**	**3.968**	**0.026**[a]	
Residual	29	2.72×10^{-6}			
b) Living plants					
Covariate	1	4.05×10^{-4}	0.951	0.32	
CO_2	2	1.65×10^{-4}	0.386	0.70	
Light	1	9.56×10^{-4}	2.248	0.12	
CO_2 × Light	2	3.40×10^{-5}	0.080	0.93	
Residual	29	4.25×10^{-4}			

5.4.4 Seagrass physiology

Light had a strong physiological effect on seagrass, which was consistent across all levels of CO_2. Under HL, ETR_{max} increased compared to LL (Fig. 4a,b,c), while the light harvesting capabilities of leaves generally decreased (i.e. QY_{max}) (Table 4). In contrast, the physiological effects of CO_2 were not as consistent (Fig. 4a,b,d). While ETR was generally higher where CO_2 was enriched, this pattern was not-significant (Fig. 4). Interestingly, while the QY_{max} was lower in HL compared to LL plants at the halfway point of the experiment, after 12 weeks this difference was absent (Table 4). It is important to remember these measurments were taken on the younger leaves that were realtively free of epiphytes, therefore they largely reflect the direct benefits of CO_2 and light, rather than changes caused by epiphytes on the older leaves. Taking measurements on older leaves would have required destroying epiphyte assemblages and associated confounding effects.

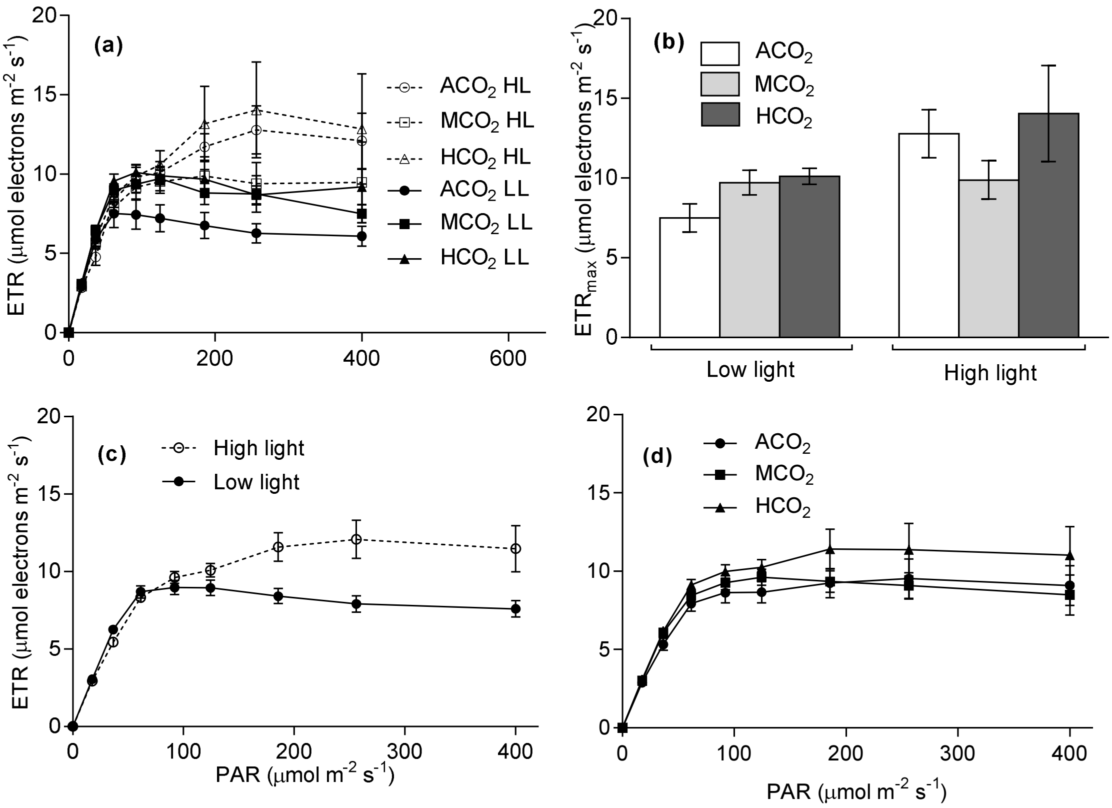


Figure 5.4 a) Rapid Light Curves (RLC) (n = 6), b) ETR_{max} (n = 6), c) RLC pooled by light treatment (n = 18) and d) RLC pooled by CO_2 treatment (n = 12) for juvenile seagrass plants grown at different CO_2 and light levels for 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. ETR calculated at nine different successive irradiances (0, 18, 37, 62, 92, 125, 186, 256 & 400 μmol m^{-2} s^{-1}). Bars represent Mean ± SE. (ANOVA for ETR_{max}, CO_2: $F_{2,30} = 1.14$, $p = 0.364$, Light: $F_{1,30} = 5.62$, $p = 0.033$, CO_2 × Light: $F_{2,30} = 2.23$, $p = 0.137$)

5.4.5 Seagrass biochemistry

Variations to the tissue chemistry of seagrass were driven largely by light conditions that caused changes in % N, while the % C of seagrasses did not change significantly between the treatments. These changes resulted in C:N ratios increasing under HL, as well as CO_2 (Table 4). An inverse relationship also emerged between seagrass % N and the biomass of both seagrass and algal epiphytes in the experimental microcosms. This was supported by regression analysis of % N with total seagrass biomass (r^2 = 0.280) and total epiphyte biomass (r^2 = 0.378) (Fig. S3a,b).

Table 5.4 Average values and ANOVA for QY_{max}, % N, % C & C:N of juvenile seagrass *Amphibolis antarctica* grown at different CO_2 and light levels for 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light; gdw = gram of dry weight. Mean ± SE (*n* = 6). Significant effects from ANOVA are highlighted in bold

		QY_{max} (6 weeks)		**QY_{max} (12 weeks)**		**% N (gdw)**		**% C (gdw)**		**C:N**	
ACO_2 & LL		0.683	± 0.016	0.656	± 0.033	1.45	± 0.08	62.61	± 3.06	43.34	± 1.39
ACO_2 & HL		0.567	± 0.029	0.646	± 0.021	1.29	± 0.04	61.69	± 2.41	47.85	± 1.46
MCO_2 & LL		0.672	± 0.016	0.658	± 0.012	1.38	± 0.02	64.01	± 1.50	46.40	± 0.94
MCO_2 & HL		0.588	± 0.019	0.638	± 0.024	1.16	± 0.05	63.06	± 2.23	55.06	± 3.13
HCO_2 & LL		0.671	± 0.018	0.683	± 0.017	1.30	± 0.07	62.01	± 2.24	47.85	± 1.46
HCO_2 & HL		0.538	± 0.015	0.631	± 0.023	1.26	± 0.06	65.27	± 2.20	52.22	± 1.95
	df	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
CO_2	2	2.14	0.13	0.08	0.92	2.10	0.13	0.29	0.76	**3.67**	**0.044**[a]
Light	1	**33.17**	**0.001**	2.17	0.18	**4.59**	**0.049**	0.05	0.83	**7.27**	**0.012**
CO_2 × Light	29	1.92	0.15	0.46	0.64	0.70	0.52	0.57	0.56	0.01	0.99

[a] **CO_2** Pairwise C:N $ACO_2 < MCO_2 = HCO_2$.

5.5 DISCUSSION

The opposing effects of CO_2 on seagrasses under different irradiances highlights that forecasts of direct effects under global change might be modified where primary producers interact. We found that the generally positive effects of CO_2 enrichment on seagrass growth and biomass (Campbell and Fourqurean 2013; Jiang et al. 2010; Palacios and Zimmerman 2007; Russell et al. 2013) became negligible and even unfavourable for growth under high light, which was accompanied by greater proliferation of filamentous algal epiphytes. While physiology predicts the benefits of CO_2 enrichment for seagrass could exceed those for algae (Beer and Koch 1996), purely physiological studies can have temporal constraints that limit their reconciliation with biotic interactions. Our findings build upon this physiological theory by showing the positive responses of filamentous epiphytes to CO_2 under high irradiances were concomitant with reduced seagrass biomass and leaf balance. Importantly, all seagrass plants maintained positive leaf growth throughout the experiment, indicating growth was inhibited by the experimental conditions (e.g. the combination of high light and CO_2), but not arrested entirely.

Similar biotic interactions occur across biomes, where seemingly beneficial abiotic factors might indirectly inhibit certain taxa. Under CO_2 enrichment in terrestrial systems, species lower in the canopy can be disadvantaged in comparison to those species with a greater canopy height that acquire more light (Reekie and Bazzaz 1989). On sub-tidal rocky reef, canopy-forming kelp can limit the response of opportunistic understorey turf algae species to abiotic changes in nutrients and CO_2 (Falkenberg et al. 2012; Russell and Connell 2005) and in seagrass meadows eutrophication can favour opportunistic species (i.e. epiphytes), that overgrow and inhibit the response of habitat formers (i.e. seagrass) (Bryars et al. 2011; Tomasko and Lapointe 1991). Collectively, these examples and our study help to build an understanding that biotic interactions have the potential to modify direct physiological predictions associated with global change.

In all of these examples the species that limits the response of another appears to dominate resource acquisition, most likely due to their physical position. Epiphytes can inhibit seagrass physiological performance in several ways, including direct shading, expansion of the diffusive boundary layer, as well as providing a physical barrier and competition for both nutrient and C_i acquisition (Borowitzka et al. 2006; Nayar et al. 2009; Nayar et al. 2010; Sand-Jensen 1977). Epiphyte photosynthesis can expand the area depleted in C_i that surrounds a leaf, while simultaneously increasing O_2 concentrations and, therefore, promoting greater photorespiration in the host plant (Sand-Jensen 1989; Sand-Jensen et al. 1985). Experiments within filamentous algal communities on sub-tidal rock show productivity of the algae itself decreases rapidly as a function of biomass, which is caused by self-shading and boundary layer effects (Copertino et al. 2009). It follows that living substrata, such as seagrass leaves, could be similarly impacted.

While seagrasses have significant scope to benefit from increased CO_2, algal responses are more variable (Beer and Koch 1996; Koch et al. 2013). Often algal species which already have an efficient carbon concentrating mechanisms will show no response to CO_2 enrichment (Israel and Hophy 2002). Whereas, less morphologically complex algae, such as the filamentous epiphytes in this experiment, often appear to lack an efficient carbon concentrating mechanism in contemporary CO_2 environments, thus may benefit disproportionately from greater direct diffusion of CO_2 in the future (Falkenberg et al. 2013a; Hepburn et al. 2011). Yet, there is limited information on the carbon acquisition pathways of many algae species, which can be strong competitors and in some instances nuisance species (Koch et al. 2013; Thomsen et al. 2012).

While many non-calcified algal groups benefit from CO_2 enrichment (Connell and Russell 2010; Sarker et al. 2013; Zou and Gao 2009), this contrasts with calcified epiphytes that can decrease where lower carbonate saturation favours dissolution or limits recruitment (Hall-Spencer et al. 2008; Martin et al. 2008). In accordance with this prediction, calcified epiphytes decreased at HCO_2. While lower carbonate saturation might have caused this loss of calcified epiphytes, this effect could also have been driven by greater leaf shedding from plants at HCO_2 and HL, as older leaves support greater biomass of calcified epiphytes. While calcified epiphytes can impact *A. antactica* in a negative manner (Burnell unpublished data), the strong positive co-variation between calcified epiphytes and seagrass biomass is a common association (James et al. 2009), and suggests calcified epiphytes were more likely to be a "passenger" on seagrass leaves, rather than inducing seagrass loss.

Physiological responses pointed to the direct benefits of light and CO_2 for seagrass. Greater light and CO_2 resulted in generally higher ETR_{max}, albeit this response was non-signifcant for CO_2. This increase in ETR_{max} was concomitant with a decrease in $QY_{max.}$ These responses represent predictable effects of photo-acclimation, where light limited plants might increase their harvesting capabilities (i.e. QY_{max}), but still maintain lower ETR_{max} (Kubler and Raven 1995; Schwarz and Hellblom 2002). The non-significant effect of QY_{max} after 12 weeks suggested that HL plants possibly increased the harvesting capacity of young leaves, as older leaves became overgrown and shed.

Treatment effects on seagrass growth were also evident in the changing chemical composition of plant tissues. The observed decreases in % N (increased C:N) under HL are attributable to the dilution of nitrogen from increasing accumulation of non-structural carbohydrates (Campbell and Fourqurean 2013; Gifford et al. 2000; Peralta et al. 2002; Stitt and Krapp 1999), which was supported by regression analysis of total seagrass biomass against % N of seagrass ($r^2 = 0.280$). A portion of this could also be due to increasing nitrogen demand by plants at high CO_2 where photosynthesis can increase (Alexandre et al. 2012; Stitt and Krapp 1999). While increased seagrass growth under enriched CO_2 can induce nitrogen limitation (Alexandre et al. 2012), the internal nitrogen content in plants was within natural limits sampled from other oligotrophic meadows in the Gulf St Vincent (Range 1.01–1.70%; Burnell unpublished data) and locations sampled in Western Australia (Range 0.75–2.5%; Burkholder et al. 2013). Significant nitrogen limitation restricting growth during the experiment would also seem unlikely given the greatest growth was observed where % N was lowest. The significant negative relationship between total epiphyte biomass and seagrass % N ($r^2 = 0.378$) could indicate that competition from epiphytes limited seagrass nitrogen acquisition, but this could also reflect similar growth requirements for seagrass and epiphytes.

There are a number of additional abiotic and biotic conditions not manipulated here that can also affect seagrass epiphytes (Bryars 2009; Keuskamp 2004; Lavery et al. 2007; Lavery and Vanderklift 2002). In many instances physical abrasion among seagrass leaves during periods of greater water movement might control epiphyte assemblages (Bryars 2009; Lavery et al. 2007). In accordance with this, filamentous algae are often more prevalent in sheltered locations, whilst calcified algae are associated with exposed coasts (Kendrick and Burt 1997; Lavery and Vanderklift 2002). Similarly, seasonal or global changes in temperature might increase algal growth relative to seagrass (Bulthuis and Woelkerling 1983; Eklöf et al. 2012; Short and Neckles 1999). Mesograzers, such as amphipods and gastropods, can also play an important, but variable, role in controlling algal growth on seagrass. For example, in southern Australia there is evidence mesograzers can reduce filamentous epiphyte biomass on *Posidonia* spp, but experiments on *A. antarctica* have shown limited effects (Cook et al. 2011; Keuskamp 2004). Similarly, some macro-invertebrates such as urchins can modify seagrass biomass and graze epiphytes, but are generally sparsely distributed in the region (Burnell et al. 2013a; Burnell et al. 2013b). Thus, differences in hydrodynamics, temperature and grazing assemblages can deliver variable conditions that modify epiphyte proliferation, suggesting the degree to which epiphytes inhibit seagrass might reflect the temporal and spatial variability of these conditions.

There also remains limited understanding of how predictions of the potential effects of changing global conditions based on artificial laboratory environments will translate to actual changes in natural habitats. One recent comparison of opportunistic algal growth in reef habitats between *in situ* (i.e. CO_2 vent) and laboratory settings found comparable rates of growth between these natural and artificial habitats (Connell et al. 2013). While *in situ* studies at volcanic CO_2 vents can overcome some confounding effects associated with limited water exchange and other abiotic/biotic conditions, such studies must be interpreted cautiously due to the legacy of unknown variation in these habitats and sometimes limited replication (Calosi et al., 2013, Koch et al., 2013). Importantly, a cautionary approach using the combination of multiple techniques will most effectively progress our understanding of the ecological and physiological changes associated with global change (Russell et al. 2012).

Loss of seagrass meadows places them amongst the most threatened habitats worldwide (Orth et al. 2006; Waycott et al. 2009). While elevated CO_2 is often considered in terms of its direct positive effects on the growth of photosynthetic organisms, we consider that this focus overlooks the important role of CO_2 as one of a number of resources which can combine to have contrasting effects on competitors. These contrasting effects, therefore, have the capacity to shift the dominance of opportunistic taxa (e.g. epiphytes) under favorable abiotic conditions. Consequently, understanding how resources combine to alter biotic interactions has an important role in forecasting the persistence or change of ecosystems.

5.6 SUPPORTING INFORMATION

Table 5.S1 ANOVA comparing the effects of CO_2 and Light on the biomass of calcified epiphytic algae on juvenile seagrass *Amphibolis antarctica* after 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2. Significant effects from ANOVA are highlighted in bold

Source	**df**	**MS**	***F***	**p**
a) Shed leaves				
Covariate	**1**	$\mathbf{2.01 \times 10^{-4}}$	**95.96**	**0.001**
CO_2	2	4.74×10^{-6}	2.262	0.11
Light	1	8.35×10^{-6}	3.984	0.062
CO_2 × Light	2	1.03×10^{-6}	0.491	0.61
Residual	29	2.10×10^{-6}		
b) Living plants				
Covariate	**1**	$\mathbf{6.17} \times 10^{-3}$	**183.3**	**0.001**
$\mathbf{CO_2}$	**2**	$\mathbf{1.84} \times 10^{-4}$	**5.452**	**0.006**[a]
Light	1	1.46×10^{-4}	4.335	0.055
CO_2 × Light	2	7.16×10^{-5}	2.124	0.16
Residual	29	3.37×10^{-5}		

[a] $\mathbf{CO_2}$ Pairwise for Living Plants $ACO_2 = MCO_2 > HCO_2$

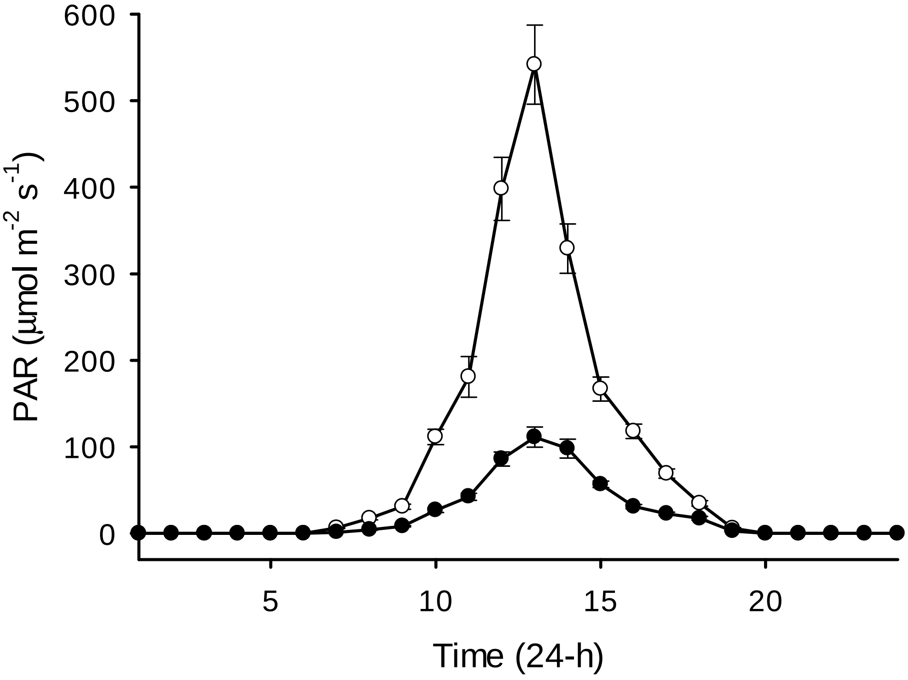


Figure 5.S1 Avergae hourly irradiance (PAR in µmol m^{-2} s^{-1}) over 24 hours for 12 weeks at different light treatments. closed circles = low light; open circles = high light. Data points represent Mean ± SE (*n* = 84)

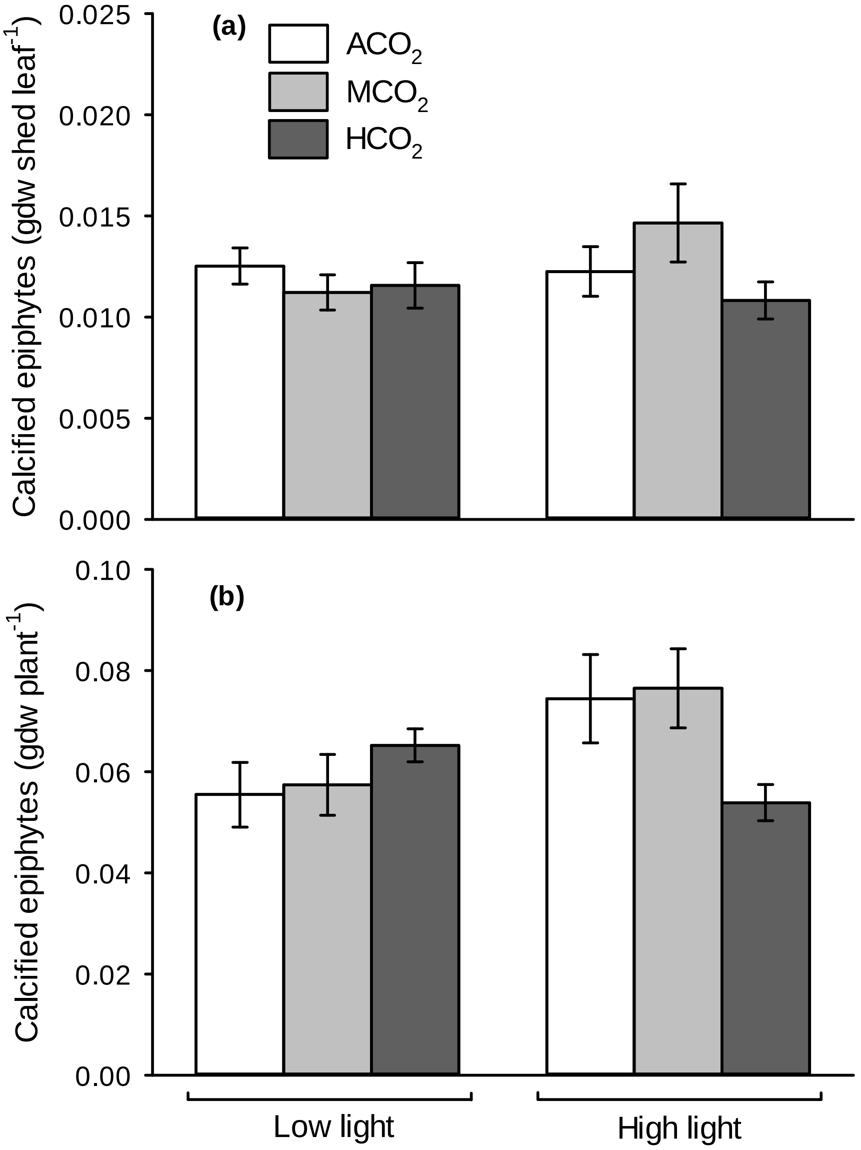


Figure 5.S2 Calcified epiphytic algae on a) shed leaves and b) living plants of juvenile seagrass grown at different CO_2 and light levels for 12 weeks. ACO_2 = ambient CO_2; MCO_2 = moderate CO_2; HCO_2 = high CO_2; LL = low light; HL = high light. Bars represent Mean ± SE (*n* = 6)

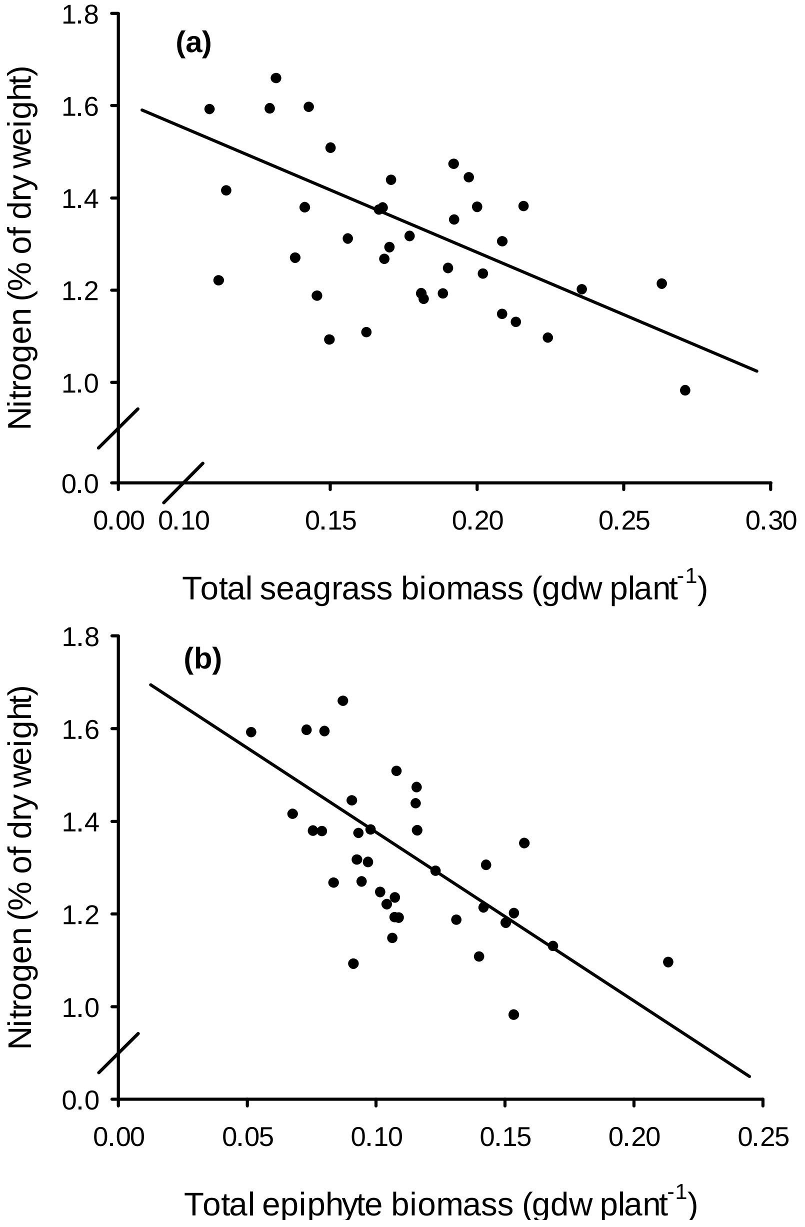


Figure 5.S3 Regression anlysis showing a) total seagrass biomass (above ground + below ground) *v.* % N in seagras tissue and b) total epiphyte biomass (filamentous alage + calcified algae) *v.* % N in seagrass tissue. Linear regression analysis for total seagrass biomass ($r^2 = 0.280$, $F_{1,35} = 13.24$, $P < 0.001$) and total epiphyte biomass ($r^2 = 0.378$, $F_{1,35} = 20.70$, $P < 0.001$)

Epiphytes and grazing on seagrass

Above: A sea-urchin consuming seagrass (*Posidonia* sp.) with few epiphytes. Below: An urchin consuming seagrass (*Posidonia* sp.) covered in calcified and filamentous epiphytes.

Photo credits - Above: Andrew Irving; Below: Owen Burnell

CHAPTER SIX

EUTROPHICATION OFFSETS INCREASED SEA-URCHIN GRAZING ON SEAGRASS CAUSED BY OCEAN WARMING AND ACIDIFICATION

Owen W. Burnell, Bayden D. Russell, Andrew D. Irving and Sean D. Connell

School of Earth and Environmental Sciences,
The University of Adelaide, Adelaide, SA 5005 Australia

Marine Ecology Progress Series 2013, 485 pp 37-46

STATEMENT OF AUTHORSHIP

In this paper I executed the laboratory experiment and analysed the data. I wrote the manuscript and collected the field samples, assisted by Bayden D. Russell, Andrew D. Irving & Sean D. Connell. All authors contributed to the experimental design. Bayden D. Russell, Sean D. Connell & I provided funding for the project.

Signatures of co-authors:

Dr Bayden D. Russell

Dr Andrew D. Irving

Professor Sean D. Connell

PREAMBLE

Chapter 6 was motivated by the many changing environmental conditions, both locally and globally, that might impact grazing upon seagrass. It uses the plant-herbivore interaction between urchins and seagrass as a model ecosystem to examine potential changes to *per capita* grazing rates. The chapter is a reproduction of a co-authored manuscript published in *Marine Ecology Progress Series,* and therefore is written in plural throughout and uses the journal formatting. The paper is written for a global audience with an interest in how grazing ecology can be modified by anthropogenic change. The manuscript can be cited as:

Burnell OW**,** Russell BD, Irving AD, Connell SD (2013) Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification. *Marine Ecology-Progress Series,* 485: 37-46.

CHAPTER SIX

EUTROPHICATION OFFSETS INCREASED SEA-URCHIN GRAZING ON SEAGRASS CAUSED BY OCEAN WARMING AND ACIDIFICATION

6.1 ABSTRACT

The accumulation of atmospheric [CO_2] continues to warm and acidify oceans concomitant with local disturbances, such as eutrophication. These changes can modify plant-herbivore grazing interactions by affecting the physiology of grazers and by altering the nutritional value of plants. However, such environmental changes are often studied in isolation, providing little understanding of their combined effects. We tested how ocean warming and acidification affect the *per capita* grazing by the sea-urchin *Amblypneustes pallidus* on the seagrass *Amphibolis antarctica*, and how such effects may differ between ambient and eutrophic nutrient conditions. Consistent with metabolic theory, grazing increased with warming, but in contrast to our expectations, acidification also increased grazing. While nutrient enrichment reduced grazing, it did not fully counterbalance the increase associated with warming and acidification. Collectively, these results suggest that ocean warming and acidification may combine to strengthen top-down pressure by herbivores. Localised nutrient enrichment could ameliorate some of the increased *per capita* grazing effect caused by warming and acidification, provided other common negative effects of

eutrophication on seagrass, including overgrowth by epiphytes and herbivore aggregation, are not overwhelming. There is value in assessing how global and local environmental change will combine, often in non-intuitive ways, to modify biological interactions that shape habitats.

6.2 INTRODUCTION

The unprecedented magnitude of anthropogenic impacts on natural environments continues to challenge our theoretical and applied understanding of ecosystem dynamics (Vitousek et al. 1997, Jackson 2001). In recent years, there has been considerable interest regarding the effects of ocean warming and acidification (i.e. reduced pH) generated by increasing atmospheric [CO_2] (Meehl et al. 2007). These global-scale changes occur concomitantly with local disturbances, such as eutrophication (Gorman et al. 2009, Russell et al. 2009), raising new questions regarding the cumulative effects of global and local disturbances on ecological processes and species interactions (Hoegh-Guldberg & Bruno 2010, Russell et al. 2012).

Understanding whether abiotic change can modify the outcome of species interactions that subsequently scale-up to the community level is of central importance to ecologists, particularly for species that have disproportionately large effects on community and habitat structure (Sanford 1999, Harley et al. 2006, Gooding et al. 2009, Kordas et al. 2011). To date, research has primarily focused on how changing abiotic conditions directly affect individual organisms (e.g. Orr et al. 2005, Parker et al. 2009, Sheppard-Brennand et al. 2010), with little insight into how changing conditions may indirectly alter whole ecosystems by modifying fundamental ecological processes such as grazing (Kordas et al. 2011, Russell et al. 2012).

Sea-urchins can play a pivotal role in controlling the abundance of habitat-forming species via their grazing (Hughes 1994, Eklöf et al. 2008). Perhaps the world's best known example is the kelp forest-sea urchin-sea otter interaction chain in the north-east Pacific Ocean, which has long been held as a classic example of top-down control on habitat abundance depending on the local control of urchin herbivores by otters (Estes et al. 1998, Steneck et al. 2002). Strong effects of urchins certainly extend to other habitats, particularly seagrass meadows where overgrazing can damage habitats on both tropical and temperate coasts (Rose et al. 1999, Valentine et al. 2000, Eklöf et al. 2008, Valentine & Edgar 2010).

Abiotic change can modify plant-herbivore interactions both directly by affecting the physiology of grazers and indirectly by altering the nutritional value and chemistry of plants (Cruz-Rivera & Hay 2000, Hillebrand et al. 2009, O'Connor 2009). Warming has a predictable effect on the metabolic rate of ectotherms, which subsequently drives proportional increases in consumption (Hillebrand et al. 2009, O'Connor 2009). Near physiological limits, this relationship often deteriorates as physiological stress reduces consumption or grazing (Sanford 2002, Lemoine & Burkepile 2012). In contrast, the relative infancy of research into ocean acidification means its effects on grazing have rarely been quantified. While emerging research suggests acidification will disrupt biological processes such that feeding ability and opportunities may be reduced (e.g. through exo-skeletal structural damage, increased predator avoidance and damage to feeding parts) (Bibby et al. 2007, Wood et al. 2008, Marchant et al. 2010, Stumpp et al. 2012), acidification can elevate grazing rates (Li & Gao 2012). While urchins can decrease consumption in response to large declines in pH (Stumpp et al. 2012), little is known regarding responses to more intermediate acidification scenarios. Increased grazing could occur because acidification boosts the metabolic rate of ectotherms through disruption of growth and physiological regulation, which are both energy-dependent processes (Brown et al. 2004, Cummings et al. 2011, Catarino et al. 2012).

Eutrophication can modify plant-herbivore interactions, as changes in water quality affect the uptake and storage of nutrients by primary producers (McGlathery 1995, Valentine & Heck 2001), but can also be impacted by temperature and [CO_2] (Touchette & Burkholder 2002, Jiang et al. 2010, Falkenberg et al. 2013a). However, the effect of the nutritional balance of plants on grazing is difficult to forecast because organisms can display both avoidance and compensatory responses to changes in perceived food quality (Cruz-Rivera & Hay 2000, Hillebrand et al. 2009, Tomas et al. 2011, de los Santos et al. 2012). Urchins can reduce *per capita* grazing when nutrient content in primary food sources are enriched, thus providing more rapid satiation (Lares & McClintock 1991, Valentine & Heck 2001), or when epiphytes and phytoplankton offer a nutrient rich alternative food (Tewfik et al. 2005).

The short-spined sea-urchin *Amblypneustes pallidus* has the potential to cause overgrazing of the seagrass *Amphibolis antarctica* to a point where virtually all of the above-ground biomass is lost (O. W. Burnell & A. D. Irving pers. obs.). To understand how this plant-herbivore interaction may be modified by changing environmental conditions, we tested the independent and cumulative effects of warming, acidification and nutrient enrichment on the grazing of seagrass by fixed densities of sea-urchins. We tested the hypothesis that increased grazing due to warming would be counteracted by decreases associated with ocean acidification and nutrient enrichment.

6.3 MATERIALS AND METHODS

Urchins *Amblypneustes pallidus* and seagrasses *Amphibolis antarctica* were maintained in experimental microcosms to test the effects of warming, acidification and nutrient enrichment on grazing. Two temperature levels (15 and 20 °C), 2 pH levels (current and forecasted) and 2 nutrient levels (ambient and enriched) were manipulated in a fully crossed design, making a total of 8 treatment combinations.

Levels of temperature and pH were selected to identify realistic future grazing responses, rather than inducing stress-related responses, which would likely affect the survival or physiological ability of urchins to function (i.e. below pH 7.5 or outside the thermal tolerance range) (Sanford 2002, Miles et al. 2007). Temperature and pH were manipulated to induce grazing effects likely under future projection scenario A1B for the year 2100 (i.e. 3°C and 0.2 pH units) (Meehl et al. 2007). For many marine invertebrates, a disproportionate increase in consumption is observed per 1°C increase at the higher end of the thermal tolerance range (Hanks 1957, Garton & Stickle 1980), particularly for small bodied sea-urchins (Siikavuopio et al. 2008). Therefore, a 5°C temperature differential from within the natural range of *Amblypneustes pallidus* (~ 12 to 25°C) was used to produce changes in consumption likely to be similar in magnitude to those that would be observed for a 3°C increase in summer maximum temperatures, as this is the period when seagrasses appear most susceptible to intense grazing (O. W. Burnell & A. D. Irving pers. obs.). This approach ensured that any stress-related reduction in grazing would not confound more realistic responses. The enriched concentration of nutrients was chosen to mimic peak levels encountered on the Adelaide metropolitan coastline based on Gorman et al. (2009).

Urchins were collected from beds of *Amphibolis antarctica* and *Posidonia* spp. from Lady Bay, South Australia (35.47645° S, 138.27570° E), while juvenile *A. antarctica* recruits were collected at Grange, South Australia (34.90415° S, 138.47079° E). Experiments were conducted in 22 l microcosms (L × W × H: 387 × 286 × 197mm), with water constantly recirculating (60 l h^{-1}) from a 180 l reservoir attached to each microcosm. Six replicate microcosms for each treatment were divided into 2 compartments with 10 seagrass recruits in each compartment (mean seagrass weight 9.07 ± 0.03 g $microcosm^{-1}$). One compartment in each microcosm contained 2 urchins (mean urchin weight 15.63 ± 0.28 g). As seagrass and any epibiota growing upon leaves were able to continue growing while being consumed, it was necessary to account for any autogenic change (i.e. change in seagrass weight due to growth or biomass loss) by maintaining seagrass in the absence of urchins. Urchins and seagrass were maintained separately under the experimental conditions for 6 d prior to the grazing experiment, which lasted for 5 d, until > 60 % of biomass was lost in the treatment with the highest grazing loss. During the pre-experimental period urchins were fed seagrass form the collection site.

To calculate grazing, a modified version of the equation $\{[H_0 \times (C_f/C_0)] - H_f\}$ from Taylor et al. (2002) was used, where H_0 is the fresh weight pre-assay, H_f is the fresh weight post-assay of seagrass exposed to urchin grazing, C_0 is the fresh weight pre-assay, C_f is the fresh weight post-assay of seagrass in control treatments. One additional variable was used to control for seagrass decline throughout the experiment in the grazing treatments. As seagrass was continually removed by the urchins, it’s autogenic change in grazing treatments is reduced relative to control treatments; therefore, the modified equation $\{[H_0 \times (C_f/C_0)] - H_f\} - \{(C_f - C_0)[1 - (H_a/100)]\}$ was used, where H_a is the average percentage of seagrass shoots surviving when exposed to urchin grazing. The percentage of surviving shoots was counted daily and averaged over the 5 d period to calculate H_a. Grazing was divided by initial urchin fresh weight to remove treatment differences as this can act as a co-variate for grazing (Underwood 1997). Seagrass and urchin fresh weight were determined by placing samples on absorbent paper for 10 s and blotting dry any excess water before weighing. At the conclusion of the experimental period, seagrasses in control treatments were frozen, and 2 individuals from each treatment were later scraped with a razor blade to estimate the percentage contribution of any epibiota to seagrass fresh weight. The ingestion of epibiota by urchins was then calculated for each experimental replicate using the equation $(C_e/C_f) \times G$, where C_e is the control fresh weight of the epibiota post-assay, C_f is the control seagrass fresh weight post-assay, and G is the grazing.

6.3.1 Temperature, pH and nutrient manipulation

Temperatures were maintained by recirculating water through heater/chiller units (TECO), while pH was controlled using probes and automatic solenoid controllers (Sera). This can be achieved because increasing CO_2 is known to decrease pH in a predictable manner. The system added CO_2 to the tanks, when required, to maintain the experimental pH level. The temperature, pH, total alkalinity and salinity were measured daily so carbon chemistry components could be calculated (Table 6.1). Seawater carbon parameters were calculated using dissociation constants K1and K2 from Roy et al. (1993) using the program CO_2Calc v. 1.0.30315.

Elemental ratios were quantified in seagrass tissue to determine changes in their nutritional value. Two seagrass individuals from each microcosm were dried for 48 h at 60°C, acid washed in 5 % HCl and scraped with a razor blade to remove epibiota, then re-dried for 48 h at 60°C. Seagrass tissue was then ground with a mortar and pestle and analysed for carbon and nitrogen (% N and % C) on an isotope ratio mass spectrometer (Hydra 2020 ANCA-GSL Version 4.0, Sercon).

Nutrient enrichment was controlled by supplying 1 g of Osmocote Plus® (Scotts) controlled release fertiliser per 15 l seawater (N:P:K = 17:4.3:8.2). Fertiliser pellets were placed in nylon mesh bags (1 mm mesh size) and submerged in the experimental reservoirs. Water samples were taken on 3 separate occasions throughout the experimental period on Days 1, 3 and 5. Water was sampled using sterile 25 ml syringes, filtered through 0.45 µm and frozen. Analysis was later done using a Lachat Quickchem 8200 Flow Injection Analyser (Hach) for nitrate and nitrite (NO_x), ammonia (NH_4) and phosphate (PO_4).

Light was provided in a 12 h light:12 h dark cycle by pairs of fluorescent lights at an average irradiance of 62.71 ± 1.51 µmol m^{-2} s^{-1}.

6.3.2 Statistical analyses

Statistical analysis of grazing was performed using seagrass tissue chemistry (i.e. C:N ratio) and epibiota percentage as potential covariates in a fully orthogonal ANCOVA design, using the program PERMANOVA+ for Primer. To further investigate any indirect contribution of food source nutritional value to seagrass grazing loss, the C:N ratio and epibiota percentage from treatment means were used as predictor variables in a distance based linear model (DISTLM) using the program PERMANOVA+ for Primer. Tissue chemistry, epibiota percentage, epibiota ingestion and seagrass autogenic change were also analysed separately using ANOVA with 2 fixed levels of temperature, pH and nutrients, in a fully orthogonal design. Average values from individual microcosms were treated as replicate samples. Pairwise comparisons were used when significant interaction terms were detected. The water chemistry was analysed using ANOVA with 2 fixed levels of temperature, pH and nutrients, in a fully orthogonal design. Average daily values from 3 sampling dates were used as replicate samples.

6.4 RESULTS

Both warming and acidification increased grazing, with the greatest change observed when they were elevated concurrently (Fig. 6.1, Table 6.2). A partially additive effect was found in which warming and acidification increased grazing to a greater extent than when either was elevated in isolation. This was considered a partially additive effect because the combined effect was smaller than the combination of the 2 independent effects added together, but larger than either of them independently (Fig. 6.2). For a detailed summary of partially additive and additive effects, see Brook et al. (2008). Nutrient enrichment offset the effect of warming and acidification by reducing grazing, though it did not fully counter the increase caused by warming and acidification (Fig. 6.1, Table 6.2). No pair-wise tests are reported for grazing as the ANCOVA only detected main effects. The contribution of seagrass autogenic change to grazing was small (between 2 and 7 %). However, a significant interaction was detected, whereby the elevation of both temperature and acidification reduced autogenic change, an effect that was not evident when either was elevated in isolation. Nutrient enrichment had no effect on autogenic change (Fig. 6.1, Tables 6.3 & 6.4).

Nutrient enrichment decreased the C:N ratio of seagrass, whilst warming and ocean acidification had no overall effect on the C:N ratio (Tables 6.3 & 6.4). Nutrient enrichment also increased the epibiota percentage of seagrass fresh weight, whilst temperature and ocean acidification had no overall effect on the epibiota percentage (Tables 6.3 & 6.4). The epibiota percentage was higher when nutrients were enriched, but when this was multiplied by grazing, the total ingestion of epibiota by urchins was not different between the experimental treatments (Fig. 6.1, Tables 6.3 & 6.4). Seagrass tissue chemistry (i.e. C:N ratio) and epibiota percentage were non-significant covariates of grazing (Table 6.2). However, when treatment means were used in a distance-based linear model, epibiota percentage was a significant predictor of grazing ($p = 0.049$, $R^2 = 0.512$), whereas tissue chemistry had no effect ($p = 0.374$, $R^2 = 0.147$).

Table 6.1 Carbonate chemistry in microcosms at different nutrient (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) levels (mean ± SE). CpH: current pH treatment; FpH: forecasted pH treatment; AN: ambient nutrients; EN: enriched nutrients. Seawater carbon parameters were calculated using dissociation constants K1and K2 from Roy et al. (1993) using the program $\mathrm{CO_2}$Calc v. 1.0.30315. TA: total alkalinity; $\mathrm{CO_3}$: carbonate; $\mathrm{HCO_3}$: bicarbonate; $p\mathrm{CO_2}$: $\mathrm{CO_2}$ partial pressure; $\mathrm{TCO_2}$: total carbon dioxide.

Treatment	**pH**	**TA (µmol kg^{-1})**	**Salinity (ppt)**	**Temp (°C)**	**$\mathrm{CO_2}$ (µmol kg^{-1})**	**$\mathrm{CO_3}$ (µmol kg^{-1})**	**$\mathrm{HCO_3}$ (µmol kg^{-1})**	**$p\mathrm{CO_2}$ (µatm)**	**$\mathrm{TCO_2}$ (µmol kg^{-1})**
15°C, CpH, AN	8.115	2682.64	38.92	15.06	14.125	236.83	2109.61	387.57	2360.57
	± 0.003	± 26.64	± 0.17	± 0.01	± 0.177	± 3.13	± 20.66	± 5.20	± 23.50
15°C, CpH, EN	8.106	2686.00	39.25	15.10	14.429	234.72	2117.58	397.08	2366.73
	± 0.001	± 6.93	± 0.14	± 0.02	± 0.059	± 0.34	± 6.61	± 1.66	± 6.97
15°C, FpH, AN	7.933	2698.67	38.58	15.03	23.381	168.17	2292.97	639.71	2484.52
	± 0.001	± 25.30	± 0.17	± 0.02	± 0.328	± 1.11	± 23.43	± 8.94	± 24.82
15°C, FpH, EN	7.925	2682.33	39.00	15.02	23.662	165.46	2282.18	648.63	2471.30
	± 0.001	± 20.33	± 0.01	± 0.02	± 0.171	± 1.35	± 17.61	± 4.57	± 19.13
20°C, CpH, AN	8.109	2686.33	39.50	19.85	12.312	275.10	2023.27	389.03	2310.68
	± 0.001	± 25.22	± 0.14	± 0.01	± 0.126	± 2.79	± 19.41	± 4.11	± 22.30
20°C, CpH, EN	8.115	2706.67	39.25	19.88	12.204	279.71	2033.97	385.38	2325.88
	± 0.001	± 10.90	± 0.01	± 0.01	± 0.061	± 1.10	± 8.79	± 1.91	± 9.92
20°C, FpH, AN	7.926	2699.00	39.33	19.88	20.604	198.12	2223.01	650.91	2441.73
	± 0.001	± 12.00	± 0.08	± 0.01	± 0.103	± 0.86	± 10.58	± 3.21	± 11.47
20°C, FpH, EN	7.921	2686.67	39.25	19.94	20.781	195.40	2217.04	657.38	2433.21
	± 0.001	± 12.33	± 0.14	± 0.02	± 0.112	± 1.04	± 10.07	± 3.83	± 11.19

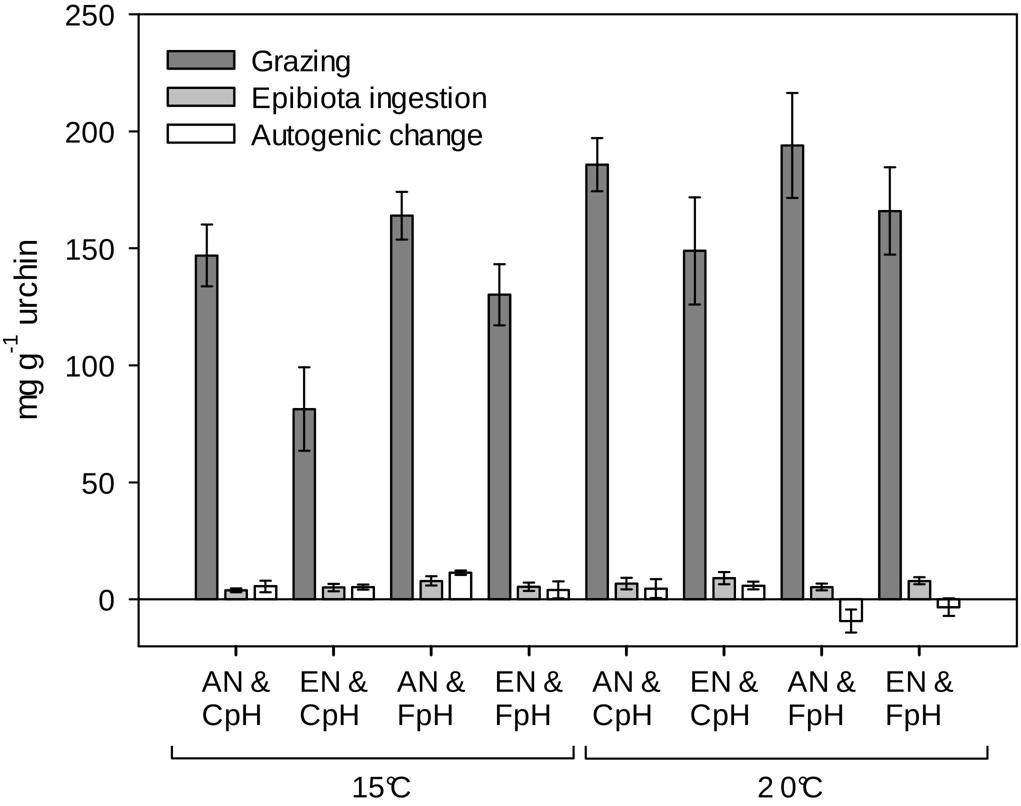


Figure 6.1 Effect of nutrients (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) on the grazing of seagrass, epibiota ingestion by urchins and autogenic change of seagrass. Bars indicate the mean ± SE for grazing, epibiota ingestion and autogenic change in seagrass fresh weight. CpH: current pH treatment; FpH: forecasted pH treatment; AN: ambient nutrients; EN: enriched nutrients.

Table 6.2 ANCOVA comparing the effect of nutrients (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) on the grazing of seagrass (*Amphibolis antarctica*) in the presence of fixed densities of a sea-urchin (*Amblypneustes pallidus*). The C:N ratio and epibiota percentage of seagrass were included in the analysis as covariates (CV) of grazing. Significant values in **bold.**

Source	df	MS	***F***	p
Grazing				
CV	2	0.004	1.39	0.244
Temperature	1	0.046	15.46	**0.001**
pH	1	0.014	4.66	**0.038**
Nutrients	1	0.036	11.29	**0.001**
Temperature × pH	1	0.004	1.16	0.255
Temperature × Nutrients	1	0.001	0.27	0.606
pH × Nutrients	1	0.002	0.80	0.351
Temperature × pH × Nutrients	1	0.003	1.06	0.318
Residual	38	0.003		

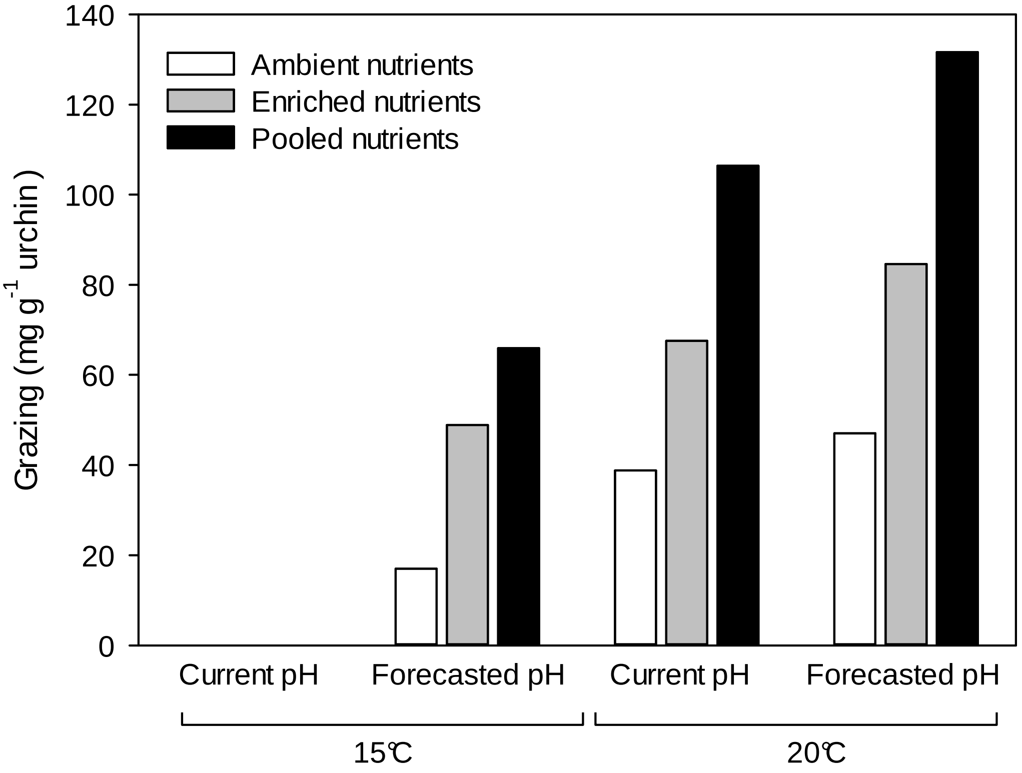


Figure 6.2 Additive effect of pH (current vs. forecasted) and temperature (15 vs. 20°C) on the grazing of seagrass by urchins at ambient nutrients, enriched nutrients and pooled nutrients.

NO_x (nitrate + nitrite) concentrations were greater (by ~7.4-fold) in enriched (0.094 ± 0.007 mg l^{-1}) than ambient (0.013 ± 0.000 mg l^{-1}) treatments ($F_{1,16}$ = 6.78, p = 0.027). Neither temperature nor ocean acidification had detectable effects on NO_x concentration ($F_{1,16}$ = 0.80, p = 0.417 and $F_{1,16}$ = 1.27, p = 0.889 respectively). NH_4 concentrations were greater (by ~2.3-fold) in enriched (0.087 ± 0.015 mg l^{-1}) than ambient (0.038 ± 0.002 mg l^{-1}) treatments ($F_{1,16}$ = 10.64, p = 0.003). Neither temperature nor ocean acidification had detectable effects on NH_4 concentration ($F_{1,16}$ = 4.07, p = 0.059 and $F_{1,16}$ = 2.74, p = 0.850, respectively). PO_4 concentrations were greater (by ~1.9-fold) in enriched (0.054 ± 0.003 mg l^{-1}) than ambient (0.029 ± 0.004 mg l^{-1}) treatments ($F_{1,16}$ = 66.41, p = 0.001). A significant interaction was detected between temperature and nutrients on PO_4 ($F_{1,16}$ = 10.99, p = 0.003), where in ambient nutrients, low temperature > high temperature, whereas in enriched nutrients, low temperature = high temperature. CO_2 had no effect ($F_{1,16}$ = 2.66, p = 0.131). Ocean acidification had no detectable effect on PO_4 concentration.

Table 6.3 Mean ± SE of C:N ratio, epibiota biomass, epibiota ingestion and autogenic change of seagrass *Amphibolis antarctica* in the presence of fixed densities of a sea-urchin *Amblypneustes pallidus* exposed to nutrients (ambient vs enriched), pH (current vs forecasted) and temperature (15 vs 20°C). CpH: current pH treatment; FpH: forecasted pH treatment; AN: ambient nutrients; EN: enriched nutrients.

Treatment	**C:N**		**Epibiota biomass (%)**		**Epibiota ingestion (mg g^{-1} urchin)**		**Autogenic change (mg g^{-1} urchin)**	
15°C, CpH, AN	16.35	± 1.18	2.53	± 0.35	3.84	± 0.78	5.54	± 2.47
15°C, CpH, EN	14.73	± 0.21	6.63	± 1.87	5.01	± 1.59	5.21	± 1.06
15°C, FpH, AN	15.65	± 0.51	4.71	± 1.19	7.89	± 2.03	11.3	± 0.91
15°C, FpH, EN	14.03	± 0.37	4.17	± 1.20	5.35	± 1.75	4.02	± 3.64
20°C, CpH, AN	15.71	± 0.15	3.37	± 1.08	6.72	± 2.49	4.59	± 4.06
20°C, CpH, EN	14.30	± 0.40	5.40	± 1.10	9.07	± 2.63	5.82	± 1.64
20°C, FpH, AN	15.30	± 1.02	2.58	± 0.62	5.29	± 1.39	-9.29	± 4.93
20°C, FpH, EN	14.67	± 0.45	4.69	± 0.80	7.86	± 1.48	-3.38	± 3.75

Table 6.4 P-values from ANOVAs comparing the effect of nutrients (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) on C:N ratio, epibiota biomass, autogenic change of seagrass *Amphibolis antarctica* and epibiota ingestion by urchins *Amblypneustes pallidus*. Autogenic change interaction term: Temperature × pH; at 15°C current pH = forecasted pH; at 20°C, current pH > forecasted pH; at current pH, 15°C = 20°C; at forecasted pH 15°C > 20°C.

Source	**C:N**	**Epibiota biomass (%)**	**Epibiota ingestion (mg g^{-1} urchin)**	**Autogenic change (mg g^{-1} urchin)**
Temperature	0.663	0.527	0.199	0.003
pH	0.419	0.579	0.738	0.044
Nutrients	**0.005**	**0.019**	0.502	0.955
Temperature × pH	0.450	0.702	0.188	**0.003**
Temperature × Nutrients	0.515	0.854	0.237	0.104
pH × Nutrients	0.677	0.155	0.510	0.797
Temperature × pH × Nutrients	0.651	0.142	0.459	0.197

6.5 DISCUSSION

Predicting the indirect effects of environmental change on habitat forming-plants requires knowledge of how their interactions with other species may be modified (Ives 1995). The complexity inherent in such tests means that indirect effects often remain unresolved relative to more conspicuous direct effects (Kordas et al. 2011). Altered species interactions can, however, have profound ecological impacts by disturbing trophic relationships within natural ecosystems (Benoît & Swain 2008, Barton et al. 2009, Connell et al. 2011). We identify the potential combination of ocean warming and acidification may combine to indirectly increase seagrass loss due to increased grazing by urchins.

Warming commonly increases herbivore grazing due to increases in metabolic demand (Hillebrand et al. 2009, O'Connor 2009). We hypothesised that the strong positive effect of warming could be ameliorated by potentially negative effects associated with acidification (Orr et al. 2005, Miles et al. 2007, Byrne et al. 2011, Cummings et al. 2011), which may indirectly reduce feeding ability or opportunities (Bibby et al. 2007, Wood et al. 2008, Marchant et al. 2010, Stumpp et al. 2012). Contrary to this hypothesis, however, we detected a positive effect of acidification on urchin grazing. Ocean acidification has been found to increase metabolic rate and in some instances grazing activity, as organisms partition more energetic resources to maintenance and repair of physiological processes (Wood et al. 2008, Cummings et al. 2011, Catarino et al. 2012, Li & Gao 2012).

Herbivores typically adjust their consumption relative to the nutritional content of their food source, which can be modified by environmental conditions (Ritchie et al. 1998, Russell & Connell 2007, Bressendorff & Toft 2011, Tomas et al. 2011). Although not ubiquitous to all macro-invertebrate groups (Hillebrand et al. 2009), we identified reduced urchin grazing when nutrients were enriched (e.g. Lares & McClintock 1991, Valentine & Heck 2001), supporting the hypothesis that nutrient enrichment could counteract increases in grazing associated with warming. Any nutrient-driven reduction in grazing pressure, however, should be interpreted cautiously given the widely documented detrimental effects of nutrient enrichment on seagrass, such as overgrowth by epiphytic algae and grazer aggregation (Silberstein et al. 1986, Ruíz et al. 2001, Orth et al. 2006, Ralph et al. 2006).

Changes in the seagrass C:N ratio were small, perhaps because the seagrass was originally sourced from a meadow intermittently exposed to high nutrient concentrations on the Adelaide metropolitan coastline, or due to the short duration of the experiment. Similarly, the effects of warming and acidification, both of which can modify C:N ratios (Touchette & Burkholder 2002, Jiang et al. 2010), had no detectable effect on the seagrass chemistry. As such, it appears that the disproportionate growth of epibiota we recorded, a common response to nutrient enrichment, may have offered a significant food resource to urchins in the enriched nutrient treatments (Tewfik et al. 2005, Bode et al. 2006, Ruíz et al. 2009). Although the epibiota biomass within each individual microcosm was not a significant covariate of grazing, the relationship between treatment means in the linear model suggests that epibiota could have contributed to nutrient driven declines in grazing. Additional declines in grazing associated with nutrient enrichment could potentially be explained by changes in nitrogen fixation through microbial activity, which can be a source of nutrients for sea-urchins (Guerinot & Patriquin 1981). While urchins appear to be able to implement fixation dependent on nutritional requirements, changes in epiphytic microflora or bacteria living on seagrass blades and ambient water quality often help aid or initiate fixation (Patriquin & Keddy 1978, Guerinot & Patriquin 1981). Alternatively to the model that urchins reduced grazing due to satiation, changes in epibiota or host plant secondary metabolites resulting from nutrient enrichment could have acted as deterrents to inhibit grazing (Wahl & Hay 1995, Weidner et al. 2004). However, this appears unlikely for the seagrass itself, given that *Amphibolis antarctica* have been found to reduce secondary phenolic compounds when nutrients are enriched (O. W. Burnell *et al.*, Chapter 3).

The *per capita* grazing we recorded does not incorporate potential population-level responses, such as consumer densities, resulting from changes in fecundity, recruitment and survival, which may occur independently under altered environments. Research in pelagic food webs suggests that warming will shift the balance of ecosystems in favour of high consumer (i.e. heterotrophs) abundance relative to producers (i.e. autotrophs), as consumption is limited primarily by temperature, whilst production has numerous other potentially limiting factors (e.g. light, [CO_2], temperature and nutrients) (Bulthuis 1987, Müren et al. 2005, O'Connor et al. 2009). A shift in favour of net heterotrophy would result in systems where over consumption is more prevalent, further compounding the *per capita* effects we identified. This may be of particular concern in habitats where urchin grazing is already prolific due to over-exploitation of herbivore predators (e.g. fish and crustaceans). In such systems, there is a reduced likelihood that increasing urchin grazing will be counteracted by similar metabolically driven increases in predation at higher trophic levels, as predator populations may already be below critical levels to achieve active control (Kordas et al. 2011). Nonetheless, microcosm experiments do overlook *per capita* compensatory effects from higher trophic organisms. For example, if temperature or CO_2 caused similar changes to predation upon urchins trophic balance may be maintained to some degree. Laboratory manipulations have identified that warming and ocean acidification will also be important in controlling future population densities of urchins via growth and calcification (Sheppard-Brennand et al. 2010, Byrne et al. 2011). Similarly, nutrient enrichment and food quality contribute to urchin population dynamics, often promoting greater densities of urchins through changes in resource availability and reproduction (Tewfik et al. 2005, Lester et al. 2007, Ruíz et al. 2009). We suggest that ecological models integrating both *per capita* responses and population ecology are needed to accurately forecast changing grazing pressure.

Changes to seagrass productivity in response to multiple disturbances will also be key to predicting their future abundance and distribution (Orth et al. 2006, Palacios & Zimmerman 2007, Eklöf et al. 2012). Although seagrass growth was not the focus of our study, the negative interactive effect of warming and acidification on autogenic change was unexpected, and the mechanism behind this decline remains uncertain. Longer-term studies will be essential in providing more accurate predictions for changes in seagrass growth and chemistry. While acidification is likely to benefit seagrasses by reducing carbon limitation (Beer & Koch 1996), this effect can be negated by other stressors, such as continuing deterioration of water quality, light conditions (Palacios & Zimmerman 2007) and warming (Eklöf et al. 2012).

The present study is one of the first to detect the positive effects of ocean acidification on grazing by an ecologically important macro-invertebrate grazer. However, we suggest that a threshold is likely to exist beyond which further decreases in pH would eventually inhibit the ability of urchins to feed (Appelhans et al. 2012, Stumpp et al. 2012), as is common for the metabolic responses to increasing temperature (Sanford 2002). Further research to establish realistic threshold levels of acidification will be important to better forecast organism responses to global change. Similarly, potential thresholds and compensatory responses associated with exposure time to acidification could exist, and highlight the need for studies incorporating longer acclimation times and opportunities for multi-generational acclimation (Sunday et al. 2011, Dupont et al. 2012).

The indirect effects from abiotic change can produce strong or unexpected outcomes that result in some of the largest effects known in ecology (Connell et al. 2011). We present some of the first indications that ocean warming and acidification could combine to intensify grazing pressure, which can strengthen top-down effects on habitat-forming plants, to unprecedented levels (Ling et al. 2009). Such change in global conditions will occur concomitantly with other alterations to the physical and biological environment, such as nutrient enrichment and over-fishing, potentially further modifying grazer abundance and their rates of consumption.

CHAPTER SEVEN

GENERAL DISCUSSION

Understanding how anthropogenic changes to environmental conditions modify habitats is a central theme of ecology (Guo et al., 2013, Vitousek et al., 1997). Worldwide, humans continue to create locally driven change in marine systems, in combination with forecasted global changes at an unprecedented rate (Halpern et al., 2008, Orth et al., 2006, Zachos et al., 2005). These established and impending changes have generated great interest in predicting how ecosystems will be modified by different abiotic conditions, which indirectly result in changing biotic interactions (Bazzaz, 1990, Guo et al., 2013, Short & Neckles, 1999). Throughout this thesis my primary focus has been to determine how the effects of anthropogenic change modify biotic interactions that are critical to the maintenance or loss of seagrass habitats. This theme of changing biotic interactions was central to all data chapters, except Chapter 4 which had an independent focus on seagrass physiology and growth. I considered changing biotic interactions primarily from two contrasting perspectives, top-down grazer effects and bottom-up resource effects, both of which have generated great interest among ecologists. While seagrass ecosystems were long believed to be driven by strong bottom-up or producer effects, top-down grazer effects continue to gain greater recognition for their biotic control of habitats (Valentine & Duffy, 2006, Valentine & Heck, 1999). The inherent complexity associated with testing biotic interactions in natural ecosystems often means there is a paucity of ecological data, particularly in relation to recently forecasted global-scale changes. In my thesis I addressed this important research area by examining how different globally driven processes might combine with local conditions to modify the biotic interactions that shape seagrass habitats.

This thesis began by examining seagrass-epiphyte-urchin interactions that are driven by changes in grazer abundance and nutrient enrichment. I found two seagrass species from the primary habitat-forming genera in temperate southern Australia have differing vulnerabilities to top-down urchin grazing, which might be based upon morphological differences, such that loss of the exposed meristem of *Amphibolis antarctica* appears to inhibit its post-grazing recovery (Chapter 2). Following this, I incorporated a second local driver of change (i.e. bottom-up nutrient enrichment) to examine grazing interactions. I subsequently found that the independent negative effects of urchins and nutrients do not combine in an additive manner, but instead act antagonistically, whereby *per capita* grazing on seagrass was reduced as urchins appear to acquire greater nutrition from increasing seagrass nitrogen content (Chapter 3). Following this, the thesis shifted to the importance of global-scale changes for seagrass. From an abiotic perspective, I presented findings on the direct benefits of forecasted CO_2 enrichment for the seagrass *A. antarctica*, stemming from its current affinity for energetically costly bicarbonate (HCO_3^-) for photosynthesis and its potential contemporary carbon limitation (Chapter 4). In the last two data chapters I highlight how changing biotic interactions could shape future seagrass habitats. In Chapter 5 I found biotic interactions with epiphytes could inhibit the predicted positive response of seagrasses to CO_2 enrichment, based upon the strong competitive ability of filamentous epiphytes under high light and enriched CO_2 (Chapter 5). In the final data chapter I found warming and enriched CO_2 might increase seagrass loss, as changing environmental conditions modify the grazing activity of consumers (Chapter 6). This final chapter concludes with a discussion of the key findings in a local and global context.

7.1 CHANGING BIOTIC INTERACTIONS OF LOCAL ORIGIN

The effects of top-down and bottom-up control in shaping natural ecosystems are of great global interest, particularly in marine environments due to the widespread spatial replication of local-scale changes along urbanised coastlines (e.g. overfishing and nutrient enrichment). While the paradigm of bottom-up influences has historically described seagrass ecosystems, evidence continues to emerge regarding top-down effects of herbivores that can drive localised seagrass loss and change ecosystem dynamics (Eklöf et al., 2008, Rose et al., 1999, Ruíz et al., 2009). I identified the seagrass species *A. antarctica* is particularly vulnerable to declines mediated by urchin grazing, primarily associated with its slower recovery when compared to *P. sinuosa*. Slower recovery rates appeared contingent upon grazing loss of the elevated meristem in *A. antarctica* meadows. Although, in some locations around temperate southern Australia *Posidonia* spp. also appear susceptible to overgrazing loss from different urchin species (see Cambridge et al., 1986, Cambridge & Hocking, 1997, Kendrick et al., 2002, Langdon et al., 2011, Larkum & West, 1990). Importantly, the urchin I studied resides elevated in the canopy, thus did not damage the protected basal meristems of *Posidonia* when compared with *Amphibolis* (Fig. 1.2 & 1.3). Analogous conclusions have been drawn regarding the detrimental effects of meristem damage for other seagrasses (Alcoverro & Mariani, 2002, Eklöf et al., 2009, Rose et al., 1999), in particular for the species *Thalassodendron ciliatum* where morphology appears to mediate its vulnerability to grazing induced meristem loss, and may even control the distribution of this species in relation to Marine Protected Areas and their ensuing maintenance of top-down control on urchins (i.e. protection of fish predators) (Alcoverro & Mariani, 2002, Alcoverro & Mariani, 2004, McClanahan et al., 1994). The similarities between these two cases are many; principally, the two seagrass genera in question (i.e. *Thalassodendron* and *Amphibolis*) are both from the family Cymodoceaceae and share a common morphology and growth pattern (Green & Short, 2003, Short & Duarte, 2001), thus suggesting species-specific and evolutionary traits can interact with grazing to mediate vulnerability to top-down control.

In southern Australia top-down control on marine primary producers has often been found to be of limited consequence in sub-tidal habitats, namely algal-forests and seagrass meadows (Bulleri et al., 2012, Edgar & Shaw, 1995, Fowler-Walker & Connell, 2002). Indeed, the overgrazing I encountered from the urchin *Amblypneustes pallidus* on seagrass appears a new phenomenon, as to my knowledge there are no previous accounts, either anecdotal or in published scientific literature, concerning significant grazing by this species. While I do not suggest this urchin currently has a widespread effect on the distribution of different seagrass species in this region, ultimately the return or recruitment interval of urchin populations in comparison with seagrass recovery will determine the persistence of overgrazing effects (Alcoverro & Mariani, 2002).

The changing nature of urchin abundance I found in meadows suggests populations boom and bust. Therefore, unlike rocky reefs where following overgrazing urchins might persist and maintain habitats in an altered barren state, by feeding on new recruits, opportunistic algae and coralline algae (Bonaviri et al., 2011, Leinaas & Christie, 1996), seagrass overgrazing by *A. pallidus* decimated all food and habitat resources, thus great mortality or migration of urchins ensued. I observed no seagrass recovery and no urchins in overgrazed areas for almost three years. As such, when overgrazing of all photosynthetic tissue occurs recovery appears slow and dependent on new recruitment or rhizome expansion from adjacent meadows. While remaining dead shoots (Fig. 1.3) might provide a substrate that aids long-term recruitment and sediment stability, many temperate seagrasses are renowned for their typically slow recovery that can take decades to centuries, if ever (Bryars & Neverauskas, 2004, Irving, 2013, Kirkman & Kuo, 1990).

Given the legacy of nutrient enrichment in creating marine habitat loss worldwide and emerging local urchin effects, in Chapter 3 I investigated how these processes (i.e. top-down vs. bottom-up) might interact to shape the maintenance or loss of seagrass. Bottom-up effects (i.e. nutrient enrichment) are renowned for their ability to drive widespread loss in seagrass habitats, commonly from epiphyte overgrowth (Bryars et al., 2011, Walker & McComb, 1992), but changes in nutrient resources can also concomitantly modify food quality and quantity for consumers, and therefore, interact with grazing on seagrass (Anton et al., 2011, McGlathery, 1995). In seagrass systems the combination of nutrient enrichment and urchin grazing often appears to culminate in overgrazing (Eklöf et al., 2008, Ruíz et al., 2009), but somewhat counter-intuitively to this observation previous lab-based studies have found nutrient enrichment might moderate *per capita* grazing (Lares & McClintock, 1991, Valentine & Heck, 2001). In natural meadows I also found nutrient enrichment and associated changes in seagrass biochemistry and epiphyte cover moderated *per capita* grazing effects on seagrass (Chapter 3), a finding which was replicated again in laboratory manipulations during Chapter 6. Although top-down effects of urchins on seagrass blades remained strong, as their diet did not switch exclusively to epiphytes, this reduction in *per capita* grazing supports the notion that significant grazing effects observed worldwide in eutrophic meadows are more likely associated with expanding urchin populations rather than *per capita* grazing effects (Eklöf et al., 2008, Ruíz et al., 2009).

7.2 CHANGING ABIOTIC CONDITIONS OF GLOBAL ORIGIN

Despite the substantive concern regarding the deleterious effects of global change on many organisms (Orr et al., 2005, Thomas et al., 2004, Thuiller et al., 2005), increasing CO_2 is anticipated to deliver direct benefits to primary producers in many terrestrial and aquatic habitats (Curtis & Wang, 1998, Koch et al., 2013, Wand et al., 1999). While directly evolved from terrestrial plants ~90 million years ago, aquatic angiosperms like seagrasses may derive greater benefits from global change than their terrestrial relatives due to the extremely slow diffusion of CO_2 in water compared to air, which has resulted in potential carbon limitation and/or the evolution of energetically intensive HCO_3^- acquisition mechanisms. These potential benefits of CO_2 were evident as all the seagrass species I tested were reliant to some degree on energetically costly HCO_3^- acquisition (Chapter 4), which translated to a beneficial growth response and greater photosynthetic efficiency in *A. antarctica* under forecasted CO_2. In accordance with previous findings for both terrestrial and aquatic plants this effect that was greater on below ground resources than above ground photosynthetic tissue (Bader et al., 2013, Campbell & Fourqurean, 2013a, Russell et al., 2013). Nonetheless, global uncertainty still surrounds the extent to which forecasted CO_2 will deliver long lasting benefits to many primary producers (Bader et al., 2013, DeLucia et al., 1999), particularly as a number of other abiotic limitations and biotic interactions can emerge to modify these responses (Alexandre et al., 2012, Eklöf et al., 2012, Palacios & Zimmerman, 2007, Chapters 5 & 6).

It was interesting to find HCO_3^- use was much greater in the two *Amphibolis* spp. compared to *P. sinuosa*. While carbon acquisition strategies seem to vary greatly between species and genera, there are environmental factors that might drive these differences (Koch et al., 2013, Uku et al., 2005). Depth and meadow density might be key variables in controlling carbon dynamics in seagrass meadows, as dense meadows with a high level of productivity can create localised depletion of inorganic carbon (C_i), in particular dissolved CO_2 (Buapet et al., 2013, Duarte et al., 2013, Koch et al., 2013, Semesi et al., 2009). Similarly, lower flow through meadows also significantly increases boundary layer effects at the leaf-scale (Fonseca & Kenworthy, 1987, Koch, 1994), thus a greater ability to acquire CO_2 from HCO_3^- might be beneficial for seagrass exposed to conditions with limited water exchange and a high ratio of productivity (i.e. photosynthesis) to water.

While both *Amphibolis* spp. and *Posidonia* spp. can form dense meadows (Shepherd & Sprigg, 1976), there is evidence that the canopy of *Amphibolis* might have a greater effect in slowing water movement than *Posidonia* (van Keulen & Borowitzka, 2002), therefore, it could be surmised that this might reduce the CO_2 replenishment rate and therefore increase dependence on alternative C_i forms. However, *Amphibolis* spp. also naturally inhabit areas of greater wave exposure than *Posidonia* spp., which would promote greater water exchange. Other factors, such as the greater photosynthetic rate of *Amphibolis* spp. compared with *Posidonia* spp. (Masini & Manning, 1997, Chapter 4) might also expose plants to greater relative depletion of CO_2, which could promote the evolution of HCO_3^- acquisition mechanisms. Similar differences might also exist within genera. For example, I found lower HCO_3^- reliance for *P. sinuosa* than has been reported for *Posidonia australis* (James & Larkum, 1996), the latter of which commonly occurs higher in the intertidal zone and thus might be exposed to greater CO_2 depletion. While I have suggested some potential drivers of HCO_3^- reliance, the understanding of how dissolved CO_2 varies in natural seagrass meadows is poorly defined, and would be dependent on the many unique environmental conditions that vary intra- and inter-specifically.

7.3 CHANGING BIOTIC INTERACTIONS OF GLOBAL ORIGIN

The greater photosynthesis and growth that can be exhibited by seagrass under elevated CO_2 conditions (Jiang et al., 2010, Palacios & Zimmerman, 2007, Chapter 4), was tempered at higher irradiances, where I found damaging growth of epiphytic algae increased (Chapter 5). Numerous studies report variable responses of seagrass to CO_2 enrichment (see Alexandre et al., 2012, Campbell & Fourqurean, 2013a, Jiang *et al.*, 2010, Palacios & Zimmerman, 2007), but there are few published accounts regarding non-calcified algal epiphytes, which might reflect the ephemeral nature of their growth. In one previous study forecasted CO_2 increased the dominance of seagrass over free-living and epiphytic algae, but this effect was ameliorated under warming scenarios where algal growth was favoured (Eklöf et al., 2012). In southern Australia epiphyte overgrowth is believed to have played a great role in historical seagrass loss, often associated with eutrophication (Bryars et al., 2011, Walker & McComb, 1992). Similarly to eutrophication and temperature, I found shallow well-illuminated conditions might also create seagrass loss by promoting disproportionate epiphyte growth under forecasted CO_2. Indeed other accounts of seagrass loss in southern Australia display the potential for epiphyte induced damage in shallow well-illuminated locations (Bryars et al., 2011, Bulthuis & Woelkerling, 1983).While shading is often implicated as the mechanism behind epiphyte driven seagrass loss, given such effects occurred in high light treatments it would seem possible other mechanisms besides shading also contributed to seagrass decline, such as algal competition for nutrients or C_i.

There is limited understanding of how CO_2 driven changes from artificial laboratory environments will translate to real-world CO_2 increases over scales of 10s to 100s of years. Volcanic CO_2 vents that naturally decrease pH on highly localised scales are one relatively new tool being used to gain insight into future environments. For seagrass, findings suggest they will derive large benefits from enriched CO_2 by increasing below ground energy stores (Hall-Spencer et al., 2008, Russell et al., 2013). Forecasted CO_2 might also decrease calcified epiphytes growing upon leaves (Martin et al., 2008), but few studies have reported on filamentous seagrass epiphytes under natural CO_2 enrichment. In one naturally acidified reef habitat, algal turf species decreased in cover (Porzio et al., 2011), however, this contrasts with another study of sub-tidal rock following disturbance that found small filamentous species colonise rapidly and persist under acidified conditions (Kroeker et al., 2013). It follows that seagrasses leaves which continuously produce new substrate might favour such algal groups that rapidly colonise under high CO_2 as I found in Chapter 5. Greater spatial and temporal replication at CO_2 vent sites would be required to provide meaningful data on the interactions between seagrass and non-calcified epiphytes, given the great number of conditions other than CO_2 that can modify epiphyte proliferation (e.g. light, temperature, nutrients & hydrodynamics) (Borowitzka et al., 2006, Lavery et al., 2007).

Biotic interactions under forecasted CO_2 will not only be modified between primary producer groups (i.e. epiphyte-seagrass), but also between different trophic levels (i.e. urchin-seagrass). To date, a great amount of research has focussed on the deleterious effects of global abiotic changes on the survival and growth of marine organisms, in particular calcifiers. While some studies report seemingly dire consequences (Byrne et al., 2011, Hoegh-Guldberg et al., 2007), other variable outcomes suggest calcifying organisms might still play an important ecological role in future environments (Calosi et al., 2013, Ries et al., 2009). In Chapter 6 I found sub-lethal changes to ocean warming and acidification might combine to increase *per capita* grazing rates of urchins on seagrass. This finding for warming is consistent with metabolic theory for other marine ectotherms that commonly increase grazing under higher temperatures (Hillebrand et al., 2009, O'Connor, 2009), however, in contrast to my expectations, acidification also increased grazing, possibly to compensate for increased energy required for physiological maintenance under acidified conditions (Cummings et al., 2011, Li & Gao, 2012). Evidence for such trade-offs and compensatory effects continue to emerge. For example, in sea-stars Gooding et al., (2009) found enriched CO_2 increased consumption, but still resulted in a lower percentage of calcified mass, whereas, Wood et al., (2008) found enriched CO_2 increased calcification, but this was associated with wasting of muscle tissue. As such, it appears organisms might be forced to partition resources differently in the future, but ultimately still appear to suffer under acidification, supporting the notion that the future survival and ecological roles of calcifiers is uncertain (Sheppard-Brennand et al., 2010, Byrne et al., 2011, Gooding et al., 2009, Kroeker et al., 2013, Wood et al., 2008). Although, recent mutli-generational studies (Dupont et al., 2012, Sunday et al., 2011) and observations of urchins at naturally acidified CO_2 vent sites (Calosi et al., 2013) suggest they can adapt to live under acidified conditions. The urchin I studied resides within dense meadows of primary producers, and therefore, might be particularly accustomed to large diurnal variation in autotroph-driven pH (i.e. from photosynthesis and respiration) (Koch et al., 2013, Semesi et al., 2009), which might modify their ability to tolerate acidified conditions (Dupont et al., 2010). Thus, it appears urchins might remain and even increase their grazing significance in a higher CO_2 world. However, if such rapid global changes continue to surpass worst case predictions, conditions that exceed physiological thresholds and organism functioning would seem inevitable (Miles et al., 2007, Sanford, 2002).

7.4 FUTURE RESEARCH

I highlight top-down grazer control can modify seagrass meadows in southern Australia, but both spatial and temporal replication would be required to provide wider inferences of these results. Perhaps a more widespread synthesis of the distribution of the urchin *A. pallidus* would also shed light upon the drivers behind population expansions and therefore the localities of seagrass meadows that are most vulnerable. The generally inconspicuous nature of *A. pallidus* is one factor that might have contributed to the paucity of information regarding its ecology (Fig. 7.1).

While my thesis was approached primarily from the perspective of biotic interactions that mediate seagrass loss, it uncovers many areas of interest regarding urchin physiology and nutrition. Future studies focussed on urchins could deliver a greater mechanistic understanding of some of my results. Firstly, isotope analysis will provide a powerful tool to determine whether urchins derive the majority of their nutritional requirements from seagrass or their epiphytes, by comparing individuals acclimated to different food sources (e.g. Tewfik et al., 2005). Secondly, identifying changes to urchin fitness that result from differing food quality, such as fecundity and reproduction will give greater interpretive value to the *per capita* responses I identified here. Likewise, *per capita* grazing responses to warming and acidification will be strengthened by integrating findings with modelling of population level responses to better define top-down processes under global change.

In reference to the effect of forecasted CO_2 on seagrass physiology and ecology, studies must continue to break new methodological boundaries to examine these plants *in situ*, where natural abiotic limitations and biotic interactions are present. A great legacy of laboratory experimentation using CO_2 has provided an in depth understanding of seagrass carbon physiology, when compared with the limited knowledge of enriched CO_2 on biotic interactions that occur in nature. While truly *in situ* CO_2 experimentation might better reconcile such ecological effects, research in this area has been limited due to the financial and technical hurdles associated with manipulating CO_2 in natural settings. However, *in situ* research is rapidly expanding and much can already be gleaned by combining knowledge from current attempts, such as naturally enriched meadows at volcanic CO_2 vents (e.g. Hall-Spencer et al., 2008, Russell et al., 2013), microcosm and mesocosm experiments (e.g. Eklöf et al., 2012, Palacios & Zimmerman, 2007) and recent advances in benthic CO_2 manipulation techniques (e.g. Campbell & Fourqurean, 2011, Campbell & Fourqurean, 2013a). While any technique that limits water exchange or organism movements is likely to modify natural abiotic and biotic conditions, other methods such as CO_2 vents must be interpreted cautiously due to the legacy of unknown variation in these habitats and limited replication (Calosi et al., 2013, Koch et al., 2013). Nonetheless, the combination of these techniques continues to enhance our understanding of the physiology and ecology associated with global change.

Figure 7.1. The inconspicuous urchin. Above: A short-spined sea-urchin in a meadow of *Posidonia sp*. Below: An urchin in a meadow of *Amphibolis antarctica*, but photographed from beneath the canopy. Photo credits, Above: Andrew Irving; Below: Owen Burnell

7.5 CONCLUSIONS

Predicting the cumulative impacts of anthropogenic change on habitat-forming primary producers is inherently complex due to opposing direct effects, as well changing competitive and trophic interactions (Harley et al., 2012). I found these changing biotic interactions can certainly overwhelm direct effects of abiotic change on seagrass, particularly where local conditions favour organisms that inhibit seagrass (e.g. epiphytes and urchins). While it can often be difficult to attribute exact cause for stochastic events that drive habitat loss in nature (e.g. urchin population expansions or epiphyte overgrowth), understanding how anthropogenic changes can combine to promote such ecological effects can help foresee and, therefore, prevent such outcomes. While some local-scale changes can be abated through effective management strategies (i.e. over-fishing and nutrient enrichment), curtailing global-scale changes requires significant political will from society as a whole (i.e. CO_2).

I add to the growing body of evidence for top-down grazer effects on seagrass, highlighting that changing biotic interactions under different environmental conditions (e.g. resource and consumer shifts) should preclude the application of any single paradigm from the dichotomy of top-down vs. bottom-up control to seagrass meadows. In fact, there is merit in the recognition that top-down and bottom-up control may be more effectively assessed as a continuum of competing consumer versus producer effects (Connell et al., 2011). Strong biotic interactions that control habitats in the face of anthropogenic change will not be limited to seagrass ecosystems, but span terrestrial (Reekie & Bazzaz, 1989), coral reef (Diaz-Pulido et al., 2011) and sub-tidal kelp systems (Falkenberg et al., 2012).

Although, the widespread occurrence and legacy of seagrass loss along urbanised coastlines suggests they will remain particularly vulnerable to the combination of many human mediated changes in the future (Duarte, 2002, Orth et al., 2006, Short & Neckles, 1999). If humans are to continue reaping the many ecosystem services from optimally functioning seagrass habitats, a greater understanding of biotic control under altered states is required.

REFERENCES

Agostini S, Desjobert J-M, Pergent G (1998) Distribution of phenolic compounds in the seagrass *Posidonia oceanica*. *Phytochemistry,* **48**, 611-617.

Alcoverro T, Mariani S (2002) Effects of sea urchin grazing on seagrass (*Thalassodendron ciliatum*) beds of a Kenyan lagoon. *Marine Ecology-Progress Series,* **226**, 255-263.

Alcoverro T, Mariani S (2004) Patterns of fish and sea urchin grazing on tropical Indo-Pacific seagrass beds. *Ecography,* **27**, 361-365.

Alcoverro T, Mariani S (2005) Shoot growth and nitrogen responses to simulated herbivory in Kenyan seagrasses. *Botanica Marina,* **48**, 1-7.

Alexandre A, Silva J, Buapet P, Björk M, Santos R (2012) Effects of CO_2 enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*. *Ecology and Evolution,* **2**, 2620-2630.

Anderson WB, Polis GA (1999) Nutrient fluxes from water to land: seabirds affect plant nutrient status on Gulf of California islands. *Oecologia,* **118**, 324-332.

Anton A, Cebrián J, Heck KL, Duarte CM, Sheehan KL, Miller M-EC, Foster D (2011) Decoupled effects (positive to negative) of nutrient enrichment on ecosystem services. *Ecological Applications,* **21**, 991-1009.

Appelhans YS, Thomsen J, Pansch C, Melzner F, Wahl M (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and *Carcinus maenas*. *Marine Ecology-Progress Series,* **459**, 85-98.

Arnold T, Mealey C, Leahey H, Miller AW, Hall-Spencer JM, Milazzo M, Maers K (2012) Ocean acidification and the loss of phenolic substances in marine plants. *Plos One,* **7**, e35107.

Bader MKF, Leuzinger S, Keel SG, Siegwolf RTW, Hagedorn F, Schleppi P, Körner C (2013) Central European hardwood trees in a high-CO_2 future: synthesis of an 8-year forest canopy CO_2 enrichment project. *Journal of Ecology*, **101**, 1509-1519.

Badger MR, Andrews TJ (1982) Photosynthesis and inorganic carbon usage by the marine cyanobacterium, *Synechococcus* sp. *Plant Physiology,* **70**, 517-523.

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecological Monographs*, **81**, 169-193.

Barton BT, Beckerman AP, Schmitz OJ (2009) Climate warming strengthens indirect interactions in an old-field food web. *Ecology*, **90**, 2346-2351.

Bastyan GR, Cambridge ML (2008) Transplantation as a method for restoring the seagrass *Posidonia australis*. *Estuarine Coastal and Shelf Science*, **79**, 289-299.

Bazzaz FA (1990) The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics*, **21**, 167-196.

Beardall J, Beer S, Raven JA (1998) Biodiversity of marine plants in an era of climate change: some predictions based on physiological performance. *Botanica Marina,* **41**, 113-123.

Beardall J, Giordano M (2002) Ecological implications of microalgal and cyanobacterial CO_2 concentrating mechanisms, and their regulation. *Functional Plant Biology,* **29**, 335-347.

Beddingfield SD, McClintock JB (1998) Differential survivorship, reproduction, growth and nutrient allocation in the regular echinoid *Lytechinus variegatus* (Lamarck) fed natural diets. *Journal of Experimental Marine Biology and Ecology*, **226**, 195-215.

Beer S (1989) Photosynthesis and photorespiration of marine angiosperms. *Aquatic Botany*, **34**, 153-166.

Beer S, Björk M, Gademann R, Ralph PJ (2001) Measurements of photosynthetic rates in seagrasses. In: *Global Seagrass Research Methods.* (eds Short FT, Coles RG) pp. 393-406, Elsevier, Amsterdam.

Beer S, Björk M, Hellblom F, Axelsson L (2002) Inorganic carbon utilization in marine angiosperms (seagrasses). *Functional Plant Biology*, **29**, 349-354.

Beer S, Eshel A, Waisel Y (1977) Carbon metabolism in seagrasses .I. Utilization of exogenous inorganic carbon species in photosynthesis. *Journal of Experimental Botany*, **28**, 1180-1189.

Beer S, Koch E (1996) Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. *Marine Ecology-Progress Series*, **141**, 199-204.

Beer S, Vilenkin B, Weil A, Veste M, Susel L, Eshel A (1998) Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Marine Ecology-Progress Series*, **174**, 293-300.

Benoît HP, Swain DP (2008) Impacts of environmental change and direct and indirect harvesting effects on the dynamics of a marine fish community. *Canadian Journal of Fisheries and Aquatic Sciences*, **65**, 2088-2104.

Berner RA, Kothavala Z (2001) GEOCARB III: A revised model of atmospheric CO_2 over phanerozoic time. *American Journal of Science*, **301**, 182-204.

Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J (2007) Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biology Letters,* **3**, 699-701.

Björk M, Weil A, Semesi S, Beer S (1997) Photosynthetic utilisation of inorganic carbon by seagrasses from Zanzibar, East Africa. *Marine Biology,* **129**, 363-366.

Bode A, Alvarez-Ossorio MT, Varela M (2006) Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling estimated from stable isotopes. *Marine Ecology-Progress Series,* **318**, 89-102.

Bolser RC, Hay ME, Lindquist N, Fenical W, Wilson D (1998) Chemical defenses of freshwater macrophytes against crayfish herbivory. *Journal of Chemical Ecology,* **24**, 1639-1658.

Bonaviri C, Fernández TV, Fanelli G, Badalamenti F, Gianguzza P (2011) Leading role of the sea urchin *Arbacia lixula* in maintaining the barren state in southwestern Mediterranean. *Marine Biology,* **158**, 2505-2513.

Borowitzka MA, Lavery PS, van Keulen M (2006) Epiphytes of Seagrasses. In: *Seagrasses: Biology, Ecology and Conservation.* (eds Larkum AWD, Orth RJ, Duarte CM) pp. 441-461, Springer, Dordrecht.

Bressendorff BB, Toft S (2011) Dome-shaped functional response induced by nutrient imbalance of the prey. *Biology Letters,* **7**, 517-520.

Brook BW, Sodhi NS, Bradshaw CJA (2008) Synergies among extinction drivers under global change. *Trends in Ecology & Evolution,* **23**, 453-460.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology,* **85**, 1771-1789.

Brueggeman AJ, Gangadharaiah DS, Cserhati MF, Casero D, Weeks DP, Ladunga I (2012) Activation of the carbon concentrating mechanism by CO_2 deprivation coincides with massive transcriptional restructuring in *Chlamydomonas reinhardtii*. *Plant Cell,* **24**, 1860-1875.

Bryars S, Collings G, Miller D (2011) Nutrient exposure causes epiphytic changes and coincident declines in two temperate Australian seagrasses. *Marine Ecology-Progress Series,* **441**, 89-103.

Bryars S, Neverauskas V (2004) Natural recolonisation of seagrasses at a disused sewage sludge outfall. *Aquatic Botany,* **80**, 283-289.

Bryars S, Rowling K (2009) Benthic habitats of eastern Gulf St Vincent: major changes in benthic cover and composition following european settlement of Adelaide. *Trans. R. Soc. S. Aust.,* **133**, 318-338.

Bryars S, Wear R, Collings G (2008) Seagrasses of Gulf St Vincent and Investigator Strait. In: *Natural history of Gulf St Vincent.* (eds Shepherd SA, Bryars S, Kirkegaard I, Harbison P, Jennings JT). pp. 132-147, Royal Society of South Australia Inc, Adelaide.

Bryars SR (2009) Can regional nutrient status be used to predict plant biomass, canopy structure and epiphyte biomass in the temperate seagrass *Amphibolis antarctica*? *Marine and Freshwater Research,* **60**, 1054-1067.

Buapet P, Gullström M, Björk M (2013) Photosynthetic activity of seagrasses and macroalgae in temperate shallow waters can alter seawater pH and total inorganic carbon content at the scale of a coastal embayment. *Marine and Freshwater Research,* **64**, 1040-1048.

Bulleri F, Russell BD, Connell SD (2012) Context-dependency in the effects of nutrient loading and consumers on the availability of space in marine rocky environments. *Plos One,* **7**, e33825.

Bulthuis DA (1987) Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany,* **27**, 27-40.

Bulthuis DA, Woelkerling WJ (1983) Biomass accumulation and shading effects of epiphytes on leaves of the seagrass, *Heterozostera tasmanica*, in Victoria, Australia. *Aquatic Botany,* **16**, 137-148.

Burke MK, Dennison WC, Moore KA (1996) Non-structural carbohydrate reserves of eelgrass *Zostera marina*. *Marine Ecology Progress Series,* **137**, 195-201.

Burkepile DE, Hay ME (2006) Herbivore vs. nutrient control of marine primary producers: context-dependent effects. *Ecology,* **87**, 3128-3139.

Burkholder DA, Fourqurean JW, Heithaus MR (2013) Spatial pattern in seagrass stoichiometry indicates both N-limited and P-limited regions of an iconic P-limited subtropical bay. *Marine Ecology Progress Series,* **472**, 101-115.

Burnell OW, Connell SD, Irving AD, Russell BD (2013a) Asymmetric patterns of recovery in two habitat forming seagrass species following simulated overgrazing by urchins. *Journal of Experimental Marine Biology and Ecology,* **448**, 114-120.

Burnell OW, Russell BD, Irving AD, Connell SD (2013b) Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification. *Marine Ecology Progress Series,* **485**, 37-46.

Byrne M, Ho M, Wong E, Soars NA, Selvakumaraswamy P, Sheppard-Brennand H, Dworjanyn SA, Davis AR (2011) Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. *Proceedings of the Royal Society B-Biological Sciences*, **278**, 2376-2383.

Calosi P, Rastrick SPS, Graziano M, Thomas SC, Baggini C, Carter HA, Hall-Spencer JM, Milazzo M, Spicer JI (2013) Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid-base and ion-regulatory abilities. *Marine Pollution Bulletin*, **73**, 470-484.

Cambridge ML (1999) Growth strategies of Rottnest Island seagrasses. In: *The seagrass flora and fauna of Rottnest Island, Western Australia.* (eds Walker DI, Wells FE) pp. 1-24, Western Australian Museum, Perth.

Cambridge ML, Chiffings AW, Brittan C, Moore L, McComb AJ (1986) The loss of seagrass in Cockburn Sound, Western Australia. 2. Possible causes of seagrass decline. *Aquatic Botany*, **24**, 269-285.

Cambridge ML, Hocking PJ (1997) Annual primary production and nutrient dynamics of the seagrasses *Posidonia sinuosa* and *Posidonia australis* in south-western Australia. *Aquatic Botany,* **59**, 277-295.

Cambridge ML, Kuo J (1979) Two new species of seagrasses from Australia, *Posidonia sinuosa* and *P. angustifolia* (posidoniaceae). *Aquatic Botany*, **6**, 307-328.

Campbell JE, Fourqurean JW (2011) Novel methodology for *in situ* carbon dioxide enrichment of benthic ecosystems. *Limnology and Oceanography-Methods,* **9**, 97-109.

Campbell JE, Fourqurean JW (2013a) Effects of *in situ* CO_2 enrichment on the structural and chemical characteristics of the seagrass Thalassia testudinum. *Marine Biology,* **160**, 1465-1475.

Campbell JE, Fourqurean JW (2013b) Mechanisms of bicarbonate use influence the photosynthetic carbon dioxide sensitivity of tropical seagrasses. *Limnology and Oceanography,* **58**, 839-848.

Carr H, Axelsson L (2008) Photosynthetic utilization of bicarbonate in *Zostera marina* is reduced by inhibitors of mitochondrial ATPase and electron transport. *Plant Physiology,* **147**, 879-885.

Catarino AI, Bauwens M, Dubois P (2012) Acid-base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures. *Environmental Science and Pollution Research,* **19**, 2344-2353.

Cebrián J (1999) Patterns in the fate of production in plant communities. *American Naturalist,* **154**, 449-468.

Cebrián J, Duarte CM, Agawin NSR, Merino M (1998) Leaf growth response to simulated herbivory: a comparison among seagrass species. *Journal of Experimental Marine Biology and Ecology,* **220**, 67-81.

Cebrián J, Shurin JB, Borer ET, Cardinale BJ, Ngai JT, Smith MD, Fagan WF (2009) Producer nutritional quality controls ecosystem trophic structure. *Plos One,* **4**, e4929.

Chapman ARO, Johnson CR (1990) Disturbance and organization of macroalgal assemblages in the Northwest Atlantic. *Hydrobiologia,* **192**, 77-121.

Coley PD (1988) Effects of plant-growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia,* **74**, 531-536.

Collier CJ, Waycott M, Ospina AG (2012) Responses of four Indo-West Pacific seagrass species to shading. *Marine Pollution Bulletin*, **65**, 342-354.

Collings G, Bryars S, Nayar S, Miller D, Lill J, O'loughlin E (2006) Elevated nutrient responses of the meadow forming seagrasses, *Amphibolis* and *Posidonia*, from the Adelaide metropolitan coastline. *ACWS Technical Report No. 11 prepared for the Adelaide Coastal Waters Study Steering Committee.* South Australian Research and Development Institute (Aquatic Sciences), Publication No. RD01/0208-16, Adelaide.

Connell SD, Kroeker KJ, Fabricius KE, Kline DI, Russell BD (2013) The other ocean acidification problem: CO_2 as a resource among competitors for ecosystem dominance. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* **368**, 20120442.

Connell SD, Russell BD (2010) The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proceedings of the Royal Society B-Biological Sciences*, **277**, 1409-1415.

Connell SD, Russell BD, Irving AD (2011) Can strong consumer and producer effects be reconciled to better forecast 'catastrophic' phase-shifts in marine ecosystems? *Journal of Experimental Marine Biology and Ecology,* **400**, 296-301.

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

Cook K, Vanderklift MA, Poore AGB (2011) Strong effects of herbivorous amphipods on epiphyte biomass in a temperate seagrass meadow. *Marine Ecology-Progress Series,* **442**, 263-269.

Copertino MS, Cheshire A, Kildea T (2009) Photophysiology of a turf algal community: integrated responses to ambient light and standing biomass. *Journal of Phycology,* **45**, 324-336.

Costanza R, d'Arge R, ge Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature,* **387**, 253-260.

Courtney T, Westfield I, Ries JB (2013) CO_2-induced ocean acidification impairs calcification in the tropical urchin *Echinometra viridis*. *Journal of Experimental Marine Biology and Ecology,* **440**, 169-175.

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters,* **11**, 1304-1315.

Cruz-Rivera E, Hay ME (2000) Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology,* **81**, 201-219.

Cummings V, Hewitt J, van Rooyen A, Currie K, Beard S, Thrush S, Norkko J, Barr N, Heath P, Halliday NJ, Sedcole R, Gomez A, McGraw C, Metcalf V (2011) Ocean acidification at high latitudes: potential effects on functioning of the antarctic bivalve *Laternula elliptica*. *Plos One,* **6**, e16069.

Curtis PS, Wang XZ (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia,* **113**, 299-313.

Darling ES, McClanahan TR, Côté IM (2010) Combined effects of two stressors on Kenyan coral reefs are additive or antagonistic, not synergistic. *Conservation Letters,* **3**, 122-130.

Darnell KM, Heck KL (2013) Species-specific effects of prior grazing on the palatability of turtlegrass. *Journal of Experimental Marine Biology and Ecology,* **440**, 225-232.

de los Santos CB, Brun FG, Onoda Y, Cambridge ML, Bouma TJ, Vergara JJ, Pérez-Lloréns JL (2012) Leaf-fracture properties correlated with nutritional traits in nine Australian seagrass species: implications for susceptibility to herbivory. *Marine Ecology-Progress Series,* **458**, 89-102.

DeLucia EH, Hamilton JG, Naidu SL, Thomas RB, Andrews JA, Finzi AC, Lavine M, Matamala R, Mohan JE, Hendrey GR, Schlesinger WH (1999) Net primary production of a forest ecosystem with experimental CO_2 enrichment. *Science,* **284**, 1177-1179.

Dennison WC (1987) Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquatic Botany,* **27**, 15-26.

Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KRN (2011) High CO_2 enhances the competitive strength of seaweeds over corals. *Ecology Letters,* **14**, 156-162.

Duarte CM (1990) Seagrass nutrient content. *Marine Ecology-Progress Series,* **67**, 201-207.

Duarte CM (1991) Seagrass depth limits. *Aquatic Botany,* **40**, 363-377.

Duarte CM (2002) The future of seagrass meadows. *Environmental Conservation,* **29**, 192-206.

Duarte CM, Hendriks IE, Moore TS, Olsen YS, Steckbauer A, Ramajo L, Carstensen J, Trotter JA, McCulloch M (2013) Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts*, **36**, 221-236.

Duarte CM, Middelburg JJ, Caraco N (2005) Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences,* **2**, 1-8.

Ducker SC, Foord NJ, Knox RB (1977) Biology of Australian seagrasses: genus *Amphibolis* C Agardh (Cymodoceaceae). *Australian Journal of Botany* **25**, 67-95.

Dumay O, Costa J, Desjobert JM, Pergent G (2004) Variations in the concentration of phenolic compounds in the seagrass *Posidonia oceanica* under conditions of competition. *Phytochemistry,* **65**, 3211-3220.

Dupont S, Dorey N, Stumpp M, Melzner F, Thorndyke M (2012) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology,* ***160***, 1835-1843.

Dupont S, Ortega-Martínez O, Thorndyke M (2010) Impact of near-future ocean acidification on echinoderms. *Ecotoxicology,* **19**, 449-462.

Edgar GJ, Shaw C (1995) The production and trophic ecology of shallow-water fish assemblages in southern Australia .3. General relationships between sediments, seagrasses, invertebrates and fishes. *Journal of Experimental Marine Biology and Ecology*, **194**, 107-131.

Eklöf JS, Alsterberg C, Havenhand JN, Sundbäck K, Wood HL, Gamfeldt L (2012) Experimental climate change weakens the insurance effect of biodiversity. *Ecology Letters*, **15**, 864-872.

Eklöf JS, de la Torre-Castro M, Gullström M, Uku J, Muthiga N, Lyimo T, Bandeira SO (2008) Sea urchin overgrazing of seagrasses: a review of current knowledge on causes, consequences, and management. *Estuarine Coastal and Shelf Science,* **79**, 569-580.

Eklöf JS, McMahon K, Lavery PS (2009) Effects of multiple disturbances in seagrass meadows: shading decreases resilience to grazing. *Marine and Freshwater Research,* **60**, 1317-1327.

Estes JA, Palmisano JF (1974) Sea otters: their role in structuring nearshore communities. *Science,* **185**, 1058-1060.

Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JBC, Marquis RJ, Oksanen L, Oksanen T, Paine RT, Pikitch EK, Ripple WJ, Sandin SA, Scheffer M, Schoener TW, Shurin JB, Sinclair ARE, Soulé ME, Virtanen R, Wardle DA (2011) Trophic downgrading of planet earth. *Science,* **333**, 301-306.

Estes JA, Tinker MT, Williams TM, Doak DF (1998) Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science,* **282**, 473-476.

Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine Pollution Bulletin,* **50**, 125-146.

Falk S, Palmqvist K (1992) Photosynthetic light utilization efficiency, Photosystem II heterogeneity, and fluorescence quenching in *Chlamydomonas reinhardtii* during the induction of the CO_2 concentrating mechanism. *Plant Physiology,* **100**, 685-691.

Falkenberg LJ, Russell BD, Connell SD (2012) Stability of Strong Species Interactions Resist the Synergistic Effects of Local and Global Pollution in Kelp Forests. *Plos One,* **7**, e33841.

Falkenberg LJ, Russell BD, Connell SD (2013a) Contrasting resource limitations of marine primary producers: implications for competitive interactions under enriched CO_2 and nutrient regimes. *Oecologia,* **172**, 575-583.

Falkenberg LJ, Russell BD, Connell SD (2013b) Future herbivory: the indirect effects of enriched CO_2 may rival its direct effects. *Marine Ecology Progress Series,* **492**, 85-95.

Fernandes M, Benger S, Sharma SK, Gaylard S, Kildea T, Hoare S, Braley M, Irving AD (2012) The use of delta N-15 signatures of translocated macroalgae to map coastal nutrient plumes: improving species selection and spatial analysis of metropolitan datasets. *Journal of Environmental Monitoring,* **14**, 2399-2410.

Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. *Limnology and Oceanography,* **44**, 864-877.

Fonseca MS, Kenworthy WJ (1987) Effects of current on photosynthesis and distribution of seagrasses. *Aquatic Botany,* **27**, 59-78.

Foster GG, Hodgson AN, Balarin M (1999) Effect of diet on growth rate and reproductive fitness of *Turbo sarmaticus* (Mollusca: Vetigastropoda: Turbinidae). *Marine Biology,* **134**, 307-315.

Fowler-Walker MJ, Connell SD (2002) Opposing states of subtidal habitat across temperate Australia: consistency and predictability in kelp canopy-benthic associations. *Marine Ecology-Progress Series,* **240**, 49-56.

Fridlyand L, Kaplan A, Reinhold L (1996) Quantitative evaluation of the role of a putative CO_2-scavenging entity in the cyanobacterial CO_2-concentrating mechanism. *Biosystems,* **37**, 229-238.

Garton D, Stickle WB (1980) Effects of salinity and temperature on the predation rate of *Thais haemastoma* on *Crassostrea virginica* spat. *Biological Bulletin,* **158**, 49-57.

Gifford RM, Barrett DJ, Lutze JL (2000) The effects of elevated CO_2 on the C : N and C : P mass ratios of plant tissues. *Plant and Soil,* **224**, 1-14.

Gooding RA, Harley CDG, Tang E (2009) Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *Proceedings of the National Academy of Sciences of the United States of America,* **106**, 9316-9321.

Gorman D, Russell BD, Connell SD (2009) Land-to-sea connectivity: linking human-derived terrestrial subsidies to subtidal habitat change on open rocky coasts. *Ecological Applications,* **19**, 1114-1126.

Granger S, Lizumi H (2001) Water quality measurement methods for seagrass habitat. In: *Global Seagrass Research Methods.* (eds Short FT, Coles RG) pp. 393-406, Elsevier, Amsterdam.

Green EP, Short FT eds (2003) *World Atlas of Seagrasses.* University of California Press Berkeley.

Grignon-Dubois M, Rezzonico B, Alcoverro T (2012) Regional scale patterns in seagrass defences: phenolic acid content in *Zostera noltii*. *Estuarine Coastal and Shelf Science,* **114**, 18-22.

Guerinot ML, Patriquin DG (1981) The association of N_2 fixing bacteria with sea-urchins. *Marine Biology,* **62**, 197-207.

Guinotte JM, Fabry VJ (2008) Ocean acidification and its potential effects on marine ecosystems. *Annals of the New York Academy Sciences*, **1134**, 320-342

Guo H, Zhang Y, Lan Z, Pennings SC (2013) Biotic interactions mediate the expansion of black mangrove (*Avicennia germinans*) into salt marshes under climate change. *Global Change Biology,* **19**, 2765-2774.

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia MC (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature,* **454**, 96-99.

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. *Science,* **319**, 948-952.

Hanks JE (1957) The rate of feeding of the common oyster drill, *Urosalpinx cinerea* (Say), at controlled water temperatures. *Biological Bulletin,* **112**, 330-335.

Harley CDG, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH (2012) Effects of climate change on global seaweed communities. *Journal of Phycology,* **48**, 1064-1078.

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. *Ecology Letters,* **9**, 228-241.

Heck KL, Carruthers TJB, Duarte CM, Hughes AR, Kendrick G, Orth RJ, Williams SW (2008) Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. *Ecosystems,* **11**, 1198-1210.

Hellblom F, Beer S, Björk M, Axelsson L (2001) A buffer sensitive inorganic carbon utilisation system in *Zostera marina*. *Aquatic Botany,* **69**, 55-62.

Hepburn CD, Pritchard DW, Cornwall CE, Mcleod RJ, Beardall J, Raven JA, Hurd CL (2011) Diversity of carbon use strategies in a kelp forest community: implications for a high CO_2 ocean. *Global Change Biology*, **17**, 2488-2497.

Hillebrand H, Borer ET, Bracken MES, Cardinale BJ, Cebrián J, Cleland EE, Elser JJ, Gruner DS, Harpole WS, Ngai JT, Sandin S, Seabloom EW, Shurin JB, Smith JE, Smith MD (2009) Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. *Ecology Letters*, **12**, 516-527.

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the World's marine ecosystems. *Science,* **328**, 1523-1528.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. *Science,* **318**, 1737-1742.

Hughes AR, Bando KJ, Rodriguez LF, Williams SL (2004) Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. *Marine Ecology-Progress Series,* **282**, 87-99.

Hughes BB, Eby R, Van Dyke E, Tinker MT, Marks CI, Johnson KS, Wasson K (2013) Recovery of a top predator mediates negative eutrophic effects on seagrass. *Proceedings of the National Academy of Sciences of the United States of America,* **110**, 15313-15318.

Hughes TP (1994) Catastrophes, phase-shifts, and large scale-degradation of a Caribbean coral-reef. *Science,* **265**, 1547-1551.

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. *Science,* **301**, 929-933.

Ibarra-Obando SE, Heck KL, Spitzer PM (2004) Effects of simultaneous changes in light, nutrients, and herbivory levels, on the structure and function of a subtropical turtlegrass meadow. *Journal of Experimental Marine Biology and Ecology,* **301**, 193-224.

Invers O, Pérez M, Romero J (1999) Bicarbonate utilization in seagrass photosynthesis: role of carbonic anhydrase in *Posidonia oceanica* (L) Delile and *Cymodocea nodosa* (Ucria) Ascherson. *Journal of Experimental Marine Biology and Ecology,* **235**, 125-133.

Irving AD (2009) Seagrass rehabilitation in Adelaide's coastal waters VI. refining techniques for the rehabilitation of *Amphibolis* spp. final report prepared for the Coastal Management Branch of the Department for Environment and Heritage SA. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.

Irving AD (2013) A century of failure for habitat recovery. *Ecography,* **36**, 414-416.

Irving AD, Connell SD, Russell BD (2011) Restoring coastal plants to improve global carbon storage: reaping what we sow. *Plos One,* **6**, e18311.

Israel A, Hophy M (2002) Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biology,* **8**, 831-840.

Ives AR (1995) Predicting the response of populations to environmental change. *Ecology,* **76**, 926-941.

Jackson EL, Rowden AA, Attrill MJ, Bossey SJ, Jones MB (2001) The importance of seagrass beds as a habitat for fishery species. *Oceanography and Marine Biology, **39*** 269-304.

Jackson JBC (2001) What was natural in the coastal oceans? *Proceedings of the National Academy of Sciences of the United States of America,* **98**, 5411-5418.

James NP, Bone Y, Brown KM, Cheshire A (2009) Calcareous epiphyte production in cool-water carbonate seagrass depositional environments - southern Australia. In: *Perspectives in Carbonate Geology: A Tribute to the Career of Robert Nathan Ginsburg.* (eds Swart PK, Eberli GP, Mckenzie JA). Wiley, DOI: 10.1002/9781444312065.ch9.

James PL, Larkum AWD (1996) Photosynthetic inorganic carbon acquisition of *Posidonia australis*. *Aquatic Botany,* **55**, 149-157.

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls A, B, C1 and C2 in higher plants, algae and natural phytoplankton. *Biochemie Und Physiologie Der Pflanzen,* **167**, 191-194.

Jiang ZJ, Huang XP, Zhang JP (2010) Effects of CO_2 Enrichment on photosynthesis, growth, and biochemical composition of seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. *Journal of Integrative Plant Biology,* **52**, 904-913.

Jorda G, Marbà N, Duarte CM (2012) Mediterranean seagrass vulnerable to regional climate warming. *Nature Climate Change,* **2**, 821-824.

Kelaher BP, Van Den Broek J, York PH, Bishop MJ, Booth DJ (2013) Positive responses of a seagrass ecosystem to experimental nutrient enrichment. *Marine Ecology Progress Series*, **487**, 15-25.

Kendrick G, Lavery P (2001) Assessing biomass, assemblage structure and productivity of algal epiphytes on seagrasses. In: *Global Seagrass Research Methods.* (eds Short FT, Coles RG) pp. 199-222, Elsevier, Amsterdam.

Kendrick GA, Aylward MJ, Hegge BJ, Cambridge ML, Hillman K, Wyllie A, Lord DA (2002) Changes in seagrass coverage in Cockburn Sound, Western Australia between 1967 and 1999. *Aquatic Botany,* **73**, 75-87.

Kendrick GA, Burt JS (1997) Seasonal changes in epiphytic macro-algae assemblages between offshore exposed and inshore protected *Posidonia sinuosa* Cambridge et Kuo Seagrass Meadows, Western Australia. *Botanica Marina,* **40**, 77-85.

Keuskamp D (2004) Limited effects of grazer exclusion on the epiphytes of *Posidonia sinuosa* in South Australia. *Aquatic Botany*, **78**, 3-14.

Kirkman H, Kuo J (1990) Pattern and process in southern Western Australian seagrasses. *Aquatic Botany,* **37**, 367-382.

Kirkman H, Young PC (1981) Measurement of health, and echinoderm grazing on *Posidonia oceanica* (L.) Delile. *Aquatic Botany*, **10**, 329-338.

Koch EW (1994) Hydrodynamics, diffusion-boundary layers and photosynthesis of the seagrasses *Thalassia testudinum* and *Cymodocea nodosa*. *Marine Biology,* **118**, 767-776.

Koch M, Bowes G, Ross C, Zhang XH (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology,* **19**, 103-132.

Koike I, Mukai H, Nojima S (1987) The role of sea urchin *Tripneustes gratilla* Linnaeus in decomposition and nutrient cycling in a tropical seagrass bed. *Ecological Research,* **2**, 19-30.

Kordas RL, Harley CDG, O'Connor MI (2011) Community ecology in a warming world: the influence of temperature on interspecific interactions in marine systems. *Journal of Experimental Marine Biology and Ecology,* **400**, 218-226.

Kroeker KJ, Gambi MC, Micheli F (2013) Community dynamics and ecosystem simplification in a high-CO_2 ocean. *Proceedings of the National Academy of Sciences of the United States of America,* **110**, 12721-12726.

Kübler JE, Raven JA (1995) The interaction between inorganic carbon acquisition and light supply in *Palmaria palmata* (Rhodophyta). *Journal of Phycology,* **31**, 369-375.

La Nafie YA, de los Santos CB, Brun FG, van Katwijk MM, Bouma TJ (2012) Waves and high nutrient loads jointly decrease survival and separately affect morphological and biomechanical properties in the seagrass *Zostera noltii*. *Limnology and Oceanography,* **57**, 1664-1672.

Langdon MW, Paling EI, van Keulen M (2011) The development of urchin barrens in seagrass meadows at Luscombe Bay, Western Australia from 1985 to 2004. *Pacific Conservation Biology,* **17**, 48-53.

Lares MT, McClintock JB (1991) The effects of food quality and temperature on the nutrition of the carnivorous sea-urchin *Eucidaris-tribuloides*. *Journal of Experimental Marine Biology and Ecology,* **149**, 279-286.

Larkum AWD, Drew EA, Ralph PJ (2006) Photosynthesis and metabolism in seagrasses at the cellular level. In: *Seagrasses; Biology, Ecology and Conservation.* (eds Larkum AWD, Orth RJ, Duarte CM) pp. 323-345, Springer, Dordrecht.

Larkum AWD, Roberts G, Kuo J, Strother S (1989) Gaseous movement in seagrasses. In: *Biology of seagrasses: A treatise of the biology of seagrasses with a specialreference to the Australian region.* (eds Larkum AWD, McComb AJ, Shepherd SA) pp. 686-722, Elsevier, Amsterdam.

Larkum AWD, West RJ (1990) Long-term changes of seagrass meadows in Botany Bay, Australia. *Aquatic Botany*, **37**, 55-70.

Lavery PS, McMahon K, Mulligan M, Tennyson A (2009) Interactive effects of timing, intensity and duration of experimental shading on *Amphibolis griffithii*. *Marine Ecology Progress Series,* **394**, 21-33.

Lavery PS, Reid T, Hyndes GA, Van Elven BR (2007) Effect of leaf movement on epiphytic algal biomass of seagrass leaves. *Marine Ecology-Progress Series*, **338**, 97-106.

Lavery PS, Vanderklift MA (2002) A comparison of spatial and temporal patterns in epiphytic macroalgal assemblages of the seagrasses *Amphibolis griffithii* and *Posidonia coriacea*. *Marine Ecology Progress Series,* **236**, 99-112.

Lawrence J, Régis MB, Delmas P, Gras G, Klinger T (1989) The effect of quality of food on feeding and digestion in *Paracentrotus lividus* (Lamarck) (Echinodermata: Echinoidea). *Marine Behaviour and Physiology,* **15**, 137-144.

Lee KS, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology,* **350**, 144-175.

Leinaas HP, Christie H (1996) Effects of removing sea urchins (*Strongylocentrotus droebachiensis*): stability of the barren state and succession of kelp forest recovery in the east Atlantic. *Oecologia,* **105**, 524-536.

Lemoine NP, Burkepile DE (2012) Temperature-induced mismatches between consumption and metabolism reduce consumer fitness. *Ecology,* **93**, 2483-2489.

Lester SE, Gaines SD, Kinlan BP (2007) Reproduction on the edge: large-scale patterns of individual performance in a marine invertebrate. *Ecology,* **88**, 2229-2239.

Li J, Jiang Z, Zhang J, Qiu J-W, Du M, Bian D, Fang J (2013) Detrimental effects of reduced seawater pH on the early development of the Pacific abalone. *Marine Pollution Bulletin*.

Li W, Gao K (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. *Marine Pollution Bulletin,* **64**, 699-703.

Ling SD, Johnson CR, Frusher SD, Ridgway KR (2009) Overfishing reduces resilience of kelp beds to climate-driven catastrophic phase shift. *Proceedings of the National Academy of Sciences of the United States of America,* **106**, 22341-22345.

Littler MM, Littler DS (1980) The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *The American Naturalist,* **116**, 25-44.

Livore JP, Connell SD (2012a) Effects of food origin and availability on sea urchin condition and feeding behaviour. *Journal of Sea Research,* **68**, 1-5.

Livore JP, Connell SD (2012b) Fine-scale effects of sedentary urchins on canopy and understory algae. *Journal of Experimental Marine Biology and Ecology,* **411**, 66-69.

Maberly SC, Madsen TV (2002) Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. *Functional Plant Biology,* **29**, 393-405.

Mabrouk L, Hamza A, Ben Brahim M, Bradai M-N (2013) Variability in the structure of epiphyte assemblages on leaves and rhizomes of *Posidonia oceanica* in relation to human disturbances in a seagrass meadow off Tunisia. *Aquatic Botany,* **108**, 33-40.

Mackey P, Collier CJ, Lavery PS (2007) Effects of experimental reduction of light availability on the seagrass *Amphibolis griffithii*. *Marine Ecology Progress Series,* **342**, 117-126.

Marbà N, Cebrián J, Enríquez S, Duarte CM (1996) Growth patterns of western Mediterranean seagrasses: species-specific responses to seasonal forcing. *Marine Ecology Progress Series,* **133**, 203-215.

Marbà N, Hemminga MA, Duarte CM (2006) Resource translocation within seagrass clones: allometric scaling to plant size and productivity. *Oecologia,* **150**, 362-372.

Marbà N, Walker DI (1999) Growth, flowering, and population dynamics of temperate Western Australian seagrasses. *Marine Ecology-Progress Series,* **184**, 105-118.

Marchant HK, Calosi P, Spicer JI (2010) Short-term exposure to hypercapnia does not compromise feeding, acid-base balance or respiration of *Patella vulgata* but surprisingly is accompanied by radula damage. *Journal of the Marine Biological Association of the United Kingdom,* **90**, 1379-1384.

Martin S, Rodolfo-Metalpa R, Ransome E, Rowley S, Buia MC, Gattuso JP, Hall-Spencer J (2008) Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biology Letters,* **4**, 689-692.

Masini RJ, Manning CR (1997) The photosynthetic responses to irradiance and temperature of four meadow-forming seagrasses. *Aquatic Botany,* **58**, 21-36.

Matsuda Y, Colman B (1995) Induction of CO_2 and bicarbonate transport in the green alga *Chlorella ellipsoidea*: 1. Time course of induction of the two systems. *Plant Physiology,* **108**, 247-252.

Mattson WJ (1980) Herbivory in relation to plant nitrogen-content. *Annual Review of Ecology and Systematics,* **11**, 119-161.

McClanahan TR, Nugues M, Mwachireya S (1994) Fish and sea urchin herbivory and competition in Kenyan coral reef lagoons: the role of reef management. *Journal of Experimental Marine Biology and Ecology,* **184**, 237-254.

McGlathery KJ (1995) Nutrient and grazing influences on a subtropical seagrass community. *Marine Ecology-Progress Series,* **122**, 239-252.

McMillan C (1984) The condensed tannins (proanthocyanidins) in seagrasses. *Aquatic Botany,* **20**, 351-357.

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C (2007) Global climate projections. In: *Climate change 2007: The physical science basis. Contribution of the Working Group I to the Forth Assesment Report of the Intergovernmental Panel on Climate Change.* (eds Soloman S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL) Cambridge University Press, Cambridge and New York.

Menge BA (2000) Top-down and bottom-up community regulation in marine rocky intertidal habitats. *Journal of Experimental Marine Biology and Ecology,* **250**, 257-289.

Miles H, Widdicombe S, Spicer JI, Hall-Spencer J (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Marine Pollution Bulletin,* **54**, 89-96.

Millhouse J, Strother S (1986) The effect of pH on the inorganic carbon source for photosynthesis in the seagrass *Zostera muelleri* irmisch ex aschers. *Aquatic Botany,* **24**, 199-209.

Müren U, Berglund J, Samuelsson K, Andersson A (2005) Potential effects of elevated sea-water temperature on pelagic food webs. *Hydrobiologia,* **545**, 153-166.

Nayar S, Collings G, Pfennig P, Royal M (2012) Managing nitrogen inputs into seagrass meadows near a coastal city: flow-on from research to environmental improvement plans. *Marine Pollution Bulletin,* **64**, 932-940.

Nayar S, Collings GJ, Miller DJ, Bryars S, Cheshire AC (2009) Uptake and resource allocation of inorganic carbon by the temperate seagrasses *Posidonia* and *Amphibolis*. *Journal of Experimental Marine Biology and Ecology,* **373**, 87-95.

Nayar S, Collings GJ, Miller DJ, Bryars S, Cheshire AC (2010) Uptake and resource allocation of ammonium and nitrate in temperate seagrasses *Posidonia* and *Amphibolis*. *Marine Pollution Bulletin,* **60**, 1502-1511.

Neighbors MA, Horn MH (1991) Nutritional quality of macrophytes eaten and not eaten by 2 temperate-zone herbivorous fishes - a multivariate comparison. *Marine Biology,* **108**, 471-476.

Neverauskas VP (1987) Accumulation of periphyton biomass on artificial substrates deployed near a sewage-sludge outfall in South Australia. *Estuarine Coastal and Shelf Science,* **25**, 509-517.

Niell FX (1976) C/N ratio in some marine macrophytes and its possible ecological significance. *Botanica Marina,* **19**, 347-350.

Niu S, Zhang P, Liu J, Guo D, Zhang X (2012) The effect of temperature on the survival, growth, photosynthesis, and respiration of young seedlings of eelgrass *Zostera marina* L. *Aquaculture,* **350**, 98-108.

O'Connor MI (2009) Warming strengthens an herbivore-plant interaction. *Ecology,* **90**, 388-398.

O'Connor MI, Piehler MF, Leech DM, Anton A, Bruno JF (2009) Warming and resource availability shift food web structure and metabolism. *Plos Biology,* **7**, e1000178.

Ooi JLS, Kendrick GA, van Niel KP (2011) Effects of sediment burial on tropical ruderal seagrasses are moderated by clonal integration. *Continental Shelf Research,* **31**, 1945-1954.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner GK, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig MF, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature,* **437**, 681-686.

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006) A global crisis for seagrass ecosystems. *Bioscience,* **56**, 987-996.

Orth RJ, Moore KA (1983) Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. *Science,* **222**, 51-53.

Palacios SL, Zimmerman RC (2007) Response of eelgrass *Zostera marina* to CO_2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats. *Marine Ecology-Progress Series,* **344**, 1-13.

Parker LM, Ross PM, O'Connor WA (2009) The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). *Global Change Biology,* **15**, 2123-2136.

Patriquin DG, Keddy C (1978) Nitrogenase activity (Acetylene-reduction) in a Novia Scotian salt marsh: its association with angiosperms and influence of some edaphic factors. *Aquatic Botany,* **4**, 227-244.

Pearse JS (2006) Ecological role of purple sea urchins. *Science*, **314**, 940-941.

Pearse JS, Hines AH (1987) Long-term population dynamics of sea urchins in a central California kelp forest: rare recruitment and rapid decline. *Marine Ecology-Progress Series,* **39**, 275-283.

Pedersen MF, Paling EI, Walker DI (1997) Nitrogen uptake and allocation in the seagrass *Amphibolis antarctica*. *Aquatic Botany,* **56**, 105-117.

Pennings SC, Paul VJ (1992) Effect of plant toughness, calcification, and chemistry on herbivory by *Dolabella auricularia*. *Ecology,* **73**, 1606-1619.

Pennings SC, Svedberg JM (1993) Does $CaCO_3$ in food deter feeding by sea-urchins. *Marine Ecology Progress Series,* **101**, 163-167.

Peralta G, Pérez-Lloréns JL, Hernández I, Vergara JJ (2002) Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. *Journal of Experimental Marine Biology and Ecology*, **269**, 9-26.

Porzio L, Buia MC, Hall-Spencer JM (2011) Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology*, **400**, 278-287.

Power ME (1992) Top-down and bottom-up forces in food webs: do plants have primacy. *Ecology,* **73**, 733-746.

Prado P, Alcoverro T, Romero J (2010) Influence of nutrients in the feeding ecology of seagrass (*Posidonia oceanica* L.) consumers: a stable isotopes approach. *Marine Biology (Berlin),* **157**, 715-724.

Prins HBA, Elzenga JTM (1989) Bicarbonate utilization: function and mechanism. *Aquatic Botany,* **34**, 59-83.

Przeslawski R, Davis AR, Benkendorff K (2005) Synergistic effects associated with climate change and the development of rocky shore molluscs. *Global Change Biology,* **11**, 515-522.

Ralph PJ, Durako MJ, Enríquez S, Collier CJ, Doblin MA (2007) Impact of light limitation on seagrasses. *Journal of Experimental Marine Biology and Ecology,* **350**, 176-193.

Ralph PJ, Tomasko D, Moore K, Seddon S, Macinnis-Ng CMO (2006) Human impacts on seagrasses: Eutrophication, sedimentation, and contamination. In: *Seagrasses: Biology, ecology and conservation.* (eds Larkum AWD, Orth RJ, Duarte CM) pp. 567-593, Springer, Dordrecht.

Raven JA (1991) Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO_2 and temperature. *Plant Cell and Environment,* **14**, 779-794.

Raven JA, Giordano M, Beardall J, Maberly SC (2011) Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. *Photosynthesis Research,* **109**, 281-296.

Raven JA, Lucas WJ (1985) Energy costs of carbon acquisition. In: *Inorganic carbon uptake by aquatic photosynthetic organisms.* (eds Lucas WJ, Berry JA) pp. 305-324, American Society of Plant Physiologists, Maryland.

Reekie EG, Bazzaz FA (1989) Competition and patterns of resource use among seedlings of five tropical trees grown at ambient and elevated CO_2. *Oecologia,* **79**, 212-222.

Reynaud S, Leclercq N, Romaine-Lioud S, Ferrier-Pagés C, Jaubert J, Gattuso JP (2003) Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biology,* **9**, 1660-1668.

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology,* **37**, 1131-1134.

Ritchie ME, Tilman D, Knops JMH (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology,* **79**, 165-177.

Rivers DO, Short FT (2007) Effect of grazing by Canada geese *Branta canadensis* on an intertidal eelgrass *Zostera marina* meadow. *Marine Ecology-Progress Series,* **333**, 271-279.

Rose CD, Sharp WC, Kenworthy WJ, Hunt JH, Lyons WG, Prager EJ, Valentine JF, Hall MO, Whitfield PE, Fourqurean JW (1999) Overgrazing of a large seagrass bed by the sea urchin *Lytechinus variegatus* in Outer Florida Bay. *Marine Ecology-Progress Series,* **190**, 211-222.

Rosemond AD, Mulholland PJ, Elwood JW (1993) Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. *Ecology,* **74**, 1264-1280.

Roy RN, Roy LN, Vogel KM, Portermoore C, Pearson T, Good CE, Millero FJ, Campbell DM (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. *Marine Chemistry,* **44**, 249-267.

Ruíz JM, Pérez M, Romero J (2001) Effects of fish farm loadings on seagrass (*Posidonia oceanica*) distribution, growth and photosynthesis. *Marine Pollution Bulletin,* **42**, 749-760.

Ruíz JM, Pérez M, Romero J, Tomas F (2009) The importance of herbivory in the decline of a seagrass (*Posidonia oceanica*) meadow near a fish farm: an experimental approach. *Botanica Marina,* **52**, 449-458.

Russell BD, Connell SD (2005) A novel interaction between nutrients and grazers alters relative dominance of marine habitats. *Marine Ecology-Progress Series,* **289**, 5-11.

Russell BD, Connell SD (2007) Response of grazers to sudden nutrient pulses in oligotrophic versus eutrophic conditions. *Marine Ecology-Progress Series,* **349**, 73-80.

Russell BD, Connell SD, Uthicke S, Muehllehner N, Fabricius KE, Hall-Spencer JM (2013) Future seagrass beds: can increased productivity lead to increased carbon storage? *Marine Pollution Bulletin,* **73**, 463-469.

Russell BD, Harley CDG, Wernberg T, Mieszkowska N, Widdicombe S, Hall-Spencer JM, Connell SD (2012) Predicting ecosystem shifts requires new approaches that integrate the effects of climate change across entire systems. *Biology Letters,* **8**, 164-166.

Russell BD, Passarelli CA, Connell SD (2011) Forecasted CO_2 modifies the influence of light in shaping subtidal habitat. *Journal of Phycology,* **47**, 744-752.

Russell BD, Thompson JAI, Falkenberg LJ, Connell SD (2009) Synergistic effects of climate change and local stressors: CO_2 and nutrient-driven change in subtidal rocky habitats. *Global Change Biology,* **15**, 2153-2162.

Sand-Jensen K (1977) Effect of epiphytes on eelgrass photosynthesis. *Aquatic Botany,* **3** 55--63.

Sand-Jensen K (1989) Environmental variables and their effect on photosynthesis of aquatic plant communities. *Aquatic Botany*, **34**, 5-25.

Sand-Jensen K, Revsbech NP, Jørgensen BB (1985) Microprofiles of oxygen in epiphyte communities on submerged macrophytes. *Marine Biology*, **89**, 55-62.

Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. *Science,* **283**, 2095-2097.

Sanford E (2002) Water temperature, predation, and the neglected role of physiological rate effects in rocky intertidal communities. *Integrative and Comparative Biology,* **42**, 881-891.

Sarker YM, Bartsch I, Olischläger M, Gutow L, Wiencke C (2013) Combined effects of CO_2, temperature, irradiance and time on the physiological performance of *Chondrus crispus* (Rhodophyta). *Botanica Marina,* **56**, 63-74.

Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. *Nature,* **413**, 591-596.

Schiel DR, Lilley SA (2011) Impacts and negative feedbacks in community recovery over eight years following removal of habitat-forming macroalgae. *Journal of Experimental Marine Biology and Ecology,* **407**, 108-115.

Schupp PJ, Paul VJ (1994) Calcium carbonate and secondary metabolites in tropical seaweeds: variable effects on herbivorous fishes. *Ecology,* **75**, 1172-1185.

Schwarz AM, Björk M, Buluda T, Mtolera H, Beer S (2000) Photosynthetic utilisation of carbon and light by two tropical seagrass species as measured *in situ*. *Marine Biology,* **137**, 755-761.

Schwarz AM, Hellblom F (2002) The photosynthetic light response of *Halophila stipulacea* growing along a depth gradient in the Gulf of Aqaba, the Red Sea. *Aquatic Botany,* **74**, 263-272.

Semesi IS, Beer S, Björk M (2009) Seagrass photosynthesis controls rates of calcification and photosynthesis of calcareous macroalgae in a tropical seagrass meadow. *Marine Ecology-Progress Series,* **382**, 41-47.

Shepherd SA, Sprigg RC (1976) *Substrate, sediments and subtidal ecology of Gulf St. Vincent and Investigator Strait*. Royal Society of South Australia Inc., Adelaide.

Shepherd SA, Womersley HBS (1981) The algal and seagrass ecology of Waterloo Bay, South Australia. *Aquatic Botany,* **11**, 305-371.

Sheppard-Brennand H, Soars N, Dworjanyn SA, Davis AR, Byrne M (2010) Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. *Plos One,* **5**, e11372.

Short FT, Duarte CM (2001) Methods for measurement of seagrass growth and production. In: *Global Seagrass Research Methods.* (eds Short FT, Coles RG) pp. 155-182, Elsevier, Amsterdam.

Short FT, Neckles HA (1999) The effects of global climate change on seagrasses. *Aquatic Botany,* **63**, 169-196.

Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. *Environmental Conservation,* **23**, 17-27.

Siikavuopio SI, Mortensen A, Christiansen JS (2008) Effects of body weight and temperature on feed intake, gonad growth and oxygen consumption in green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture,* **281**, 77-82.

Silberstein K, Chiffings AW, McComb AJ (1986) The loss of seagrass in Cockburn Sound, Western Australia. 3. The effect of epiphytes on productivity of *Posidonia australis* Hook. F. *Aquatic Botany,* **24**, 355-371.

Silva J, Sharon Y, Santos R, Beer S (2009) Measuring seagrass photosynthesis: methods and applications. *Aquatic Biology,* **7**, 127-141.

Smith JE, Hunter CL, Smith CM (2010) The effects of top-down versus bottom-up control on benthic coral reef community structure. *Oecologia,* **163**, 497-507.

Sogard SM (1992) Variability in growth-rates of juvenile fishes in different estuarine habitats. *Marine Ecology-Progress Series,* **85**, 35-53.

Spalding MH, Ogren WL (1982) Photosynthesis is required for induction of the CO_2 concentrating system in *Chlamydomonas reinhardii*. *Febs Letters,* **145**, 41-44.

Steele L, Valentine JF (2012) Idiosyncratic responses of seagrass phenolic production following sea urchin grazing. *Marine Ecology Progress Series,* **466**, 81-92.

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation,* **29**, 436-459.

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell and Environment,* **22**, 583-621.

Strauss SY, Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution,* **14**, 179-185.

Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012) Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. *Aquatic Toxicology*, **110**, 194-207.

Sunday JM, Crim RN, Harley CDG, Hart MW (2011) Quantifying rates of evolutionary adaptation in response to ocean acidification. *Plos One*, **6**, e22881.

Taylor RB, Sotka E, Hay ME (2002) Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. *Oecologia,* **132**, 68-76.

Terborgh J, Lopez L, Nunez P, Rao M, Shahabuddin G, Orihuela G, Riveros M, Ascanio R, Adler GH, Lambert TD, Balbas L (2001) Ecological meltdown in predator-free forest fragments. *Science,* **294**, 1923-1926.

Tewfik A, Rasmussen JB, McCann KS (2005) Anthropogenic enrichment alters a marine benthic food web. *Ecology,* **86**, 2726-2736.

Thayer GW, Bjorndal KA, Ogden JC, Williams SL, Zieman JC (1984) Role of larger herbivores in seagrass communities. *Estuaries*, **7**, 351-376.

Thimijan RW, Heins RD (1983) Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. *Hortscience*, **18**, 818-822

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. *Nature,* **427**, 145-148.

Thomsen MS, Wernberg T, Engelen AH, Tuya F, Vanderklift MA, Holmer M, McGlathery KJ, Arenas F, Kotta J, Sillimann BR (2012) A meta-analysis of seaweed impacts on seagrasses: generalities and knowledge gaps. *Plos One,* **7**, e28595.

Thuiller W, Lavorel S, Araujo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 8245-8250.

Tomas F, Abbott JM, Steinberg C, Balk M, Williams SL, Stachowicz JJ (2011) Plant genotype and nitrogen loading influence seagrass productivity, biochemistry, and plant-herbivore interactions. *Ecology,* **92**, 1807-1817.

Tomasko DA, Lapointe BE (1991) Productivity and biomass of *Thalssaia testudinum* as related to water column nutient availability and epiphyte levels: field observations and experimental studies. *Marine Ecology Progress Series,* **75**, 9-17.

Tomlinson P (1974) Vegetative morphology and meristem dependence – the foundation of productivity in seagrasses. *Aquaculture,* **4**, 107-130.

Touchette BW, Burkholder JM (2000) Overview of the physiological ecology of carbon metabolism in seagrasses. *Journal of Experimental Marine Biology and Ecology,* **250**, 169-205.

Touchette BW, Burkholder JM (2002) Seasonal variations in carbon and nitrogen constituents in eelgrass (*Zostera marina* L.) as influenced by increased temperature and water-column nitrate. *Botanica Marina,* **45**, 23-34.

Townsend EC, Fonseca MS (1998) Bioturbation as a potential mechanism influencing spatial heterogeneity of North Carolina seagrass beds. *Marine Ecology Progress Series,* **169**, 123-132.

Uku J, Beer S, Björk M (2005) Buffer sensitivity of photosynthetic carbon utilisation in eight tropical seagrasses. *Marine Biology,* **147**, 1085-1090.

Underwood AJ (1997) *Experiments in ecology: their logical design and interpretation using analysis of variance.* Cambridge University Press, Cambridge.

Valentine JF, Duffy JE (2006) The central role of grazing in seagrass ecology. In: *Seagrasses: Biology, Ecology and Conservation.* (eds Larkum AWD, Orth RJ, Duarte CM) pp. 463-501, Springer, Dordrecht.

Valentine JF, Heck KL (1991) The role of sea urchin grazing in regulating subtropical seagrass meadows: evidence from field manipulations in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology,* **154**, 215-230.

Valentine JF, Heck KL (1999) Seagrass herbivory: evidence for the continued grazing of marine grasses. *Marine Ecology Progress Series,* **176**, 291-302.

Valentine JF, Heck KL (2001) The role of leaf nitrogen content in determining turtlegrass (*Thalassia testudinum*) grazing by a generalized herbivore in the northeastern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology,* **258**, 65-86.

Valentine JF, Heck KL, Kirsch KD, Webb D (2000) Role of sea urchin *Lytechinus variegatus* grazing in regulating subtropical turtlegrass *Thalassia testudinum* meadows in the Florida Keys (USA). *Marine Ecology-Progress Series,* **200**, 213-228.

Valentine JP, Edgar GJ (2010) Impacts of a population outbreak of the urchin *Tripneustes gratilla* amongst Lord Howe Island coral communities. *Coral Reefs,* **29**, 399-410.

Valentine JP, Johnson CR (2005) Persistence of sea urchin (*Heliocidaris erythrogramma*) barrens on the east coast of Tasmania: inhibition of macroalgal recovery in the absence of high densities of sea urchins. *Botanica Marina*, **48**, 106-115.

van Keulen M, Borowitzka MA (2002) Comparison of water velocity profiles through morphologically dissimilar seagrasses measured with a simple and inexpensive current meter. *Bulletin of Marine Science,* **71**, 1257-1267.

van der Meijden E, Wijn M, Verkaar HJ (1988) Defense and regrowth, alternative plant strategies in the struggle against herbivores. *OIKOS,* **51**, 355-363.

van Montfrans J, Wetzel RL, Orth RJ (1984) Epiphyte-grazer relationships in seagrass meadows: consequences for seagrass growth and production. *Estuaries,* **7***,* 289-309.

Vaughan GM, Corballis MC (1969) Beyond tests of significance: estimating strength of effects in selected ANOVA designs. *Psychological Bulletin,* **72**, 204-&.

Vergeer LHT, Aarts TL, de Groot JD (1995) The ‘wasting disease’ and the effect of abiotic factors (light intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. *Aquatic Botany*, **52**, 35-44.

Vergeer LHT, Develi A (1997) Phenolic acids in healthy and infected leaves of *Zostera marina* and their growth-limiting properties towards *Labyrinthula zosterae*. *Aquatic Botany,* **58**, 65-72.

Vergés A, Alcoverro T, Romero J (2011) Plant defences and the role of epibiosis in mediating within-plant feeding choices of seagrass consumers. *Oecologia,* **166**, 381-390.

Vergés A, Becerro MA, Alcoverro T, Romero J (2007a) Experimental evidence of chemical deterrence against multiple herbivores in the seagrass *Posidonia oceanica*. *Marine Ecology-Progress Series,* **343**, 107-114.

Vergés A, Becerro MA, Alcoverro T, Romero J (2007b) Variation in multiple traits of vegetative and reproductive seagrass tissues influences plant-herbivore interactions. *Oecologia,* **151**, 675-686.

Vergés A, Pérez M, Alcoverro T, Romero J (2008) Compensation and resistance to herbivory in seagrasses: induced responses to simulated consumption by fish. *Oecologia,* **155**, 751-760.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494-499.

Vonk JA, Pijnappels MHJ, Stapel J (2008) *In situ* quantification of *Tripneustes gratilla* grazing and its effects on three co-occurring tropical seagrass species. *Marine Ecology Progress Series,* **360**, 107-114.

Wahl M, Hay ME (1995) Associational resistance and shared doom: effects of epibiosis on herbivory. *Oecologia*, **102**, 329-340.

Walker D (1987) *The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis.* Oxygraphics, Sheffield.

Walker DI (1985) Correlations between salinity and growth of the seagrass *Amphibolis antarctica* (Labill) sonder and aschers in Shark Bay, Western Australia, using a new method for measuring production-rate. *Aquatic Botany*. **23**, 13-26.

Walker DI, Cambridge ML (1995) An experimental assessment of the temperature responses of two sympatric seagrasses, *Amphibolis antarctica* and *Amphibolis griffithii*, in relation to their biogeography. *Hydrobiologia,* **302**, 63-70.

Walker DI, Lukatelich RJ, Bastyan G, McComb AJ (1989) Effect of boat moorings on seagrass beds near Perth, Western Australia. *Aquatic Botany,* **36**, 69-77.

Walker DI, McComb AJ (1992) Seagrass degradation in Australian coastal waters. *Marine Pollution Bulletin,* **25**, 191-195.

Wand SJE, Midgley GF, Jones MH, Curtis PS (1999) Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology,* **5**, 723-741.

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America,* **106**, 12377-12381.

Weidner K, Lages BG, da Gama BAP, Molis M, Wahl M, Pereira RC (2004) Effect of mesograzers and nutrient levels on induction of defenses in several Brazilian macroalgae. *Marine Ecology Progress Series,* **283**, 113-125.

Williams WE, Garbutt K, Bazzaz FA, Vitousek PM (1986) The response of plants to elevated CO_2 II Deciduous-forest tree communities. *Oecologia,* **69**, 454-459.

Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. *Proceedings of the Royal Society B-Biological Sciences,* **275**, 1767-1773.

Wressnig A, Booth DJ (2007) Feeding preferences of two seagrass grazing monacanthid fishes. *Journal of Fish Biology,* **71**, 272-278.

Wressnig A, Booth DJ (2008) Patterns of seagrass biomass removal by two temperate Australian fishes (Monacanthidae). *Marine and Freshwater Research,* **59**, 408-417.

Yamamuro M (1999) Importance of epiphytic cyanobacteria food sources for heterotrophs in a tropical seagrass bed. *Coral Reefs,* **18**, 263-271.

Zachos JC, Röhl U, Schellenberg SA, Sluijs A, Hodell DA, Kelly DC, Thomas E, Nicolo M, Raffi I, Lourens LJ, McCarren H, Kroon D (2005) Rapid acidification of the ocean during the Paleocene-Eocene thermal maximum. *Science,* **308**, 1611-1615.

Zimmerman RC, Kohrs DG, Steller DL, Alberte RS (1997) Impacts of CO_2 enrichment on productivity and light requirements of eelgrass. *Plant Physiology,* **115**, 599-607.

Zou DH, Gao KS (2009) Effects of elevated CO_2 on the red seaweed *Gracilaria lemaneiformis* (Gigartinales, Rhodophyta) grown at different irradiance levels. *Phycologia,* **48**, 510-517.

APPENDIX A

PREAMBLE

Appendix A is a co-authored manuscript published in the journal *PLoS ONE,* with Bayden D. Russell as the senior author and Sean D. Connell, Camille Mellin, Barry W. Brook, myself and Damien A. Fordham as co-authors. It is, therefore, written in plural throughout and uses the journal formatting. The manuscript can be cited as:

Russell BD, Connell SD, Mellin C, Brook BW, Burnell OW, Fordham DA (2012) Predicting the distribution of commercially viable invertebrate stocks under future climate. *PLoS ONE,* 7(12): e46554. doi:10.1371/journal.pone.0046554.

OPEN ACCESS Freely available online

PLOS | ONE

Predicting the Distribution of Commercially Important Invertebrate Stocks under Future Climate

Bayden D. Russell[1]*, **Sean D. Connell**[1], **Camille Mellin**[2,3], **Barry W. Brook**[3], **Owen W. Burnell**[1], **Damien A. Fordham**[3]

1 Southern Seas Ecology Laboratories, School of Earth & Environmental Sciences, University of Adelaide, Adelaide, South Australia, Australia, **2** Australian Institute of Marine Science, Townsville, Queensland, Australia, **3** The Environment Institute and School of Earth & Environmental Sciences, University of Adelaide, Adelaide, South Australia, Australia

Abstract

The future management of commercially exploited species is challenging because techniques used to predict the future distribution of stocks under climate change are currently inadequate. We projected the future distribution and abundance of two commercially harvested abalone species (blacklip abalone, *Haliotis rubra* and greenlip abalone, *H. laevigata*) inhabiting coastal South Australia, using multiple species distribution models (SDM) and for decadal time slices through to 2100. Projections are based on two contrasting global greenhouse gas emissions scenarios. The SDMs identified August (winter) Sea Surface Temperature (SST) as the best descriptor of abundance and forecast that warming of winter temperatures under both scenarios may be beneficial to both species by allowing increased abundance and expansion into previously uninhabited coasts. This range expansion is unlikely to be realised, however, as projected warming of March SST is projected to exceed temperatures which cause up to 10-fold increases in juvenile mortality. By linking fine-resolution forecasts of sea surface temperature under different climate change scenarios to SDMs and physiological experiments, we provide a practical first approximation of the potential impact of climate-induced change on two species of marine invertebrates in the same fishery.

Citation: Russell BD, Connell SD, Mellin C, Brook BW, Burnell OW, et al. (2012) Predicting the Distribution of Commercially Important Invertebrate Stocks under Future Climate. PLoS ONE 7(12): e46554. doi:10.1371/journal.pone.0046554

Editor: Kimberly Patraw Van Niel, University of Western Australia, Australia

Received March 14, 2012; **Accepted** September 2, 2012; **Published** December 12, 2012

Funding: The research was partly funded by Australian Research Council Grants to D. A. Fordham, B. W. Brook and S. D. Connell. C. Mellin was funded by the Marine Biodiversity Hub, a collaborative partnership supported through funding from the Australian Government's National Environmental Research Program (NERP). NERP Marine Biodiversity Hub partners include the Institute for Marine and Antarctic Studies, University of Tasmania; CSIRO, Geoscience Australia, Australian Institute of Marine Science, Museum Victoria, Charles Darwin University and the University of Western Australia. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding received for this study.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: bayden.russell@adelaide.edu.au

Introduction

The distributions of many marine species are strongly related to environmental conditions, making them susceptible to both medium- and long-term changes in abiotic conditions (e.g. medium-term changes to El Niño cycles: [1]; and long-term climate change: [2],[3]). Commercially exploited populations may be particularly susceptible to changes in environmental conditions, as many of the world's fisheries stocks are over-exploited, with benthic habitats also being damaged by fishing operations [4], and consequently the capacity of their populations to withstand multiple and interacting environmental changes is eroded [5].

Current projections suggest that multi-decadal increases in ocean temperatures are likely to cause commercially harvested species to become increasingly vulnerable to overfishing at lower latitudes, where species are closer to thermal-tolerance limits and have been forecast to undergo population declines [6]. Such predictions are supported by observations of different populations of the same fish species at high and low latitudes alternately experiencing either population increase or decrease with historical warming (e.g. Norwegian cod; [7],[8]). Current modelling and empirical techniques can be used to predict the geographical range of marine species at coarse scales [9,10] and in rare instances have been used to detect present-day spatial abundance patterns at finer resolutions (e.g. [11]). Yet, future projections of changes in range and abundance have been limited by uncertainties arising from choice of species distribution model (SDM) and global climate model (GCM), a lack of consideration of model-selection uncertainty, and a failure to integrate relevant biological detail (e.g., species interactions, connectivity and dispersal). All of these factors influence projections of species' range movement and extinction risk [12,13]. Therefore, efforts to address these model-related uncertainties should lead to improvements in the capacity to predict the abundance of commercially harvested species under changing environmental conditions, and may enhance the capability of management to ensure harvest can be maintained into the future.

Research on the causes of and changes in the distribution of marine species, to date, has been limited by a paucity of high-resolution and broad-scale environmental data in the marine realm [14,15]. In cases where climate-change impacts have been assessed, they have mostly focused on bioclimatic envelop or single species distribution model (SDM) approaches [14], sometimes integrating demographic information [6]. While these approaches can predict present-day distributions reasonably well, projections of future distributions can vary widely among SDMs [16,17,18].

Recent advances in multi-model ensemble techniques may provide more robust and realistically bounded estimates of future distributions [19], but have only recently been applied in marine systems (e.g. [11]).

Further complicating such predictions are uncertainties in the climate projections themselves. The degree of confidence that we can place on future climate change projections depends on GCM performance and uncertainties that need to be assessed rigorously, via climate model evaluation [20]. GCMs are helpful in providing physically realistic representations of global-climate dynamics [21], yet they tend to provide less reliable descriptions of local and regional climates [22], partly because processes that occur at scales smaller than the GCM resolution (such as cloud and topographic influences) cannot be modelled explicitly and must be parameterised [23].

Here, we use two species of commercially harvested abalone with overlapping ranges, blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*), to demonstrate a novel correlative and experimental approach for predicting the future distribution and abundance of marine invertebrates. While present-day management of the fishery is appropriate [24,25], forecast changes in key environmental requirements may mean that fine-scale estimates of population densities under future conditions will be required to successfully manage the long-term persistence of abalone stocks. At the moment climate change is not being considered in the fishery management plans. We hypothesise that green and blacklip abalone will respond differently to future ocean warming, because the two species have different biological responses to temperature, with blacklip exhibiting a lower optimal temperature than greenlip (17.0°C *vs.* 18.3°C, respectively; [26]), but above a certain thermal tolerance this difference is likely to disappear. While recently developed SDMs have identified the most likely environmental factors which drive population densities, including temperature [11], there are currently no forecasts of how these populations will change under future climates.

Thus, the objectives of this study were to: (1) project changes in the range and abundance of *H. rubra* and *H. laevigata* in southern Australia at decadal time slices under different climate-change scenarios; (2) develop forecasts of sea surface temperature which better account for inter-model variation in GCM projections, downscaled to biologically relevant resolutions; and (3) determine whether demographic processes need to be incorporated in models by experimentally assessing juvenile mortality at projected temperatures.

Materials and Methods

Species distribution models

Blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone occur on the southern coast of Australia and have overlapping distributions (greenlip abalone occupying approximately the central 2/3 of the distribution of blacklip [27]). For the specific purpose of this study, a species' range refers to its area of occupancy within the study area, i.e. corresponding to the spatial extent of the fishery in southern Australia. Distribution and abundance data for both species within their ranges were collated from multiple fisheries monitoring and regional biodiversity surveys between 1980 and 2009. All surveys were done using SCUBA on rocky substrate between 5–30 m depth across South Australia (approximately 130–142°E). In previous work, we modelled the present-day distribution and spatial abundance of both species individually using SDMs [11]. To summarise (as background to the current forecast-based paper), a multi-model ensemble averaging technique was used to weight SDM projections. Generalized linear models (GLM) and boosted regression trees (BRT) were used to generate the model-averaged forecasts of abundance, because both techniques demonstrated good skill in forecasting present-day occurrence and abundance patterns for *H. rubra* and *H. laevigata* (see [11] for more comprehensive detail on modelling abundance with these SDMs). We constrained model development and training to the region with the most comprehensive abundance survey data available (South Australia). Out-of-sample validation included an assessment of spatial transferability of model predictions; SDMs were validated using independent data from similar surveys done across several hundred km of coast to the east of the study area (approximately 142–147°E).

For both abalone species, the best primary predictors of abundance were mean August (winter) sea surface temperature (SST) and its standard deviation (a linear correlative relationship for *H. rubra*, quadratic for *H. laevigata*), harvest intensity, water depth and distance from the nearest boat launch point. While sea bottom temperature may be slightly cooler than SST ($<2°C$ at depths less than 30 m [mean difference $= 0.27°C$]) in our study region, bottom temperature was strongly correlated with SST ($r = 0.825$) and was not a better predictor of abalone abundance than SST [11]. Therefore, we chose to use SST as our primary temperature predictor in the model. To determine whether anticipated change in future SST can be expected to influence the range dynamics of either abalone species, we developed downscaled-decade forecasts of August SST (2010–2100), according to different greenhouse gas emissions scenarios. All other predictors were fixed to the values used for model fitting [11] for a particular location.

Sea Surface Temperature projections

The SST data were extracted from satellite images focused on southern Australia at a 4.6-km resolution (AVHRR Pathfinder product version 5.0), for separate day and night passes. While sea surface temperatures are often higher than those near the sea bottom, AVHRR satellite derived SST data correlate well with bottom temperatures [28]. Mean monthly day and night SST data from 1985 to 2004 were used to calculate a 20-year monthly day/night average for August and March SST [11]; a period that closely resembles the baseline period used to validate GCMs (1980–2000). Thin-plate–spline surface-smoothing techniques were then used to downscale the coarse-resolution data to a 0.01° latitude/longitude grid-cell resolution [11]. The degree of smoothness of the fitted function was determined by minimizing a measure of predictive error of the fitted surface given by the generalised cross validation [29]. Moreover, out-of-source sampling on a subset of the Pathfinder data, specifically retained for validation, was used to evaluate model fit ($<\pm 0.5°C$) [11]. MAGICC/SCENGEN 5.3 (http://www.cgd.ucar.edu/cas/wigley/magicc), a coupled gas cycle/aerosol/climate model used in the IPCC Fourth Assessment Report [30], was used to generate future changes in August and March SST at the turn of each decade (2010–2100) using an ensemble of five GCMs, chosen according to their superior skill in globally forecasting March, August and annual SST and their consistency with other GCMs [20,31]. The skill of the full suite of GCMs used for the Fourth Assessment Report (AR4) of the Intergovernmental Panel on Climate Change (IPCC) can be assessed directly in MAGICC/SCENGEN according to their ability to simulate observed conditions using different variables, and different statistical-validation metrics, over any user-specified region.

The comparison metrics that we used for validation of GCM outputs were: (i) model bias (i.e., the difference between model and observed spatial means averaged over a user-specified area); (ii) pattern correlation; and (iii) standard and centred root-mean-square errors. Rather than using actual values of these various statistics, we placed them on a level playing field by using only model ranks for each statistic. Our key overall comparison metric was the cumulative rank [31]. MAGGICC/SCENGEN also allows for an outlier analysis to be computed which compares future projections based on individual models with the average projection of all other models. Although, GCM data from the Coupled Model Intercomparison Project 3 (CMIP3) archived GCM database (www-pcmdi.llnl.gov/) could be used to generate ensemble averaged forecasts directly, a key features that makes working within the MAGICC/SCENEGEN framework superior is the way in which MAGICC/SCENEGEN standardises different GCMs to align to user-specified selected climate sensitivities, meaning inter-model differences in future climate forecasts can be studied without being obfuscated by differences in climate sensitivity [31].

The five best-ranked models were CCSM3, MIROC 3.2 (hires), ECHAM5/MPI-OM, MRI-CGCM2.3.2 and GFDC-CM2.1 (model terminology follows that used in the CMIP3 model data base). These five models were used to generate multi-model averaged climate forecasts – change in average daily SST (°C) in August and March (2.5×2.5° latitude/longitude grid cell resolution). With less than five models the results are more sensitive to the number and choice of models, while for more than five models, the additional information has a relatively small effect on the average forecast [32]. Climate forecasts were generated according to two emission scenarios: a high-CO_2-concentration stabilising Reference scenario (WRE750) and a more conservative Policy emissions scenario, assuming substantive intervention (LEV1) [33,34].

These climate anomalies were downscaled to an ecologically relevant spatial scale (0.01×0.01° longitude/latitude), using the "change factor" method, where the low-resolution change from a GCM is added directly to a high-resolution baseline observed climatology [35]. Bi-linear interpolation of the GCM data (2.5×2.5°) to a resolution of 0.5×0.5° longitude/latitude was used to reduce discontinuities in the perturbed climate at the GCM grid-box boundaries [20]. While there is a range of alternative approaches, the simple "change factor" approach that we advocate is easily implemented in such a way that uncertainties arising through the generation of the baseline layer and overlay process can be easily documented.

Abalone range projections

To better understand the potential impact of forecast changes in August SST on the range and abundance of abalone, we used our already established ensemble SDM modelling approach [11] to project the spatial abundance of *H. rubra* and *H. laevigata* at the turn of each decade (2010–2100). We present forecast change in abundance (number of individuals per 100 m^2), and mean percent change in abundance above a minimum threshold of 20 individuals/100 m^2, for both *H. rubra* and *H. laevigata* for 2100. The 20 individuals/100 m^2 threshold was chosen because this is the minimum density needed to maintain the rates of recruitment required to sustain catches [36,37]. We also map changes in potential fishing grounds for *H. rubra* and *H. laevigata* in 2100 according to both emission scenarios.

Juvenile mortality - experimental methods

To test whether realised range expansion was likely to be reduced by elevated March SST, the effects of elevated temperature on juvenile *H. laevigata* mortality was experimentally tested. Mortality of juvenile abalone was recorded in a laboratory experiment spanning one month to match the monthly average temperature used in model projections. Two temperatures were used in the experiment, 17°C and 20°C, as they represent the lower and upper March temperature categories across the current distribution of *H. laevigata*; densities decline above and below these thresholds (see results below). Experiments were done on *H. laevigata* because juveniles were readily available (KIAB aquaculture, Kangaroo Island, South Australia) and data were available for *H. rubra* [38].

Experiments were conducted in 44 L aquaria with water constantly recirculating from a 200 L reservoir beneath each tank. There were 4 replicate aquaria per temperature and 8 replicate individual abalone per tank. A pump moved water from the reservoir at a constant flow rate of 200 L hr^{-1} to the tank. To maintain good water quality (i.e. nutrients and salinity), 50% of the water in each set-up was replaced weekly with fresh seawater. Light was provided in a 12:12 light dark cycle by pairs of fluorescent lights above each tank. Each light had one "grow light" which incorporated the UV spectrum (Sylvania® Gro-lux) and one "daylight" (Luxling® Daylight deluxe). Each tank contained rocks covered in coralline crusts and turf-forming algae to represent a natural environment. Abalone were fed a 1–3 mm formulated feed (EP Aquafeeds, Lonsdale, South Australia) every second day (17:00 hrs). Any unconsumed feed was removed from tanks the following morning (09:00 hrs). All tanks were aerated, with a constant flow of 10 L min^{-1}. Temperature levels remained constant throughout the treatment period. Elevated temperature (20°C±0.5°C) was controlled using Aqua One aquarium heaters. Ambient temperature (17°C±0.5°C) was controlled by recirculating water through chilling units (TECO-Ravenna). Experiments were done in a constant temperature laboratory to ensure no external inputs created temperature fluctuations and temperature was measured daily to ensure treatment levels were achieved.

Results

Under the Reference scenario (WRE 750; higher-CO_2-emission stabilisation), August (winter) SST is predicted to consistently increase over the next century to be ~1.1°C higher by 2100 (Fig. S1a). Under the Policy emissions scenario (LEV1) which assumes strong greenhouse-gas mitigation, however, average August SST in the study region is projected to increase by ~0.46°C by 2080 and then reduce to ~0.44°C above 1994 temperatures by 2100 (Fig. S1a). Warming will not be spatially consistent across the study area, however, with temperatures being between 0.87–1.77°C and 0.37–0.85°C higher in 2100 than 1994 for the Reference and Policy scenarios, respectively.

SDM future projections indicate that under the Reference scenario, both species are predicted to increase in abundance in response to increased August SST, albeit blacklip to a greater degree. Under this scenario, blacklip abundance is predicted to increase by up to 2.5 individuals m^{-2} across approximately the western two-thirds of their distribution in South Australia, while areas of substantial increases in abundance of greenlip abalone (approximately 2.5–3 indiv. m^{-2}) were more restricted to the south-eastern part of their range (Fig. 1). Elevated August SST can be expected to increase blacklip harvestable biomass by >40% by 2100 under this Reference scenario (Fig. S1b). In contrast, the more geographically restricted increase in forecasted abundance for greenlip abalone resulted in a relatively small projected increase in harvestable biomass by 2100, approximately 15% above present day levels (Fig. 1). Under the lower emission Policy scenario, SDM projections indicate that neither species of abalone will show substantial changes in abundance by 2100 (Fig. 1). This small predicted increase from current-day abundance would translate into little change in the harvestable biomass by 2100, with only marginal increases above a critical sustainable harvesting density (0.2 reproductive adults m^{-2}) of approximately 20% and <10% for blacklip and greenlip, respectively (Fig. S1b).

Overall, the SDMs predict expansion of potential fishing grounds for both species by 2100 based on predicted increases to August SST (Fig. 2). Blacklip fishing grounds would be gained across much of their range in South Australia, with greater expansion under the Reference scenario (Fig. 2). Expansion would be less for greenlip abalone and there would also be a loss of a small area of fishing grounds in the western part of South Australia (Fig. 2).

The present-day density of both species of abalone varies with summer (as well as winter) maximum monthly temperature across their current distribution. Density is greater in locations (1 km×1 km grid cells) where average March SST exceeds 17°C, but then declines substantially in locations where March temperatures are at or above 20°C (Fig. 3 a & b). In the laboratory experiment, mortality of juvenile greenlip abalone was over 10 times greater at 20°C (mean ± SE; 58.12%±8.6) than at 17°C (3.1%±3.1) (one-way ANOVA: $F_{1,6} = 35.96$, $p = 0.001$). This result is supported by the mean March SST at the distribution edges of both species for the same period used to train the SDMs. Mean temperatures at the north-eastern and north-western distribution limits of blacklip were 23.6°C and 19.9°C, respectively, with greenlip being 19.2°C and 19.0°C, respectively.

Within a large proportion of the study area (~33%), average March temperatures are currently at or below 17°C with less than 20% of the area having temperatures at or above 20°C (Fig. 4a). Under the Reference scenario, the majority of the current distribution of both species is predicted to be at or above 20°C (~78% and ~86% for green- and blacklip, respectively), with only a small percentage of their distribution at or below 17°C (~5% and ~2%, respectively; Fig. 4a). Of the locations (1 km×1 km grid cells) which the SDMs predicted would show expansion of abalone populations, ~94% and 86% are predicted to have average March SST of at or above 20°C by 2100 for green- and blacklip, respectively (Fig. 4b).

Discussion

We predict, based on correlative SDM projections, that both blacklip and greenlip abalone could increase in abundance and expand into new locations in response to warming winter temperatures under both high and low high CO_2 concentration stabilisation scenarios. However, projections of temperatures in warmer months (March), viewed in conjunction with experimental data, suggests that much of the predicted area of population expansion would be too hot, thus limiting realised range expansion through elevated juvenile mortality. Thus, to accurately predict the potential future distribution of species, we need to understand physiological responses of species across all of their life stages [39]

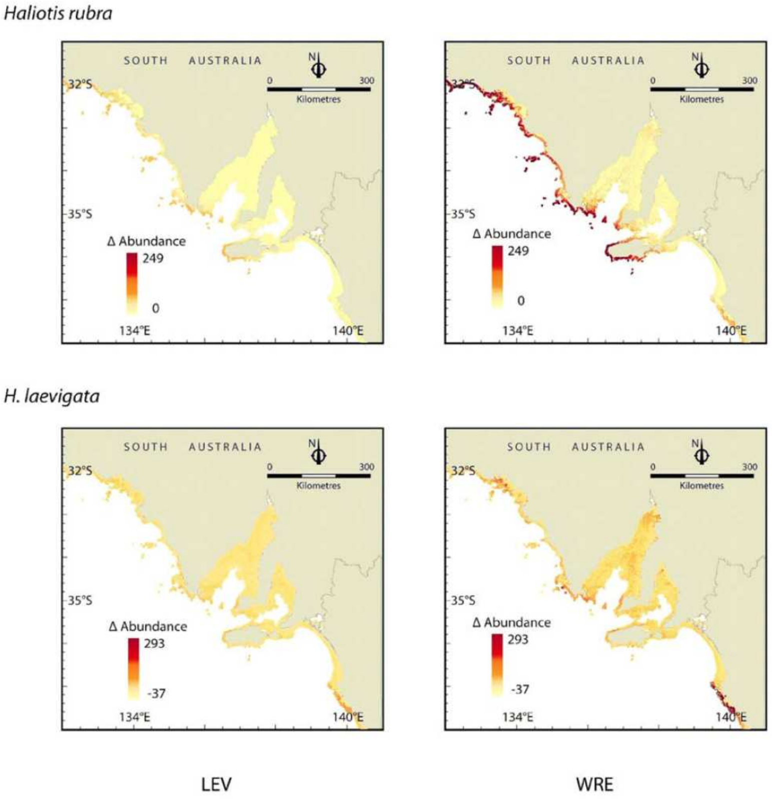


Figure 1. Forecast change in the abundance (number of individuals per 100 m^2) of *Haliotis rubra* (blacklip abalone) and *H. laevigata* (greenlip abalone) by 2100 based on projections of August SST according to two climate change emissions scenarios: a high CO_2 concentration stabilising scenario (WRE750) and a heavy mitigation Policy option (LEV1).
doi:10.1371/journal.pone.0046554.g001

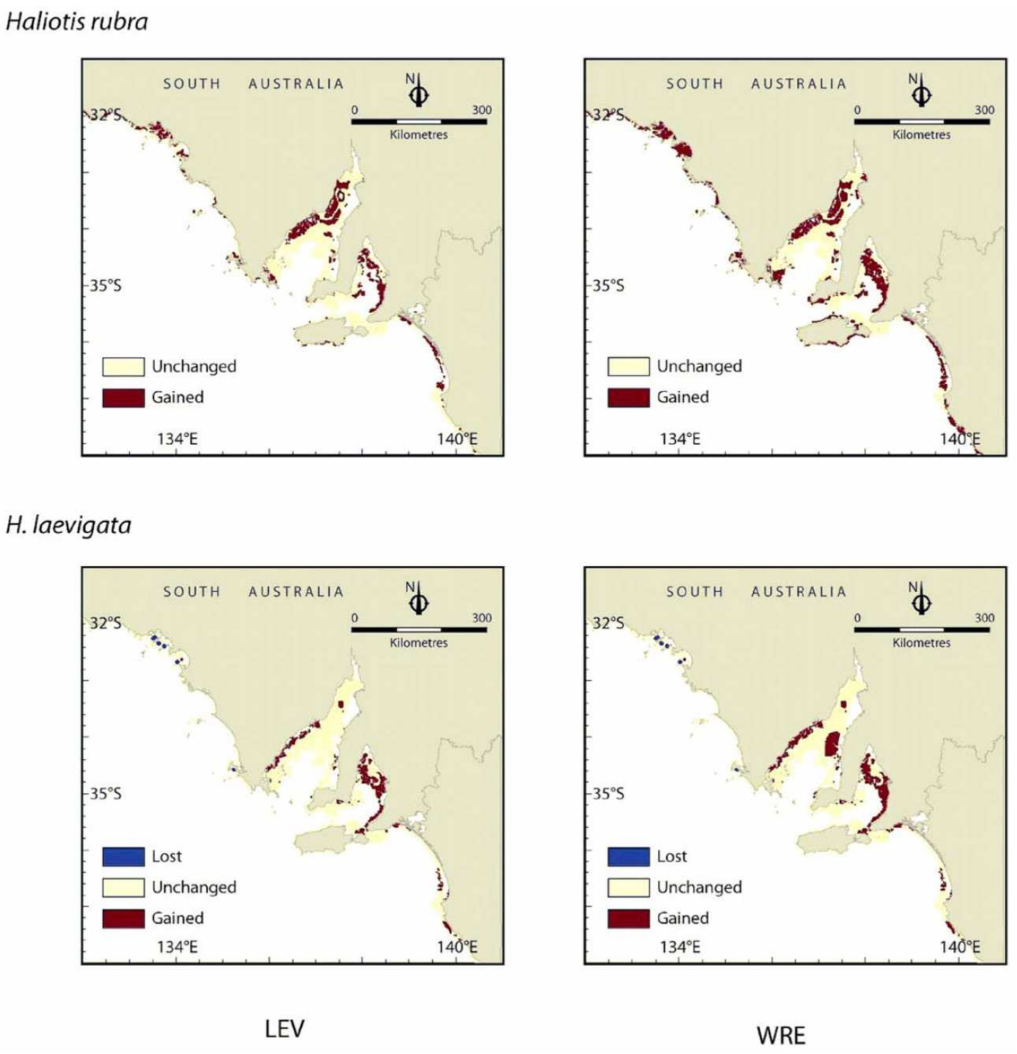


Figure 2. Changes in potential fishing grounds for *Haliotis rubra* (blacklip abalone) and *H. laevigata* (greenlip abalone) in 2100 based on projections of August SST according to two climate change scenarios: a high CO_2 concentration stabilising scenario (WRE750) and an alternative scenario that assumes strong mitigation (LEV1). Potential fishing grounds are defined based on a minimum abundance of 20 individuals/100 m^2.
doi:10.1371/journal.pone.0046554.g002

and develop modeling techniques that can make the best use of this information.

By using SDMs coupled to downscaled multi-model ensemble averaged climate projections we were also able to identify that there will be species-specific responses to warming, with blacklip populations predicted to expand across their South Australia but greenlip ones to a lesser degree, only expanding in the eastern part of the South Australian fishery. These predictions of range expansion are not isolated to abalone. Increasing winter temperatures in the North Sea over the past three decades correlated well with the northward movement of a range of taxonomically diverse species, including deep-sea fish [9], intertidal [3,40] and subtidal invertebrates [41] and their algal habitats [42]. Our models suggest an increase in winter temperatures could allow both blacklip and greenlip abalone to increase abundance within their distribution in South Australia, as well as expand into unoccupied habitat. However, the SDMs did not incorporate the concomitant increase in March SST (because March SST was not a strong predictor of present-day abundance; [11]), yet projections of these warmer temperatures suggest that the majority of the predicted area of population expansion would be too hot, thus limiting realised range expansion (Figure 4).

Reproductive output and survival of recruits are key factors that determine the range of a species. In our case study, August (winter) temperature had the largest (positive) influence on the distribution of both species of abalone [11], which is expected to increase in response to a low-, as well as high-CO_2 concentration stabilizing scenario, promoting range expansion, albeit to a lesser degree for the more conservative emission Policy scenario. While recognising that the techniques we employed were correlative, we can

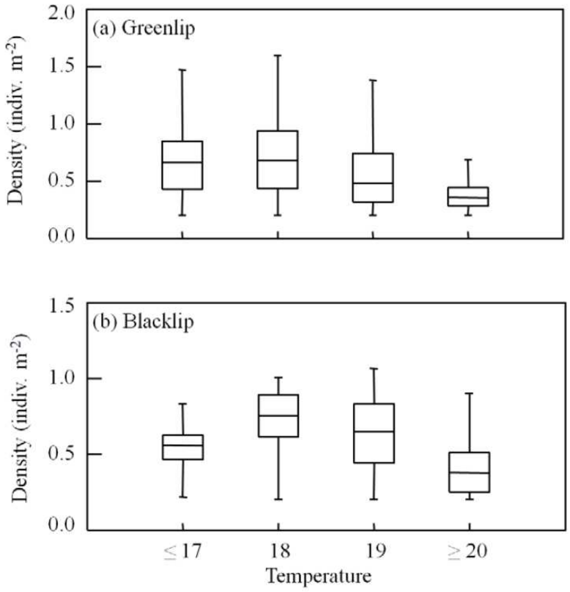


Figure 3. Box plots of the density of (a) greenlip and (b) blacklip abalone within their current distribution in South Australia, categorised according to average March Sea Surface Temperatures. Note the decrease in density of individuals for both species at 20°C and above.
doi:10.1371/journal.pone.0046554.g003

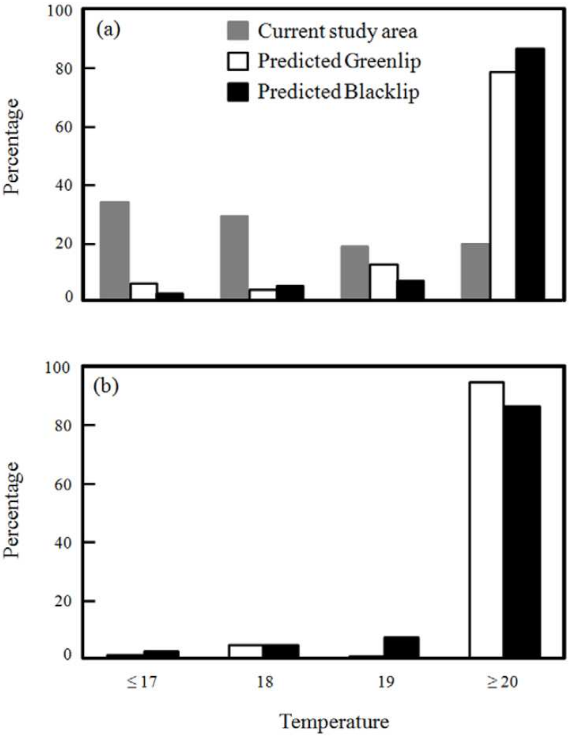


Figure 4. The percentage of South Australian waters at different mean-March temperatures. (a). Grey bars show current-day temperatures across the entire area, while the white and black bars show the percentage of the current distributions of greenlip (white bars) and blacklip abalone (black bars),at predicted temperatures for 2100 under the high-CO_2 Reference scenario and (b) the percentage of predicted abalone distributions (based on SDMs using August SST) which would be at different March temperatures in 2100. The current day temperatures (grey bars) are the same for both (a) and (b). Also note for both (a) and (b) the increase percentage of the area at or above 20°C, meaning that these predicted distributions may not be realised.
doi:10.1371/journal.pone.0046554.g004

speculate regarding possible elements of causation underpinning the model projections. The most likely mechanisms for the SDMs predicting population expansion under future warming would be increases in both reproductive potential of the adults and survival of recruits in winter. Both species of abalone used in this study show a linear relationship between the rate of gonad development and temperature between 12–18°C [43], leading to a greater reproductive output and development of larvae [44], promoting faster settlement and increased chance of survival. Concurrently, higher winter and spring temperatures improve survival in juveniles of many species of mollusc (e.g. scallops, [41]; abalone, [45], [46]).

It is unlikely, however, that the trend in global warming will be consistent among the seasons [47], and the same warming that may increase the reproductive output and recruitment of abalone in winter may also drive them above thermal tolerances in summer. This context dependence has been recognised in temperate oceans of the northern hemisphere; warming of the ocean off the Norwegian coast in spring increases growth and survival of juvenile cod, while warmer temperatures in summer increase metabolic costs and reduces growth [8]. Further, this relationship is likely to vary with latitude, as most species tend to be more susceptible to increasing temperatures in lower latitudes because they are closer to their physiological limits (e.g. [7].

Under current seasonal temperature ranges, the summer maximum temperature in the study region is below the thermal maxima of both species [26]. However, projections of March (warmer) sea surface temperatures suggest that the majority of the areas of predicted population expansion for both species would be above 20°C (Figure 4b). Water temperatures >20°C in laboratory experiments (greenlip, this study; blacklip, [38]) caused a 10-fold increase in juvenile mortality. While temperature close to the sea bed would be lower than that of the sea surface, potentially reducing mortality below that seen in experiments at 20°C, this is offset by mortality tending to be higher in the natural environment than experimental studies [48]. Additionally, mean March SST at the current distribution limits of greenlip abalone (19.2°C and 19.0°C) support the idea that increasing March water temperatures may counter the biological benefits of increasing winter temperatures. This interpretation is supported by the current densities of both species, which are low in areas with average March SST above 20°C (Figure 3).

Since abundance-type model projections are often used to inform fisheries managers of sustainable commercial catches, it is important that projection uncertainties are explored and, where possible, minimized. Our SDMs predict both an expansion into unoccupied habitats and an increase in the density of individuals within current fishing grounds, which could be interpreted as increased harvest in these locations or greater population densities at current harvest levels. In other abalone species years of higher temperature anomalies (increases) have meant greater catches because of greater recruitment [49]. However, because a large proportion of the populations of both species in South Australia are likely to experience March SST above 20°C in the future (Figure 4), we expect that any positive effect of warmer winters on abalone abundance would be nullified by a concomitant increase in March temperatures causing greater juvenile mortality. As such, the SDM projections may represent an overestimation of the potential expansion of abalone into new fishing grounds, with any realised range expansion being substantially less.

By downscaling multi-model-averaged climate forecasts to a fine resolution and generating annual projections, we improved forecasts of the influence of climate change on green and blacklip abalone range and abundance (i.e., through more robust forecasts of SST at local spatial- and short temporal-scales [50]). Recent laboratory research (greenlip, this study; blacklip, [38]), however, shows the importance of explicitly incorporating demographic processes (e.g. juvenile mortality and recruitment) into climate impact assessments. We suggest that predictions of the effect of future climate on abalone range dynamics would be strengthened by using a simulation framework that couples SDM forecasts to structured spatial population models (e.g. Figure 5). A caveat is that these sorts of models are data intensive, requiring a strong understanding of the population dynamics of the focal species (typically linked to long-term monitoring programmes, including

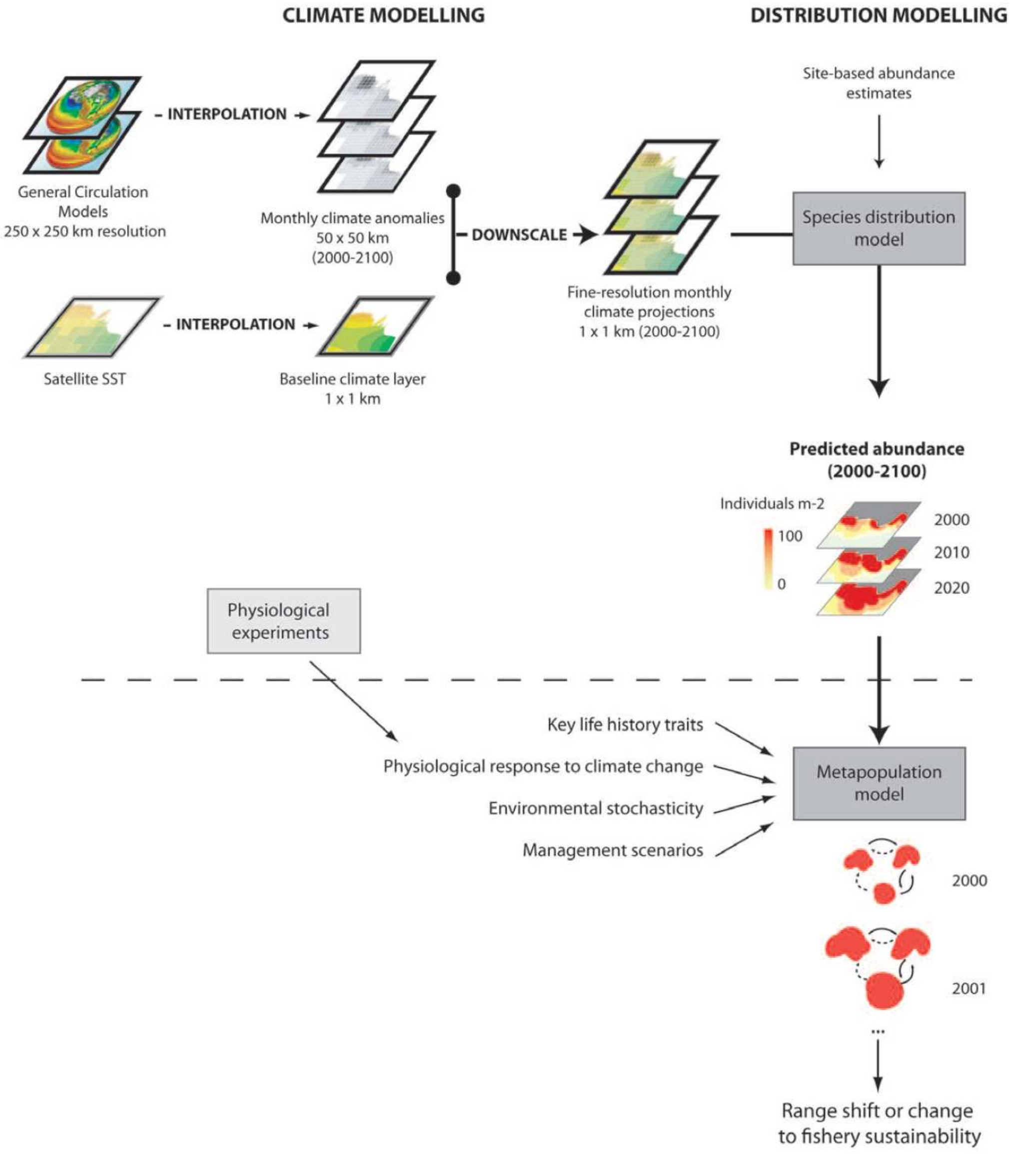


Figure 5. Schematic diagram of a hybrid-modelling approach to identify potential climate-driven changes in the distribution and abundance of commercially harvested species, and to test different fisheries management scenarios. The modelling steps that have been completed are located above the dotted line. The next step is to couple this approach with spatially explicit stochastic-demographic models, to capture some of the complexities and uncertainties underlying biological mechanisms driving species distribution and abundance patterns in response to forecasts of future climate change and harvest pressure.

doi:10.1371/journal.pone.0046554.g005

measurements of vital rates), as well as high-resolution distributional data [51] which needs to be ground-truthed to validate the accuracy of underlying distribution models. In this respect, commercially harvested species provide a good test case because the intensive study of their biology over many years generally makes this data available.

In this case study, the environmental conditions which best predicted the distribution and abundance of abalone in the SDMs under current conditions (August SST) may not accurately predict future populations if concomitant warming of summer temperatures reduces the realised distribution. Thus, robust forecasts of fisheries species need to incorporate metapopulation processes, such as spatially and temporally variant recruitment rates [52]. While other marine studies have found similar results on large scales [e.g. 30′×30′ grids; 6], we provide an important advance towards predictions on a scale that is relevant to management of fisheries (i.e. 1 km×1 km grids).

Supporting Information

Figure S1 (a) Forecasts of mean August sea surface temperature across the study area based on two climate change scenarios: a high CO_2 concentration stabilising scenario (WRE750) and a more conservative scenario, assuming heavy CO_2 mitigation (LEV1). The mean SST in the study area for 1994 (baseline for the forecasts) is also shown. Error bars show the standard deviation within the study area. Note that the WRE750 data points have been offset for clarity. (b) Forecast mean precent change in the abundance of *Haliotis rubra* (blacklip abalone) and *H. laevigata* (greenlip abalone) above a minimum threshold of 20 individuals/100 m^2, according to a high CO_2 concentration stabilization Reference scenario (WRE 750) and a heavy mitigation Policy option (LEV1).
(DOCX)

Acknowledgments

We thank S.A. Shepherd and S. Mayfield for discussion about abalone in South Australia and S.M. for comments on the manuscript. J. Tanner kindly supplied habitat data for the reef base layer.

Author Contributions

Conceived and designed the experiments: BDR SDC CM BWB OWB DAF. Performed the experiments: BDR CM OWB DAF. Analyzed the data: BDR CM DAF. Wrote the paper: BDR SDC CM BWB DAF.

References

1. Barry JP, Baxter CH, Sagarin RD, Gilman SE (1995) Climate-related, long-term faunal changes in a California rocky intertidal community. Science 267: 672–675.
2. Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. Science 308: 1912–1915.
3. Hawkins SJ, Moore PJ, Burrows MT, Poloczanska E, Mieszkowska N, et al. (2008) Complex interactions in a rapidly changing world: responses of rocky shore communities to recent climate change. Climate Research 37: 123–133.
4. Berkes F, Hughes TP, Steneck RS, Wilson JA, Bellwood DR, et al. (2006) Ecology - Globalization, roving bandits, and marine resources. Science 311: 1557–1558.
5. Sumaila UR, Cheung WWL, Lam VWY, Pauly D, Herrick S (2011) Climate change impacts on the biophysics and economics of world fisheries. Nature Climate Change 1: 449–456.
6. Cheung WWL, Lam VWY, Sarmiento JL, Kearney K, Watson R, et al. (2010) Large-scale redistribution of maximum fisheries catch potential in the global ocean under climate change. Global Change Biology 16: 24–35.
7. Planque B, Fredou T (1999) Temperature and the recruitment of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 56: 2069–2077.
8. Rogers LA, Stige LC, Olsen EM, Knutsen H, Chan KS, et al. (2011) Climate and population density drive changes in cod body size throughout a century on the Norwegian coast. Proceedings of the National Academy of Sciences of the United States of America 108: 1961–1966.
9. Hiddink JG, ter Hofstede R (2008) Climate induced increases in species richness of marine fishes. Global Change Biology 14: 453–460.
10. Sequeira A, Mellin C, Rowat D, Meekan MG, Bradshaw CJA (2011) Ocean-scale prediction of whale shark distribution. Diversity and Distributions in press.
11. Mellin C, Russell BD, Connell SD, Brook BW, Fordham DA (2012) Geographic range determinants of two commercially important marine molluscs. Diversity and Distributions 18: 133–146.
12. Beaumont LJ, Hughes L, Pitman AJ (2008) Why is the choice of future climate scenarios for species distribution modelling important? Ecology Letters 11: 1135–1146.
13. Buisson L, Thuiller W, Casajus N, Lek S, Grenouillet G (2010) Uncertainty in ensemble forecasting of species distribution. Global Change Biology 16: 1145–1157.
14. Robinson LM, Elith J, Hobday AJ, Pearson RG, Kendall BE, et al. (2011) Pushing the limits in marine species distribution modelling: lessons from the land present challenges and opportunities. Global Ecology and Biogeography. In press.
15. Watts MJ, Li Y, Russell BD, Mellin C, Connell SD, et al. (2011) A novel method for mapping reefs and subtidal rocky habitats using artificial neural networks. Ecological Modelling 222: 2606–2614.
16. Pearson RG, Thuiller W, Araujo MB, Martinez-Meyer E, Brotons L, et al. (2006) Model-based uncertainty in species range prediction. Journal of Biogeography 33: 1704–1711.
17. Buisson L, Grenouillet G (2009) Contrasted impacts of climate change on stream fish assemblages along an environmental gradient. Diversity and Distributions 15: 613–626.
18. Convertino M, Welle P, Munoz-Carpena R, Kiker GA, Chu-Agor ML, et al. (2012) Epistemic uncertainty in predicting shorebird biogeography affected by sea-level rise. Ecological Modelling 240: 1–15.
19. Araujo MB, New M (2007) Ensemble forecasting of species distributions. Trends in Ecology & Evolution 22: 42–47.
20. Fordham DA, Wigley TML, Brook BW (2011) Multi-model climate projections for biodiversity risk assessment. Ecological Applications doi:10.1890/11-0314.1.
21. Randall DA, Wood RA, Bony S, Colman R, Fichefet T, et al. (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M et al., editors. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press. pp. 589–662.
22. Wilby RL, Hay LE, Gutowski WJ, Arritt RW, Takle ES, et al. (2000) Hydrological responses to dynamically and statistically downscaled climate model output. Geophysical Research Letters 27: 1199–1202.
23. Wilby R, Charles S, Qorita E, Timbal B, Whetton PH, et al. (2004) Guidelines for use of climate scenarios developed from statistical downscaling methods. Intergovernmental Panel on Climate Change. Available: http://www.ipcc-data.org/guidelines/dgm_no2_v1_09_2004.pdf. Accessed 2012 Oct 30.
24. Mayfield S, Chick RC, Carlson IJ, Ward TM (2011) Invertebrate Dive Fisheries Can Be Sustainable: Forty Years of Production from a Greenlip Abalone Fishery off Southern Australia. Reviews in Fisheries Science 19: 216–230.
25. Mayfield S, Mundy C, Gorfine H, Hart A, Worthington D Fifty years of sustained production from Australia's abalone fisheries. Reviews in Fisheries Science. In press.
26. Gilroy A, Edwards SJ (1998) Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and greenlip abalone, *Haliotis laevigata* (Leach). Aquaculture Research 29: 481–485.
27. Shepherd SA (1973) Studies on Southern Australian Abalone (Genus-Haliotis). 1. Ecology of 5 Sympatric Species. Australian Journal of Marine and Freshwater Research 24: 217–257.
28. Smale DA, Wernberg T (2009) Satellite-derived SST data as a proxy for water temperature in nearshore benthic ecology. Marine Ecology-Progress Series 387: 27–37.
29. Hutchinson M (2006) Anusplin version 4.36. Canberra, Australia: Centre for Resource and Environmental Studies, The Australian National University.
30. IPCC (2007) Intergovernmental Panel on Climate Change: The AR4 Synthesis Report. Available: http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf. Accessed 2012 Oct 30.
31. Fordham DA, Wigley TML, Watts MJ, Brook BW (2011) Strengthening forecasts of climate change impacts with multi-model ensemble averaged projections using MAGICC/SCENGEN 5.3. Ecography doi:10.1111/j.1600-0587.2011.07398.x
32. Pierce DW, Barnett TP, Santer BD, Gleckler PJ (2009) Selecting global climate models for regional climate change studies. Proceedings of the National Academy of Sciences of the United States of America 106: 8441–8446.
33. Wigley TML, Clarke LE, Edmonds JA, Jacoby HD, Paltsev S, et al. (2009) Uncertainties in climate stabilization. Climatic Change 97: 85–121.

34. Wigley TML, Richels R, Edmonds JA (1996) Economic and environmental choices in the stabilization of atmospheric CO_2 concentrations. Nature 379: 240–243.
35. Hulme M, Raper SCB, Wigley TML (1995) An integrated framework to address climate-change (ESCAPE) and further developments of the global and regional climate modules (MAGICC). Energy Policy 23: 347–355.
36. Shepherd SA, Brown LD (1993) What is an abalone stock: implications for the role of refugia in conservation. Canadian Journal of Fisheries and Aquatic Science 50: 2001–2009.
37. Shepherd SA, Partington D (1995) Studies on southern Australian abalone (genus *Haliotis*). XVI. Recruitment, habitat and stock relations. Marine and Freshwater Research 46: 669–680.
38. Harris JO, Burke CM, Edwards SJ, Johns DR (2005) Effects of oxygen supersaturation and temperature on juvenile greenlip, *Haliotis laevigata* Donovan, and blacklip, *Haliotis rubra* Leach, abalone. Aquaculture Research 36: 1400–1407.
39. Russell BD, Harley CDG, Wernberg T, Mieszkowska N, Widdicombe S, et al. (2012) Predicting ecosystem shifts requires new approaches that integrate the effects of climate change across entire systems. Biology Letters 8: 164–166.
40. Hawkins SJ, Sugden HE, Mieszkowska N, Moore PJ, Poloczanska E, et al. (2009) Consequences of climate-driven biodiversity changes for ecosystem functioning of North European rocky shores. Marine Ecology-Progress Series 396: 245–259.
41. Shephard S, Beukers-Stewart B, Hiddink JG, Brand AR, Kaiser MJ (2010) Strengthening recruitment of exploited scallops *Pecten maximus* with ocean warming. Marine Biology 157: 91–97.
42. Wernberg T, Russell BD, Thomsen MS, Gurgel CFD, Bradshaw CJA, et al. (2011) Seaweeds in mass retreat from ocean warming. Current Biology 21: 1828–1832.
43. Grubert MA, Ritar AJ (2004) Temperature effects on the dynamics of gonad and oocyte development in captive wild-caught blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone. Invertebrate Reproduction & Development 45: 185–196.
44. Grubert MA, Ritar AJ (2004) The effect of temperature on the embryonic and larval development of blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone. Invertebrate Reproduction & Development 45: 197–203.
45. Searle T, Roberts RD, Lokman PM (2006) Effects of temperature on growth of juvenile blackfoot abalone, *Haliotis iris* Gmelin. Aquaculture Research 37: 1441–1449.
46. Takami H, Saido T, Endo T, Noro T, Musashi T, et al. (2008) Overwinter mortality of young-of-the-year Ezo abalone in relation to seawater temperature on the North Pacific coast of Japan. Marine Ecology-Progress Series 367: 203–212.
47. Kamenos NA (2010) North Atlantic summers have warmed more than winters since 1353, and the response of marine zooplankton. Proceedings of the National Academy of Sciences of the United States of America 107: 22442–22447.
48. Rossetto M, De Leo GA, Bevacqua D, Micheli F (2012) Allometric scaling of mortality rates with body mass in abalones. Oecologia 168: 989–996.
49. Shepherd SA, Turrubiates-Morales JR, Hall K (1998) Decline of the abalone fishery at La Natividad, Mexico: Overfishing or climate change? Journal of Shellfish Research 17: 839–846.
50. Stock CA, Alexander MA, Bond NA, Brander KM, Cheung WWL, et al. (2011) On the use of IPCC-class models to assess the impact of climate on Living Marine Resources. Progress in Oceanography 88: 1–27.
51. Fordham DA, Brook BW (2010) Why tropical island endemics are acutely susceptible to global change. Biodiversity and Conservation 19: 329–342.
52. Brook BW, Akcakaya HR, Keith DA, Mace GM, Pearson RG, et al. (2009) Integrating bioclimate with population models to improve forecasts of species extinctions under climate change. Biology Letters 5: 723–725.

APPENDIX B

PREAMBLE

Appendix B is a co-authored manuscript published in the journal *Sustainability*, with Laura J. Falkenberg as the senior author and myself, Sean D. Connell and Bayden D. Russell as co-authors. It is, therefore, written in plural throughout and uses the journal formatting. The manuscript can be cited as:

Falkenberg LJ, Burnell OW, Connell SD, Russell BD (2010) Sustainability in Near-shore Marine Systems: Promoting Natural Resilience. *Sustainability* **2**, 2593-2600.

Sustainability **2010**, *2*, 2593-2600; doi:10.3390/su2082593

OPEN ACCESS

sustainability

ISSN 2071-1050

www.mdpi.com/journal/sustainability

Communication

Sustainability in Near-shore Marine Systems: Promoting Natural Resilience

Laura J. Falkenberg, Owen W. Burnell, Sean D. Connell and Bayden D. Russell *

Southern Seas Ecology Laboratories, School of Earth & Environmental Sciences, University of Adelaide, DX650 418, South Australia, 5005, Australia; E-Mails: laura.falkenberg@adelaide.edu.au (L.J.F.); owen.burnell@adelaide.edu.au (O.W.B.); sean.connell@adelaide.edu.au (S.D.C.)

* Author to whom correspondence should be addressed; E-Mail: bayden.russell@adelaide.edu.au; Tel.: +61-8-8303-6587; Fax: +618-8303-6224.

Received: 1 July 2010; in revised form: 6 August 2010 / Accepted: 13 August 2010 / Published: 16 August 2010

Abstract: Accumulation of atmospheric CO_2 is increasing the temperature and concentration of CO_2 in near-shore marine systems. These changes are occurring concurrently with increasing alterations to local conditions, including nutrient pollution and exploitation of selected biota. While the body of evidence for the negative effects of climate change is rapidly increasing, there is still only limited recognition that it may combine with local stressors to accelerate degradation. By recognizing such synergies, however, it may be possible to actively manage and improve local conditions to ameliorate the effects of climate change in the medium-term (e.g., by reducing nutrient pollution or restoring populations of herbivores). Ultimately, however, the most effective way to increase the sustainability of near-shore marine systems into the future will be to decrease our reliance on carbon-based sources of energy to reduce the negative effects of climate change.

Keywords: ecosystem shift; climate change; carbon dioxide; algae; amelioration

---

1. Introduction

As the human population has grown, so too has the extent and rate at which the environment is modified [1]. While it has long been recognized that human activities directly alter local-scale processes, such as changes to primary productivity through nutrient-pollution or its consumption through fishing of foragers [2-4], it is only recently that global-scale influences have started to receive recognition [5,6]. We now recognize that human activity is driving unprecedented change to climate beyond that attributable to natural variation [7-9]. While the body of evidence for the negative effects of climate change on natural systems is rapidly increasing, there is still only limited understanding of how multiple stressors, such as increasing CO_2 and temperature, may combine to accelerate degradation [10-13]. More worrying, however, is that the impact of climate change will manifest at local scales, and as such, will also interact with local stressors that have been degrading the environment for decades, potentially accelerating change to natural systems.

2. Synergistic Effects and Accelerated Degradation

The oceans currently absorb ~30% of the CO_2 emitted into the atmosphere. It is now well established that the resulting reduction in pH (or ocean acidification) has negative effects on calcifying organisms [14-18]. An important recognition, however, is that in marine systems multiple climate parameters (e.g., temperature and pH) are inherently linked to one another via atmospheric CO_2 concentration [19,20], and may create complex outcomes which cannot be predicted through studying variables independently [13,21,22].

Most research into the effects of climate change on marine systems has considered acidification or temperature in isolation and there is a lack of knowledge about the extent of these interactions [23,24]. The few studies that have focussed on the interactions between these two parameters have demonstrated conflicting responses, showing possibilities of both synergistic negative effects on organisms [11,12] and others where the effects are not of a multiplicative nature [25]. Importantly, while organisms may show some resistance to independent stressors, their sensitivity is often altered under the concurrent application of multiple changes, resulting in effects of a larger magnitude than anticipated from the study of independent stressors [11-13,22]. Further, there is recent evidence that combinations of climate factors, such as elevated CO_2 and temperature, may have synergistic positive effects on some non-calcareous algae which facilitate ecosystem shifts, thus producing a negative ecological outcome [10].

Of even greater concern is how changing climates will combine with local environmental impacts. Numerous stressors, particularly nutrient pollution and exploitation of selected biota, have resulted from human activities and produced environmental conditions distinct from those experienced at any other time in history [26]. As the effects of climate change will manifest at similar scales to these local stressors, it is likely that they will combine to alter conditions that maintain system function through amplifying feedbacks, compounding effects and synergies [27,28]. For example, in temperate marine waters canopies of algae form forests analogous to tropical rainforests. These canopies are a foundation for marine systems, providing structure that enables stabilization of physical environments, survival of associated species and economic benefit for human societies [29,30]. On many coasts of the world, however, these canopies are being replaced by small filamentous algal turfs [31-33], causing massive loss of biodiversity and ecological function. This current decline is being driven by elevated nutrients from land-derived sources [34-36]. Furthermore, it has only recently been recognized that increasing [CO_2] may increase the productivity of non-calcareous algae, particularly the opportunistic species which facilitate system shifts, suggesting that the decline of algal canopies will be accelerated into the future as local nutrient pollution interacts with increasing [CO_2] to increase the abundance of turf-forming algal species which inhibit the recovery of algal forests [10,37].

Nutrient-driven loss of the dominant habitat forming taxa is also well documented in tropical ecosystems, where macroalgae overgrow and smother reef-building corals [38]. As with kelp dominated systems, this habitat loss is accelerated under a combination of perturbations such as increased nutrients and harvesting of herbivores [39,40]. With the increasing body of literature demonstrating the negative effects of increasing [CO_2] on corals, it is likely that the interaction between increasing [CO_2] and nutrients will cause a synergistic negative effect on coral reefs from two directions, the negative effect on corals and the positive effect on non-calcareous macroalgae. Therefore, while the study of climate stressors on individual organisms provides insights into species level responses and adaptation, an understanding of local-to-global scale interactions between multiple stressors on communities is required to identify mechanisms of increasing resilience of systems into the future.

3. Promoting Natural System Resilience

Current projections of climate driven change to oceanic pH are based primarily upon ocean physics, with the biological components of these predictions currently lacking in sophistication [20]. Subsequently, the current understanding of the potential for natural biological interactions to provide both regional and global resilience to ocean acidification remains limited. Increasing CO_2 and the associated reduction in pH is influenced by the photosynthesis and respiration of marine organisms [18,41-43]. For example, diurnal pH fluctuations in coastal sub-tidal zones caused by photosynthesis and respiration can be in the range of 0.2–0.3 units [44]. However, it is the very cause of this variation that may ameliorate some of the negative effects of climate change; it has been predicted that future CO_2 may enhance the productivity of marine plants and potentially some non-calcifying algae [24,45-47]. Implementing conservation measures to protect large habitat-forming algae and seagrasses (c.f. algal turfs which reduce habitat complexity) could allow this adaptive capacity to moderate ocean acidification and buffer against some of the negative effects associated with increased [CO_2]. While this buffering effect is likely to provide global benefits via increased carbon sequestration, additional local actions to maintain biomass of photosynthetic organisms in systems (e.g., kelp forests or seagrass meadows) may help decrease the effects of elevated CO_2 within these localities.

Another management tool to enhance the resilience of systems to climate change would be to remove the potential for synergies between climate change and local conditions that have been altered by human activities. The presence of herbivores can moderate the effect of local pollution (*i.e.*, elevated nutrients) through consumption of bloom-forming macroalgae that drive ecosystem shifts at the expense of complex habitat-formers [40,48-51]. There is also evidence that the presence of herbivores may increase the resilience of systems to climate-related stressors, as seen with the recovery of coral reefs from bleaching events [52]. Therefore, protection of herbivores from harvesting, such as through the establishment of Marine Protected Areas, could increase the resilience of natural systems to both local and global stressors.

This moderating effect of herbivory can, however, be overwhelmed by longer-term eutrophication events [48,50,53]. Further, it can be reasonably predicted that ocean acidification may disrupt the feeding biology of some herbivores [54,55], restricting their ability to effectively control the nutrient or climate-enhanced growth of algae. Therefore, reducing the potential for synergies between local conditions and climate change may only be possible by reducing the nutrient load entering marine waters from terrestrial sources [37]. Recognition of the need to increase resilience in natural systems has lead to more proactive management of local stressors in some regions. In South Australia, local government now recognizes the global-local connections of future change, and has implemented long-term policy to upgrade wastewater treatment plants and to recapture storm-water to produce recycled water for residential and industrial use. While concerned mainly with the provision of freshwater for human use, this policy also reduces the nutrient rich discharge that has primarily contributed to phase shifts on metropolitan reefs from kelp to turf-dominated [32,34].

While recent experimental work indicates that the reduction of pollution or supporting populations of herbivores may increase resilience of near-shore marine systems [37,52], it is likely that the regional biological context will be an important consideration. In regions which have naturally eutrophic waters (e.g., upwelling zones) and strong herbivory it may be more appropriate to use Marine Protected Areas to support natural populations of herbivores. Conversely, elevated nutrients can have disproportionately large effects in regions which have oligotrophic waters and weak herbivory [56] so restricting terrestrial based sources of nutrient pollution may be more affective in maintaining system resilience under future climates. Further, regional assessments of the potential impact of climate change show that it's effects will vary geographically and that actions to ameliorate climate change will need to differ among regions [57,58]. Therefore, to maximize the effectiveness of actions to increase the resilience of natural systems to climate change, it will be necessary to identify the regional drivers of systems (e.g., nutrients *vs.* herbivory) and the likely drivers of future change (e.g., temperature *vs.* increasing [CO_2])

What we are yet to identify is whether local mitigation measures are likely to be more effective when implemented before forecasted climates arrive [59]. Recruitment of habitat-forming species is key to ecosystem resilience [60], and both local and global perturbations tend to promote species which inhibit recruitment of habitat-forming species [31,34,40,52]. Therefore, it is likely that locations which have already undergone loss of habitats driven by anthropogenic perturbations will be less likely to recover to their "natural" state if local amelioration actions are implemented after global stressors have passed a threshold where recruitment of habitat-forming species is inhibited.

4. Conclusions

The anticipated synergies between local conditions and increasing temperature and CO_2 have clear implications for the function of marine systems globally. In the medium-term, it may be possible to promote the resilience of natural systems by either reducing local stressors or supporting populations of organisms which will alter the effects of these stressors. Management which limits nutrient addition (*i.e.*, stops waste water outfall) or protects herbivores from fishing (e.g., Marine Protected Areas) will restrict the growth of bloom-forming algae and possibly prevent system shifts to less desirable states, especially if such strategies are implemented in unison. Such management actions cannot, however, ameliorate the effect of negative synergies between climate factors (e.g., CO_2 and temperature) and ultimately the only way to mitigate the effects of climate change is to reduce reliance on carbon-based sources of fuel [61]. This understanding is particularly important as managing global-scale changes is inherently difficult because their sheer magnitude requires an international effort to implement policy change and because their effects are so long-lasting, if not permanent.

Acknowledgements

Financial support for this research was provided by an ARC Linkage Grant for BDR and SDC and an ARC Future Fellowship for SDC.

References

1. Vitousek, P.M.; Mooney, H.A.; Lubchenco, J.; Melillo, J.M. Human domination of earth's ecosystems. *Science* **1997**, *277*, 494-499.
2. Hatcher, B.G.; Larkum, A.W.D. An experimental analysis of factors controlling the standing crop of the epilithic algal community on a coral reef. *J. Exp. Mar. Biol. Ecol.* **1983**, *69*, 61-84.
3. Jackson, J.B.C. What was natural in the coastal oceans? *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5411-5418.
4. McElroy, M.B.; Wofsy, S.C.; Yung, Y.L. The nitrogen cycle: Perturbations due to man and their impact on atmospheric N_2O and O_3. *Phil. Trans. Roy. Soc. B-Biol. Sci.* **1977**, *277*, 159-181.
5. Gillett, N.P.; Zwiers, F.W.; Weaver, A.J.; Stott, P.A. Detection of human influence on sea-level pressure. *Nature* **2003**, *422*, 292-294.
6. Karl, T.R.; Trenberth, K.E. Modern global climate change. *Science* **2003**, *302*, 1719-1723.
7. Stott, P.A.; Tett, S.F.B.; Jones, G.S.; Allen, M.R.; Ingram, W.J.; Mitchell, J.F.B. Attribution of twentieth century temperature change to natural and anthropogenic causes. *Clim. Dynam.* **2001**, *17*, 1-21.
8. Turley, C. Impacts of changing ocean chemistry in a high-CO_2 world. *Mineral. Mag.* **2008**, *72*, 359-362.
9. Zachos, J.C.; Rohl, U.; Schellenberg, S.A.; Sluijs, A.; Hodell, D.A.; Kelly, D.C.; Thomas, E.; Nicolo, M.; Raffi, I.; Lourens, L.J.; McCarren, H.; Kroon, D. Rapid acidification of the ocean during the Paleocene-Eocene thermal maximum. *Science* **2005**, *308*, 1611-1615.
10. Connell, S.D.; Russell, B.D. The direct effects of increasing CO_2 and temperature on non-calcifying organisms: Increasing the potential for phase shifts in kelp forests. *Proc. Roy. Soc. B-Biol. Sci.* **2010**, *277*, 1409-1415.
11. Findlay, H.S.; Kendall, M.A.; Spicer, J.I.; Widdicombe, S. Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. *Mar. Biol.* **2010**, *157*, 725-735.
12. Martin, S.; Gattuso, J.P. Response of Mediterranean coralline algae to ocean acidification and elevated temperature. *Glob. Change Biol.* **2009**, *15*, 2089-2100.
13. Przeslawski, R.; Davis, A.R.; Benkendorff, K. Synergistic effects associated with climate change and the development of rocky shore molluscs. *Glob. Change Biol.* **2005**, *11*, 515-522.
14. Feely, R.A.; Sabine, C.L.; Lee, K.; Berelson, W.; Kleypas, J.; Fabry, V.J.; Millero, F.J. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* **2004**, *305*, 362-366.
15. Gattuso, J.P.; Buddemeier, R.W. Ocean biogeochemistry—Calcification and CO_2. *Nature* **2000**,

16. Gazeau, F.; Quiblier, C.; Jansen, J.M.; Gattuso, J.P.; Middelburg, J.J.; Heip, C.H.R. Impact of elevated CO_2 on shellfish calcification. *Geophys. Res. Lett.* **2007**, *34*, doi:10.1029/2006GL028554
17. Kleypas, J.A.; Buddemeier, R.W.; Archer, D.; Gattuso, J.P.; Langdon, C.; Opdyke, B.N. Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **1999**, *284*, 118-120.
18. Leclercq, N.; Gattuso, J.P.; Jaubert, J. CO_2 partial pressure controls the calcification rate of a coral community. *Glob. Change Biol.* **2000**, *6*, 329-334.
19. Hoegh-Guldberg, O.; Mumby, P.J.; Hooten, A.J.; Steneck, R.S.; Greenfield, P.; Gomez, E.; Harvell, C.D.; Sale, P.F.; Edwards, A.J.; Caldeira, K.; Knowlton, N.; Eakin, C.M.; Iglesias-Prieto, R.; Muthiga, N.; Bradbury, R.H.; Dubi, A.; Hatziolos, M.E. Coral reefs under rapid climate change and ocean acidification. *Science* **2007**, *318*, 1737-1742.
20. Kleypas, J.; Feely, R.A.; Fabry, V.J.; Langdon, C.; Sabine, C.L.; Robbins, L.L. Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research. In *Proceedings of the Workshop on the Impacts of Increasing Atmospheric CO_2 on Coral Reefs and Other Marine Calcifiers*, St Petersburg, FL, USA, 18–20 April 2005.
21. Bezemer, T.M.; Knight, K.J. Unpredictable responses of garden snail (*Helix aspersa*) populations to climate change. *Acta Oecol.* **2001**, *22*, 201-208.
22. Reynaud, S.; Leclercq, N.; Romaine-Lioud, S.; Ferrier-Pages, C.; Jaubert, J.; Gattuso, J.P. Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Glob. Change Biol.* **2003**, *9*, 1660-1668.
23. Fine, M.; Franklin, L.A. Climate change in marine ecosystems. In *Marine Ecology*; Connell, S.D., Gillanders, B.M., Eds.; Oxford University Press: Melbourne, VIC, Australia, 2007; pp. 595-612.
24. Guinotte, J.M.; Fabry, V.J. Ocean acidification and its potential effects on marine ecosystems. *Ann. N. Y. Acad. Sci.* **2008**, *1134*, 320-342.
25. Langdon, C.; Atkinson, M.J. Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. *J. Geophys. Res.* **2005**, *110*, doi:10.1029/2004JC002576.
26. Lotze, H.K.; Worm, B. Complex interactions of climatic and ecological controls on macroalgal recruitment. *Limnol. Oceanogr.* **2002**, *47*, 1734-1741.
27. Nystrom, M.; Folke, C.; Moberg, F. Coral reef disturbance and resilience in a human-dominated environment. *Trends Ecol. Evol.* **2000**, *15*, 413-417.
28. Paine, R.T.; Tegner, M.J.; Johnson, E.A. Compounded perturbations yield ecological surprises. *Ecosystems* **1998**, *1*, 535-545.
29. Steneck, R.S.; Graham, M.H.; Bourque, B.J.; Corbett, D.; Erlandson, J.M.; Estes, J.A.; Tegner, M.J. Kelp forest ecosystems: Biodiversity, stability, resilience and future. *Environ. Conserv.* **2002**, *29*, 436-459.
30. Tegner, M.J.; Dayton, P.K. Ecosystem effects of fishing in kelp forest communities. *ICES J. Mar. Sci.* **2000**, *57*, 579-589.
31. Airoldi, L.; Beck, M.W. Loss, status and trends for coastal marine habitats of Europe. *Oceanogr. Mar. Biol. Ann. Rev.* **2007**, *45*, 345-405.

32. Connell, S.D.; Russell, B.D.; Turner, D.J.; Shepherd, S.A.; Kildea, T.; Miller, D.; Airoldi, L.; Cheshire, A. Recovering a lost baseline: Missing kelp forests from a metropolitan coast. *Mar. Ecol. Prog. Ser.* **2008**, *360*, 63-72.
33. Eriksson, B.K.; Johansson, G.; Snoeijs, P. Long-term changes in the macroalgal vegetation of the inner Gullmar Fjord, Swedish Skagerrak coast. *J. Phycol.* **2002**, *38*, 284-296.
34. Gorman, D.; Russell, B.D.; Connell, S.D. Land-to-sea connectivity: Linking human-derived terrestrial subsidies to subtidal habitat-change on open rocky coasts. *Ecol. Appl.* **2009**, *19*, 1114-1126.
35. Kraufvelin, P.; Christie, H.; Olsen, M. Littoral macrofauna (secondary) responses to experimental nutrient addition to rocky shore mesocosms and a coastal lagoon. *Hydrobiologia* **2002**, *484*, 149-166.
36. Pedersen, M.; Snoeijs, P. Patterns of macroalgal diversity, community composition and long-term changes along the Swedish west coast. *Hydrobiologia* **2001**, *459*, 83-102.
37. Russell, B.D.; Thompson, J.I.; Falkenberg, L.J.; Connell, S.D. Synergistic effects of climate change and local stressors: CO_2 and nutrient driven change in subtidal rocky habitats. *Glob. Change Biol.* **2009**, *15*, 2153-2162.
38. Fabricius, K.E. Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. *Mar. Poll. Bull.* **2005**, *50*, 125-146.
39. Bellwood, D.R.; Hughes, T.P.; Folke, C.; Nystrom, M. Confronting the coral reef crisis. *Nature* **2004**, *429*, 827-833.
40. Smith, J.E.; Hunter, C.L.; Smith, C.M. The effects of top-down *versus* bottom-up control on benthic coral reef community structure. *Oecologia* **2010**, *163*, 497-507.
41. Gao, K.; Aruga, Y.; Asada, K.; Ishihara, T.; Akano, T.; Kiyohara, M. Enhanced growth of the red alga porphyra-yezoensis ueda in high CO_2 concentrations. *J. Appl. Phycol.* **1991**, *3*, 355-362.
42. Gao, K.; McKinley, K.R. Use of macroalgae for marine biomass production and CO_2 remediation: A review. *J. Appl. Phycol.* **1994**, *6*, 45-60.
43. Shirayama, Y.; Thornton, H. Effect of increased atmospheric CO_2 on shallow water marine benthos. *J. Geophys. Res.* **2005**, *110*, doi:10.1029/2004JC002618.
44. Yates, K.K.; Dufore, C.; Smiley, N.; Jackson, C.; Halley, R.B. Diurnal variation of oxygen and carbonate system parameters in Tampa Bay and Florida Bay. *Mar. Chem.* **2007**, *104*, 110-124.
45. Beardall, J.; Beer, S.; Raven, J.A. Biodiversity of marine plants in an era of climate change: Some predictions based on physiological performance. *Bot. Mar.* **1998**, *41*, 113-123.
46. Short, F.T.; Neckles, H.A. The effects of global climate change on seagrasses. *Aquat. Bot.* **1999**, *63*, 169-196.
47. Duarte, C.M. The future of seagrass meadows. *Environ. Conserv.* **2002**, *29*, 192-206.
48. Lotze, H.K.; Worm, B.; Sommer, U. Strong bottom-up and top-down control of early life stages of macroalgae. *Limnol. Oceanogr.* **2001**, *46*, 749-757.
49. Miller, M.W.; Hay, M.E. Coral-seaweed-grazer-nutrient interactions on temperate reefs. *Ecol. Monogr.* **1996**, *66*, 323-344.
50. Russell, B.D.; Connell, S.D. Response of grazers to sudden nutrient pulses in oligotrophic *versus* eutrophic conditions. *Mar. Ecol. Prog. Ser.* **2007**, *349*, 73-80.

51. Worm, B.; Lotze, H.K. Effects of eutrophication, grazing, and algal blooms on rocky shores. *Limnol. Oceanogr.* **2006**, *51*, 569-579.
52. Hughes, T.P.; Rodrigues, M.J.; Bellwood, D.R.; Ceccarelli, D.; Hoegh-Guldberg, O.; McCook, L.; Moltschaniwskyj, N.; Pratchett, M.S.; Steneck, R.S.; Willis, B. Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr. Biol.* **2007**, *17*, 360-365.
53. Eriksson, B.K.; Rubach, A.; Hillebrand, H. Dominance by a canopy forming seaweed modifies resource and consumer control of bloom-forming macroalgae. *Oikos* **2007**, *116*, 1211-1219.
54. Bibby, R.; Cleall-Harding, P.; Rundle, S.; Widdicombe, S.; Spicer, J. Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biol. Lett.* **2007**, *3*, 699-701.
55. Marchant, H.K.; Calosi, P.; Spicer, J.I. Short-term exposure to hypercapnia does not compromise feeding, acid-base balance or respiration of *Patella vulgata* but surprisingly is accompanied by radula damage. *J. Mar. Biol. Assn. UK* **2010**, *June*, doi:10.1017/S0025315410000457.
56. Russell, B.D.; Elsdon, T.E.; Gillanders, B.M.; Connell, S.D. Nutrients increase epiphyte loads: Broad scale observations and an experimental assessment. *Mar. Biol.* **2005**, *147*, 551-558.
57. Poloczanska, E.S.; Hobday, A.J.; Richardson, A.J. *Report Card of Marine Climate Change for Australia; Detailed Scientific Assessment*; NCCARF Publication: Southport, QLD, Australia, 2009.
58. Baxter, J.M.; Buckley, P.J.; Wallace, C.J. *Marine Climate Change Impacts Annual Report Card 2010–2011*; MCCIP: Lowestoft, UK, 2010; p. 12.
59. Mignone, B.K.; Socolow, R.H.; Sarmiento, J.L.; Oppenheimer, M. Atmospheric stabilization and the timing of carbon mitigation. *Clim. Change* **2008**, *88*, 251-265.
60. Pickett, S.T.A.; White, P.S. *The Ecology of Natural Disturbance and Patch Dynamics*; Academic Press: San Diego, CA, USA, 1985; p. 472.
61. Russell, B.D.; Connell, S.D. Honing the geoengineering strategy. *Science* **2010**, *327*, 144-145.

APPENDIX C

PERMISSION TO REPRODUCE PUBLISHED MATERIAL

CHAPTER TWO

Subject: Thank you for your RightsLink / Elsevier transaction

From: Copyright Clearance Center rightslink@marketing.copyright.com

To: Owen Burnell owen.burnell@adelaide.edu.au

Sent: Thurs 5/12/2013 10:51 AM

Dear Mr. Owen Burnell,

Thank you for placing your order through Copyright Clearance Center's RightsLink service. Elsevier has partnered with RightsLink to license its content. This notice is a confirmation that your order was successful.

Your order details and publisher terms and conditions are available by clicking the link below:http://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=8f6e8b10-211e-4599-8b7f-df25b69643d3

Order Details

Licensee: Owen W Burnell

License Date: Dec 4, 2013

License Number: 3282170634891

Publication: Journal of Experimental Marine Biology and Ecology

Title: Asymmetric patterns of recovery in two habitat forming seagrass species following simulated overgrazing by urchins

Type Of Use: reuse in a thesis/dissertation

Total: 0.00 USD

CHAPTER SIX

Subject: Re: Permission to include article in doctoral dissertation

From: Ian Stewart ian.stewart@int-res.com

To: Owen Burnell owen.burnell@adelaide.edu.au

Sent: Tue 3/12/2013 10:03 PM

Dear Owen

This permission is granted on the basis that by "original manuscript in PDF format" you mean an author-generated manuscript of the version of the text submitted for peer review (this is the normal definition of 'original manuscript') and you do not mean a facsimile of the final PDF version issued by the publisher. If you did mean the latter, then let me know, as we have different conditions of use in that case.

You are hereby licensed to include an author-generated version of the paper Burnell et al. (2013) Mar Ecol Prog Ser 485:37–46 in your doctoral thesis, under the following conditions: (1) the original source of publication is (as you indicate already) fully referenced, and (2) the text is integrated and bound into the body of the thesis, that is, it is not to be issued as a separate electronic file or printed fascicule.

With best wishes

Ian Stewart

Permissions Manager

Inter-Research