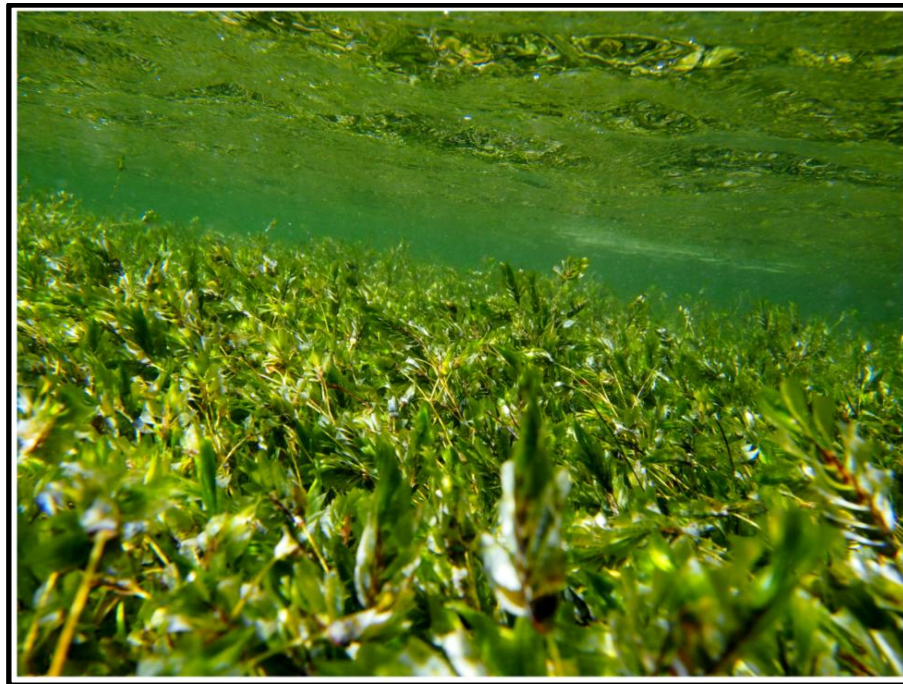


STABILITY AND RECOVERY OF COASTAL ECOSYSTEMS TO LOCAL AND GLOBAL RESOURCE ENHANCEMENT



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Presented for the degree of Doctor of Philosophy

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March 2015



Cover Image: *Amphibolis Antarctica* meadow, Lady Bay, Fleurieu Peninsula, South
Australia. Photo credit: Sam Langholz.

DECLARATION

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ABSTRACT

Human modification of the abiotic environment can cause profound change to biological communities, yet many ecosystems that face intensive anthropogenic pressure can persist without undergoing major change. To understand the inherent stability of many systems facing human driven environmental change, we need an account of the mechanisms that allow ecosystems to withstand such change. Whilst it is well known that resource enhancement favours the growth of subordinate or weedy species over habitat-forming perennials, less is known about the inherent ability of herbivores to counter this increased growth. Throughout this thesis, I assessed whether such resource enhancement can encourage herbivores to compensate for the additional productivity of opportunistic algae that can cause the decline of seagrass habitats and the displacement of kelp forests.

Human activities can modify resource availability on local (e.g. nutrients) through to global scales (e.g. carbon dioxide). Anthropogenically derived nutrients can increase local resource availability in coastal zones, stimulating the overgrowth of seagrass by epiphytic algae, leading to the decline of seagrass habitats. By experimentally manipulating nutrient concentrations and herbivore abundance, I showed that herbivores are capable of reducing the effects of local nutrient addition in a seagrass ecosystem by consuming the increased production of epiphytic algae. Importantly, this work showed that although nutrient addition increased food availability, herbivore abundance did not increase in the grazed treatments, suggesting that the greater consumption of algae was due to an increase in *per capita* grazing and not grazer abundance.

Concurrent with the local enrichment of nutrients is the global accumulation of carbon dioxide (CO₂), which can act as a resource for photosynthetic organisms that are carbon limited. Indeed, I found that experimental enrichment of both nutrients and CO₂ proved to have a greater influence on the expansion of algal turf than the provision of either resource alone, but only in the absence of herbivores. Elevated nutrients and CO₂ increased herbivore consumption, which was proportional to an increase in herbivore metabolism.

Where resource enhancement is ongoing, however, the influence of such change can overwhelm countering forces (such as herbivory) to the extent that the production of opportunistic algae escapes regulation and perennial species may be lost (e.g. seagrass decline on urbanised coasts). As the global loss of seagrass continues, efforts are made to restore lost meadows with the principle aim of restoring ecosystem function (e.g. faunal recolonisation). I used experimental restoration plots of known ages (1, 3 and 5 years) to test the rate of recovery of epifaunal composition and seagrass structure to that in an adjacent natural seagrass meadow. I found that whilst seagrass structure and epifaunal composition took three years to become similar to the natural meadow, epifaunal richness and abundance took one year. These results have suggested that recovering habitats may support similar ecosystem function as natural habitats before the full recovery of seagrass meadows themselves.

Restoration success is generally measured as the recovery of aboveground seagrass structure; which ignores the important role of the belowground element of a seagrass meadow that may not recover at the same rate. After quantifying the recovery of the aboveground components of the seagrass restoration plots, I determined the recovery of belowground components. I quantified elements of infaunal composition (e.g. richness and

abundance) and belowground seagrass structure of the same experimental restoration plots. I found that infaunal abundance and richness was similar to the natural seagrass after two years, the recovery of belowground biomass, however, took four to six years. These results have confirmed the suggestion that recovering habitats can support similar ecosystem function to natural habitats before the full recovery of seagrass *per se*.

In summary, compensatory mechanisms may play a pivotal role in enabling ecosystems to resist change and remain stable during periods of resource enhancement. Indeed, I demonstrated that compensatory responses were directly proportional to the magnitude of disturbance (or multiple disturbances) by resource enhancement. When ecosystems are lost as a result of resource enhancement, however, habitat restoration can be applied to re-establish ecosystem structure and function. I showed that recovering habitats may not need to be structurally similar to natural habitats in order to support similar ecosystem function. Recovering habitats may therefore have greater economic and social value than otherwise might have been expected.

ACKNOWLEDGEMENTS

First and foremost, I thank my supervisors Sean Connell, Bayden Russell and Jason Tanner for providing me with their time, guidance, expertise, and support and for helping me establish the basis of my scientific career. I thank Sean Connell for his enthusiasm, sharing his endless ecological knowledge, and being there for all my highs and lows. I thank Bayden Russell for his attention to detail, for providing support even when in another country and for always challenging me to think outside the square. I thank Jason Tanner for inspiring my interest in coastal ecosystems, particularly seagrass, for always being available to offer expertise, for being incredibly efficient when reviewing my work and for allowing me to use the facilities at, and to work on projects with SARDI Aquatic Sciences.

Thanks to members of the Southern Seas Ecology Laboratories and the Marine Ecosystem Program who have shared this time with me as well as sharing laboratory space and field sites. In particular, I thank Nicole Mertens for being my ‘rock’ throughout my undergraduate and honours degrees and more importantly my PhD, for providing encouragement, assistance, a good laugh and a shoulder to cry on when needed. Thanks to my colleagues and good friends Gretchen Grammar, Skye Woodcock, Laura Falkenberg and Jennie Pistevos for being so understanding, and for giving me their time and advice no matter how busy they were. Thanks to Ben Florance and Sam Langholz for their assistance in the field and without whom chapter 2 would not have been possible; you will forever be known as ‘my honours boys’.

Sincerest thanks to my incredible parents, Noelene and Alan McSkimming who have supported me both emotionally and financially through the most challenging time of my life thus far. For encouraging me to always go after what I want in life and for helping me to believe in myself. A big thank you also goes to my sister Elissa and brother-in-law Brenton for their endless support, and to my nephew Cooper and niece Grace for providing fun distractions and for sharing my love of the ocean. Thanks to my nanna Wanda and my grandma Peggy who both unfortunately passed away during the early stages of my PhD, but who have always inspired me to become an independent and strong person, I know they would both be proud.

Finally, thanks to my amazing friends, particularly Ben Feo, Joanne Rocca, Jacinta Siddall, Kerry-Anne Palzewski, Ashleagh Gillert, Katrina Redford-Brown and Jennifer Young for keeping me grounded, celebrating the highs and listening to me whinge about the lows and always pretending to be interested in my work even though the majority of the time they had no idea what I was talking about.

My sincerest thank you to you all, for without you this thesis would not have been possible.

CHAPTER ACKNOWLEDGEMENTS

Chapter 2

We thank B. Florance and S. Langholz for their valuable assistance in the field and laboratory. Thanks to L. Falkenberg for providing laboratory assistance. Financial support for this research was provided by an ARC grant to S.D. Connell and B.D. Russell, including a Future Fellowship to S.D. Connell, an APA scholarship to C.M and partly funded by the Nature Foundation of South Australia Inc. and the Dr Paris Goodsell Marine Ecology Research Grant.

Chapter 3

This manuscript was invited by the Tenth International Temperate Reefs Symposium, Perth. We thank K. Wiltshire, E. Brock, N. Mertens and L. Falkenberg for their laboratory assistance. Our research was funded by, ARC Grants to S.D.C and B.D.R, an APA scholarship to C.M and SARDI Aquatic Sciences.

Chapter 4

We thank I. Moody, A. Dobrovolskis and L. Mantilla for their field and laboratory assistance. D. Fotheringham, S. Murray-Jones (South Australian Department for Environment, Water and Natural Resources), T. Flaherty (Adelaide and Mount Lofty Ranges Natural Resources Management Board), and M. Fernandes (SA Water) provided strong support for this work, and assisted with obtaining funds from their organizations. Part of this work was conducted under Australian Research Council (ARC) Linkage Projects to J. S. Quinton (Flinders University), S. Connell and B. Russell, including a Future Fellowship to S. Connell.

Chapter 5

We thank K. Wiltshire, A. Dobrovolskis, S. Hoare and L. Mantilla for their field and laboratory assistance. D. Fotheringham, S. Murray-Jones (South Australian Department for Environment, Water and Natural Resources), T. Flaherty (Adelaide and Mount Lofty Ranges Natural Resources Management Board), and M. Fernandes (SA Water) provided strong support for this work, and assisted with obtaining funds from their organizations. Part of this work was conducted under Australian Research Council (ARC) Linkage Projects to J. S. Quinton (Flinders University), S. Connell and B. Russell, including a Future Fellowship to S. Connell.

CHAPTER 1

CHAPTER 1

GENERAL INTRODUCTION

Human activities continue to challenge the capability of ecosystems to absorb disturbances, and as anthropogenic pressures increase, an ecosystem's ability to resist change may decrease (Jackson *et al.* 2001; Folke *et al.* 2004). When resilience, being the degree of disturbance an ecosystem can withstand without undergoing a shift to a contrasting state (Holling 1973) is reduced, ecosystems may change from a desired state to an alternate or less desirable state (Scheffer *et al.* 2001; Folke *et al.* 2004). Multiple pressures can further reduce stability, making ecosystems more vulnerable to change (Paine *et al.* 1998).

Although shifts in ecosystem state (termed phase shifts) can be gradual, many shifts occur suddenly in response to a sudden large disturbance, which surpasses a particular threshold (Scheffer *et al.* 2001; Scheffer & Carpenter 2003; Folke *et al.* 2004). For example, nutrient loading in freshwater lakes shifts the ecosystem state from clear water dominated by submerged aquatic vegetation to turbid water dominated by phytoplankton (Scheffer *et al.* 1993). Nevertheless, many ecosystems that face substantial human pressure remain stable without shifting to a less desirable state (Ghedini *et al.* 2015). Developing an understanding of the compensatory responses that enable ecosystems to resist major change when faced with intensive anthropogenic pressure is, therefore, essential for improving our current knowledge on ecosystem stability.

Throughout this introduction, I discuss the enhancement of both local (e.g. nutrients) and global (e.g. CO₂ emissions) resource availability as a consequence of human activities. I then examine the liberation of resources to the marine environment, and how such resource

enhancement can shift the competitive balance between slow-growing primary producers (e.g. seagrass and kelp) and faster growing opportunistic algae (e.g. epiphytes and algal turf). I follow by exploring the circumstances that may allow herbivores to counter the effects of resource enhancement by increasing their consumption rates, potentially increasing ecosystem stability. When anthropogenic pressures degrade ecosystems to the point of habitat loss, habitat restoration can be implemented to restore the lost habitat (Elliott *et al.* 2007). I conclude by discussing the goals and success of habitat restoration, particularly seagrass restoration, and introduce the seagrass restoration site I surveyed along the coast of Adelaide, South Australia.

1.1 HUMAN ACTIVITIES ENHANCE RESOURCE AVAILABILITY

1.1.1 LOCAL ENHANCEMENT

Primary productivity is principally controlled by the availability of nitrogen, which is naturally limited throughout all ecosystems (Vitousek & Howarth 1991; Tilman & Lehman 2001). Although nitrogen is highly abundant in the atmosphere as nitrogen gas (N_2), it must be fixed before it can be utilised by plants (Delwiche 1970; Vitousek *et al.* 1997). The conversion of N_2 to a form that is available for primary production occurs both naturally and anthropogenically (Vitousek & Howarth 1991). As humanity relies on the production of plants to fulfil nutritional demands, growth in the human population has led to intensive agriculture for food production, which requires the application of large amounts of industrially created nitrogenous fertilizers (Nixon 1995; Vitousek *et al.* 1997). Humans also increase the availability and mobility of nitrogen through processes such as the combustion of fossil fuels (Vitousek *et al.* 1997; Cloern 2001; Tilman *et al.* 2001). Primary productivity can also be limited by the availability of phosphorus, derived

naturally through the weathering of rocks and enhanced by the mining of phosphate rock (Nixon 1995; Vitousek *et al.* 1997). Consequently, humans have doubled the natural supply of both nitrogen and phosphorus to terrestrial environments (Tilman *et al.* 2001).

Although the addition of fertilizers occurs globally, the application of nitrogen and phosphorus is local (Vitousek *et al.* 1997). The mobility of these elements, however, can result in them being transported to neighbouring ecosystems (Vitousek *et al.* 1997), leading to the local enrichment of the recipient system (Gorman *et al.* 2009). The discharge of nitrogen and phosphorus into the coastal zone can occur via industry, agriculture and aquaculture runoff (Nixon 1995; Cloern 2001; Ralph *et al.* 2006). Further, the global increase in urbanization associated with the growth of the human population along the coast has resulted in enhanced inputs of nutrients into the coastal zone via storm water outfalls and sewage effluent (Ralph *et al.* 2006; Gorman *et al.* 2009). For example, the concentration of dissolved inorganic nitrogen is approximately 8 – 407 times greater in urbanized than natural catchments, and this increase is primarily due to the input of sewage effluent (Gorman *et al.* 2009).

1.1.2 GLOBAL ENHANCEMENT

Atmospheric carbon dioxide (CO₂) concentrations are increasing at an unprecedented rate, driven primarily by fossil fuel combustion and deforestation (Doney *et al.* 2009).

Historically, atmospheric CO₂ levels varied between 200 and 280 parts per million (ppm) for nearly 400,000 years prior to the Industrial Revolution (Feely *et al.* 2004). In contrast, atmospheric CO₂ levels have risen by approximately 40 % in the two centuries since the Industrial Revolution to ~ 400 ppm (Doney *et al.* 2009; Schlesinger & Bernhardt 2013), and are forecasted to increase to within the range 794 – 1142 ppm by the year 2100

(Collins *et al.* 2013). The atmosphere, however, only retains 50 % of the CO₂ released via human activities, with marine waters currently absorbing approximately 30 % and terrestrial ecosystems taking up the remaining 20 % (Feely *et al.* 2004). The dissolution of CO₂ in the ocean changes the carbonate chemistry and reduces the pH of surface waters (Caldeira & Wickett 2003), and together these processes are referred to as ‘ocean acidification’ (Doney *et al.* 2009; Schlesinger & Bernhardt 2013). To date, the average pH for surface waters has already decreased by 0.1 units (Hoegh-Guldberg & Bruno 2010), with further decreases expected by the year 2100.

1.2 RESOURCE ENHANCEMENT INFLUENCES PRIMARY PRODUCTIVITY

Changes in resource availability, particularly the increase in available nitrogen, can influence plant species composition, abundance, diversity and ultimately the functioning of an ecosystem (Wedin & Tilman 1996; Vitousek *et al.* 1997). The increase in resource availability due to human activities tends to act as a disturbance that has contrasting effects on primary producers, often enabling subordinate species to become dominant (Tilman & Lehman 2001; Diaz-Pulido *et al.* 2011; Connell *et al.* 2013). For example, the application of nitrogen and phosphorus fertilizers to a wet heathland community in the Netherlands resulted in a weedy grass responding more efficiently to the increase in resource availability and becoming dominant over a perennial shrub (Aerts & Berendse 1988). Similarly, the influx of nutrients into coastal ecosystems enhances the availability of resources (Gorman *et al.* 2009), which supports a shift in the competitive balance from slow-growing primary producers to faster-growing opportunistic algal species (Worm *et al.* 1999; Connell *et al.* 2008).

As seagrass meadows often occur in areas that are nutrient limited (Duarte 1990), minor inputs of nutrients may be expected to enhance seagrass growth (Borum & Sand-Jensen 1996). Positive effects of low-level nutrient enrichment on the production of seagrass are not uncommon (Heck *et al.* 2000; Kelaher *et al.* 2013) and include, for example, faster growth rates (Wear *et al.* 1999). Nevertheless, the majority of studies that assess the effects of nutrient addition in seagrass meadows report a decline in seagrass growth, which is often associated with an increase in phytoplankton biomass and epiphytic algae cover (reviewed in Burkholder *et al.* 2007). Nutrient addition favours the later due to their position in the water column, and their highly developed nutrient uptake kinetics (Wallentinus 1984; Duarte 1995; Pedersen & Borum 1997). As such, both the increased growth of phytoplankton and epiphytic algae significantly reduces the quantity of light available to the seagrass (Wear *et al.* 1999; Burkholder *et al.* 2007). As seagrass require high levels of light for production, generally greater than 11 % and approaching 25 % irradiance for some species, shading by phytoplankton and epiphytes often leads to the decline in seagrass habitats (Duarte 2002; Orth *et al.* 2006; Burkholder *et al.* 2007).

The enhanced growth of opportunistic algae during periods of anthropogenic inputs is not limited to seagrass habitats, and has been shown in other coastal ecosystems such as kelp forests and coral reefs (McCook *et al.* 2001; Gorman *et al.* 2009). Kelp forests form morphologically complex and highly productive ecosystems along temperate coastlines (Steneck *et al.* 2002; Connell & Irving 2008). Low-lying mats of filamentous algal turfs (Connell *et al.* 2014) are natural components of these systems, and compete with kelp for available space (Gorman *et al.* 2009). Although normally ephemeral, the physiology of algal turfs enables them to rapidly absorb resources during periods of nutrient enrichment, which increases their productivity (Hein *et al.* 1995) and allows them to persist for longer

periods (Gorman *et al.* 2009). The persistence of turf beyond their normal seasonal limits inhibits the recruitment of kelp (Connell & Russell 2010), enabling them to become competitively superior, and can result in a shift from kelp-dominated landscapes to turf-dominated landscapes (Connell *et al.* 2008).

Concurrent with local increases in nutrients is the global accumulation of CO₂, which can act as a stressor to some species and as a resource to others (Beardall & Raven 2004; Feely *et al.* 2004). Initial research on CO₂ addition has primarily focused on the negative effects of ocean acidification on calcifying organisms, particularly those that form habitats such as corals (Leclercq *et al.* 2000; Feely *et al.* 2004). Whilst it is relatively well established that ocean acidification can reduce the growth and calcification of corals due to the decrease in aragonite saturation state (Gattuso *et al.* 1998; Kleypas *et al.* 1999; Hoegh-Guldberg & Bruno 2010), less consideration has been given to the influence of CO₂ on non-calcifying primary producers. CO₂ can act as a resource to photosynthetic organisms that are carbon limited by facilitating an increase in their carbon fixation rates (Hurd *et al.* 2009; Raven & Hurd 2012; Koch *et al.* 2013). The degree to which CO₂ benefits photosynthetic organisms, however, will depend on their physiology and the extent to which they are carbon limited (Koch *et al.* 2013). Most marine algae have carbon concentrating mechanisms (CCMs), which allow the active uptake of CO₂ and/or bicarbonate, are generally not carbon limited, and are thought to receive little benefit from CO₂ addition (Giordano *et al.* 2005; Hurd *et al.* 2009; Hepburn *et al.* 2011). In contrast, marine algae that take up dissolved CO₂ by diffusion are generally carbon limited and are likely to benefit from CO₂ addition (Kübler *et al.* 1999; Hepburn *et al.* 2011). An increased availability of CO₂ in coastal zones may, therefore, shift the competitive balance, allowing subordinates to displace their naturally dominant competitors (Connell *et al.* 2013).

The combination of local (e.g. nutrients) and global (e.g. CO₂ emissions) resource enhancement will further accelerate shifts in marine ecosystems (Russell *et al.* 2009; Falkenberg *et al.* 2013a). For example, the cover of turf algae is greatest when elevated nutrients and future CO₂ conditions are combined (Russell *et al.* 2009). This expansion of turf under combined CO₂ and nutrient enhancement is typical of a species that is co-limited, in that the response of turf to multiple resource enhancement was greater than the response to resources enhanced individually (see Falkenberg *et al.* 2013b).

1.3 COMPENSATORY RESPONSE OF HERBIVORES

The liberation of resources into the environment can modify the structure and function of biological communities (Hooper *et al.* 2005), yet many systems that experience considerable disturbance due to human activities remain stable, resisting environmental change (Ghedini *et al.* 2015). Understanding how compensatory processes enable ecosystems to persist in a constant state without shifting to an alternate state when faced with substantial human pressure is pivotal for increasing our knowledge of ecosystem stability. Whilst it is well known that resource enhancement can stimulate changes to primary productivity (Tilman & Lehman 2001; Connell & Russell 2010), less is known about the natural capacity for herbivores to compensate for the increased production. Importantly, if the increase in consumption by herbivores matches the increase in production by algae, then herbivores may compensate for resource enhancement and increase ecosystem stability (Ghedini *et al.* 2015).

Nutrient enhancement and herbivore consumption can interact to influence primary productivity (Burkepile & Hay 2006), however, the extent to which this occurs may vary

across ecosystems. A meta-analysis of manipulative nutrient enrichment and herbivore abundance studies showed limited interactions between nutrients and herbivores on producer biomass in terrestrial ecosystems, which was most likely due to the limited effect of herbivory on productivity. The same meta-analysis, however, showed that nutrient enrichment and herbivore removal had a positive interaction on producer biomass in marine systems (Gruner *et al.* 2008). The difference in responses between systems may be due to many marine producers such as algae lacking the effective chemical and structural defences that increase the resistance of terrestrial producers to herbivores, making algae more digestible and increasing their nutritional value (Polis & Strong 1996).

Herbivores may play a fundamental role in compensating for the effects of nutrient addition in coastal zones by preferentially consuming opportunistic algae that are nutrient-rich. Opportunistic algae can rapidly take up available nitrogen (Hein *et al.* 1995) during periods of nutrient addition, which modifies their nutritional value and stimulates consumption (Boyer *et al.* 2004; Falkenberg *et al.* 2014). Herbivores can increase consumption under periods of nutrient enrichment through a population response (i.e. increasing abundance which increases herbivory; Neckles *et al.* 1993; Karez *et al.* 2004; Roll *et al.* 2005), or via an increase in *per capita* consumption (e.g. Nicotri 1980; Falkenberg *et al.* 2014). Whether herbivores are capable of countering the effects of resource enhancement, however, is highly variable, as some studies show herbivores to increase their feeding rates under nutrient enhancement (Neckles *et al.* 1993; Williams & Ruckelshaus 1993; Russell & Connell 2007), whilst others have found no changes (Worm & Lotze 2006; Burnell *et al.* 2013).

Whilst local nutrient enhancement may increase consumption rates, the influence of global CO₂ enhancement is less clear, given the potential of CO₂ to influence both the physiology of herbivores and the production of algae. Elevated CO₂ and subsequent ocean acidification can lower the calcium carbonate saturation rate in surface waters, reducing the ability of calcifying organisms to produce their carbonate structures (Doney *et al.* 2009), potentially affecting their health, activity and feeding abilities (see Kurihara *et al.* 2004; Bibby *et al.* 2007; Miles *et al.* 2007; Siikavuopio *et al.* 2007). Recent work has, however, revealed that elevated CO₂ may actually stimulate herbivore consumption rates, which may be through a direct effect on the herbivores themselves or via an indirect effect on the food that they consume (Cummings *et al.* 2011; Burnell *et al.* 2013; Falkenberg *et al.* 2013c). Elevated CO₂ not only facilitates the growth of algal turf but also modifies the nitrogen content, which is known to increase the consumption of algae (Falkenberg *et al.* 2013c). Additionally, if CO₂ increases either the quality or quantity of food, it may stimulate an increase in herbivore metabolic rate (Lilly 1979), which is likely to be in response to the increased energetic costs of processing extra or more nutritious food (reviewed in Secor 2009).

Invertebrate mesograzers such as amphipods and gastropods, can exert strong trophic effects in temperate marine systems (Underwood 1980), by consuming opportunistic algae (Steneck & Watling 1982; Duffy & Harvilicz 2001; Hughes *et al.* 2004). Under what circumstances these herbivores can compensate for the increased growth of opportunistic algae during periods of local and global resource enhancement, potentially increasing ecosystem stability, remains unclear. If resource enhancement can stimulate these herbivores into consuming the increased productivity, then it should be possible to not only

see a proportional increase in consumption, but also a metabolic basis for the change in consumption rate.

1.4 RESTORATION TO RE-ESTABLISH ECOSYSTEM STRUCTURE AND FUNCTION

When anthropogenic pressures degrade systems to the point of habitat loss, habitat restoration can be employed to alleviate loss and to re-establish the structure and function of the lost ecosystem (Elliott *et al.* 2007; Reynolds *et al.* 2013). Habitat restoration is often implemented with the principal intent of restoring the functions and services the ecosystem supplies, such as the provision of structure for faunal communities (Dobson *et al.* 1997; Zedler & Lindig-Cisneros 2000; Simenstad *et al.* 2006). The recovery of ecosystem structure, however, does not automatically lead to the recovery of ecosystem function, and there may be a temporal and/or spatial lag between the recovery of habitat and the recolonisation of faunal communities (Zedler & Lindig-Cisneros 2000).

Restoring complex environments is inherently difficult and often results in varied levels of success (Elliott *et al.* 2007; Irving *et al.* 2010). The rate and extent of recovery of an ecosystem following the implementation of restoration often varies due to the magnitude and type of disturbance causing habitat loss and the technique used for restoration (Simenstad *et al.* 2006). As ecosystems are not stable through time, the “baseline” that should be used to measure restoration success is often uncertain, and hence setting appropriate goals to measure success based solely on attributes that were characteristic of the system prior to disturbance can be problematic (Hobbs & Harris 2001). An alternative is to use a neighbouring, undegraded habitat that is exposed to similar environmental conditions as a reference site, which represents the desired endpoint for restoration (Yates *et al.* 1994; Hobbs & Harris 2001). Restoration success can then be measured by

comparing structural and functional attributes of the restoration site to those of the reference site (Hobbs & Harris 2001; Ruiz-Jaén & Aide 2005; Benayas *et al.* 2009). This technique has been used to monitor restoration success in a variety of terrestrial (e.g. rainforests: Jansen 1997; tropical forests: Aide *et al.* 2000; and prairies: Brye *et al.* 2002), and aquatic ecosystems (e.g. salt marsh: Moy & Levin 1991; wet lands: Simenstad & Thom 1996; and seagrass: Sheridan 2004).

1.4.1 GLOBAL LOSS OF SEAGRASS ECOSYSTEMS

Seagrass meadows play a key role in the functioning of temperate, tropical and subarctic coastal ecosystems by stabilising sediment, cycling nutrients and oxygenating the water column. Additionally, seagrass meadows support complex food-webs, facilitate trophic transfer to neighbouring habitats, and exhibit greater faunal diversity and abundance than surrounding unvegetated habitats (Short & Wyllie-Echeverria 1996; Beck *et al.* 2001; Duarte 2002; Orth *et al.* 2006). Due to their coastal distribution, seagrass meadows are naturally dynamic and subject to physical and biological disturbances such as storm events and disease which can result in large-scale habitat loss (Short & Wyllie-Echeverria 1996; Duarte 2002). For example, the North Atlantic experienced substantial *Zostera marina* loss during the 1930's due to the spread of the pathogen causing eelgrass wasting disease (Short *et al.* 1987). The global loss of seagrass meadows has, however, accelerated during the last several decades due to a wide range of anthropogenic disturbances such as boat groundings, propeller scaring and particularly a decline of water quality associated with agriculture and urbanisation (Short & Wyllie-Echeverria 1996; Orth *et al.* 2006). As a result, approximately 29% of the world's seagrass habitat has been lost (Waycott *et al.* 2009), with the influx of nutrients to the coastal zone being the major contributor (Orth *et al.* 2006; Ralph *et al.* 2006; Burkholder *et al.* 2007). Importantly, the ecosystem functions

and services that seagrass meadows supply are also lost (Duarte 2002). Consequently, seagrass restoration has become a fundamental element of coastal management (Van Keulen *et al.* 2003; Bell *et al.* 2008; Cunha *et al.* 2012).

1.4.2 SEAGRASS RESTORATION

Recovery of seagrass ecosystems following loss can occur passively, being the natural recovery of the ecosystem once the perturbation causing the loss has been removed (Elliott *et al.* 2007). For example, nitrogen loading resulted in intensive seagrass loss (90 %) in Tampa Bay Florida, however, an approximately 60 % reduction in nitrogen inputs resulted in a 15 % increase in seagrass coverage (Tomasko *et al.* 2005). Although seagrass meadows may recover once the initial cause of the loss has been ameliorated, natural recovery is exceptionally slow, taking anywhere from tens to hundreds of years (Thorhaug 1986). As a result, restoration is often implemented to enhance the recovery of seagrass ecosystems (Elliott *et al.* 2007), with research increasingly focused on establishing the most effective techniques (Van Keulen *et al.* 2003; Bell *et al.* 2008; Cunha *et al.* 2012).

Seagrass restoration occurs via the introduction of adult plants or seeds/seedlings to initiate the development of a seagrass meadow (Fonseca *et al.* 1996a; Bell *et al.* 2008).

Restoration techniques will vary depending on the species of seagrass being restored, although most methods rely on harvesting seagrass and sometimes the associated sediment (e.g. plug method) from donor meadows and transplanting them to the selected restoration site (Fonseca *et al.* 1998). Transplantation, however, can be damaging to the donor population, and costs associated with harvesting, transport and transplanting can be high (Fonseca *et al.* 1998; Kenworthy *et al.* 2006).

The global success of seagrass restoration has been limited, with approximately 30 % of studies reporting success (Fonseca *et al.* 1998). Consequently, seagrass restoration is yet to reverse the global loss of seagrass ecosystems (Orth *et al.* 2006; McGlathery *et al.* 2011). Further, restoration success is often determined by short-term monitoring (< 1 year), and hence long-term success is often unclear (Cunha *et al.* 2012). Increasing the length of monitoring may therefore increase the possibility of detecting successful restoration. Additionally, restoration success is typically measured using time efficient and low cost methods such as the recovery of aboveground seagrass structure (e.g. shoot density or percentage cover, Fonseca *et al.* 2000; Di Carlo & Kenworthy 2008). These measures tell us little about the recovery of belowground structure and function, which may not recover at the same rate (Di Carlo & Kenworthy 2008).

1.4.3 SEAGRASS RESTORATION IN SOUTH AUSTRALIA

Approximately 5,200 hectares of seagrass have been lost along the coast of Adelaide, South Australia since the 1930's in response to nutrient addition and associated epiphytic algae (Neverauskas 1987; Nayar *et al.* 2012). In recent years, substantial effort has been invested in improving water quality, allowing a small amount of natural recovery in deeper waters where sediment movement is minimal (Bryars & Neverauskas 2004), suggesting that seagrass restoration may be plausible. Natural recolonisation, however, does not appear to be occurring along the inshore seagrass margin, as high wave energy and infaunal activity cause sediment movement, preventing seagrass from becoming established (Irving *et al.* 2010).

Restoration trials began in this area in 2002, using traditional methods (transplants and seedlings), however, establishment of seagrass was limited due to hydrodynamic forces

and sediment mobility (Irving *et al.* 2010). In response, a novel restoration technique was developed by Wear *et al.* (2010), using biodegradable hessian bags to stabilise the sediment and aid the natural recruitment of *Amphibolis antarctica* seedlings. *Amphibolis* seedlings possess a distinctive “grappling hook” structure at their base (Ducker *et al.* 1977), enabling seedlings to become entangled with the hessian bags and create new seagrass patches (Irving *et al.* 2013). If these restored patches continue to persist over time, it can be expected that they will provide similar structure and function to neighbouring natural seagrass meadows. It is now necessary to quantify whether ecosystem structure and function has recovered at this site. If full recovery has occurred, then we expect to see no difference in above- and belowground plant components, faunal composition (epifauna and infauna richness and abundance) and sediment physical properties (grain size distribution and nitrogen and carbon content) between the restoration site and a neighbouring natural seagrass meadow.

1.5 THESIS SCOPE AND OUTLINE

The natural ability of herbivores to compensate for the additional growth of opportunistic algae during periods of local and global resource enhancement remains unclear. In this thesis, I first aim to determine the role herbivores play in increasing the stability of coastal ecosystems during periods of resource enhancement at local (e.g. nutrient pollution) through to global scales (e.g. CO₂ emissions). When resource enhancement causes habitats to become unstable to the point of habitat loss, habitat restoration can be implemented to help alleviate loss and to re-establish the lost habitat. I then quantify the rate of recovery of ecosystem structure and function after substantial habitat loss has occurred and seagrass restoration has been implemented.

Manipulative and observational experiments were used to address the following specific aims:

1. To test whether the enhancement of nutrients stimulates an increase in herbivore consumption rates such that herbivory counters the additional productivity of algae during periods of nutrient enhancement (Chapter 2).
2. To test whether combined local and global resource enhancement (Nutrients and CO₂) stimulates an increase in the consumption and metabolic rates of herbivores, so that enhanced primary productivity of opportunistic species is countered by herbivory (Chapter 3).
3. To quantify how quickly the above- and belowground function (as measured by epifaunal and infaunal assemblages) of experimental restoration plots recovered to that of an adjacent natural seagrass meadow relative to rates of recovery of above- and belowground seagrass structure (Chapter 4 and 5, respectively).

1.5.1 THESIS SUMMARY

Each thesis chapter is outlined below.

Chapter 2

Nutrient inputs increase the growth of epiphytic algae in seagrass ecosystems, leading to seagrass decline (Duarte 1995; Burkholder *et al.* 2007), however, the extent to which herbivores can counter the excess production remains unclear. Invertebrate mesograzers can exert strong trophic effects in these systems by grazing epiphytic algae (Duffy & Harvilicz 2001), potentially increasing the survival of seagrass during nutrient enrichment. In chapter 2, I test the hypotheses that (1) nutrient addition stimulates mesograzers to

increase consumption, so that (2) the additional productivity of epiphytes during periods of nutrient addition is countered by herbivory.

Chapter 3

Concomitant with local increases in nutrients is the global accumulation of carbon dioxide (CO₂) (Feely *et al.* 2004), which in temperate coastal ecosystems can lead to the replacement of kelp by subordinates or weedy species (i.e. algal turfs) (Connell *et al.* 2013). These same resources can, however, encourage herbivores to increase their *per capita* consumption of turfs (e.g. nutrients: Russell *et al.* 2009; and carbon: Falkenberg *et al.* 2013c), as an increase in either the quality or quantity of food may stimulate an increase in metabolic rate (Lilly 1979). Consequently, in chapter 3 I test the hypotheses that (1) elevated nitrogen (N) and carbon dioxide (CO₂) not only stimulates an increase in consumption rates, but also stimulates an increase in underlying metabolic rates of gastropod herbivores, so that (2) enhanced primary productivity is countered by herbivory.

Chapter 4

Seagrass meadows provide habitat for a diverse array of animal life (Beck *et al.* 2001; Orth *et al.* 2006), and as the loss of seagrass continues globally, managers seek to restore lost meadows. Restoration is often implemented with the intent to not only restore the lost habitat *per se*, but to restore the function the habitat provides (Dobson *et al.* 1997; Simenstad *et al.* 2006). Subsequently, chapter 4 tests how quickly the epifaunal assemblage of experimental restoration plots recovered to that in an adjacent natural seagrass meadow relative to rates of recovery of seagrass *per se*.

Chapter 5

The success of restoration is generally reported as the recovery of aboveground seagrass structure (e.g. leaf biomass); however, this method may overestimate the recovery of the belowground component of the seagrass system, which may not recover at the same rate (Di Carlo & Kenworthy 2008). Following on from Chapter 4, Chapter 5 quantifies infaunal abundance, richness and composition of the same seagrass restoration plots as well as the rate of recovery of belowground seagrass biomass and sediment physical properties.

Chapter 6

Chapter 6 provides a general discussion on the key findings of the previous chapters and outlines possible directions for future research.

Thesis

Each data chapter (2 - 5) has been written in the form of an individual scientific paper and therefore uses the journal formatting. A list of co-authors and their contributions to the paper has been highlighted in the statement of authorship for each data chapter. A comprehensive reference list is included at the end of the thesis. Chapter 2 and Chapter 3 are published journal articles and Chapter 4 has been accepted for publication.

CHAPTER 2

STATEMENT OF AUTHORSHIP

Title of Paper	Compensation of nutrient pollution by herbivores in seagrass meadows		
Publication Status	<input checked="" type="checkbox"/> Published	<input type="checkbox"/> Accepted for Publication	
	<input type="checkbox"/> Submitted for Publication	<input type="checkbox"/> Publication Style	
Publication Details	McSkimming, C., Tanner, J.E., Russell, B.D. & Connell, S.D. (2015). Compensation of nutrient pollution by herbivores in seagrass meadows. <i>Journal of Experimental Marine Biology and Ecology</i> , 471 , 112-118.		

PRINCIPAL AUTHOR

Name of Principal Author	Chloe McSkimming		
Contribution to the Paper	Contributed to the conception and design of the project. Conducted the study, collected and analysed the data and drafted and revised the manuscript.		
Overall percentage (%)	80		
Signature		Date	22/07/2015

CO-AUTHOR CONTRIBUTIONS

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Jason E Tanner		
Contribution to the Paper	Contributed to the conception and design of the project and assisted with the development and revision of the manuscript.		
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Contribution to the Paper	Contributed to the conception and design of the project and assisted with the development and revision of the manuscript. Provided funding.		
Signature		Date	22/07/2015

Name of Co-Author	Sean D Connell		
Contribution to the Paper	Contributed to the conception and design of the project, assisted with the development and revision of the manuscript and acted as Corresponding author. Provided funding.		
Signature		Date	22/07/2015



Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Compensation of nutrient pollution by herbivores in seagrass meadows

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ARTICLE INFO

Article history:

Received 8 January 2015

Received in revised form 17 May 2015

Accepted 18 May 2015

Available online 8 June 2015

Keywords:

Consumption

Epiphytes

Grazing

Herbivory

Mesograzers

Nutrients

ABSTRACT

The capacity of natural systems to absorb disturbances without undergoing major change is a critical aspect of stability. While human-driven increases in resources are well known to cause profound change to habitat-forming perennials, often as an indirect effect on enhanced growth of opportunistic species, less is known about the natural capacity for consumption to compensate for this excess production. Nutrient inputs to seagrass systems increase the production of epiphytic algae and lead to seagrass decline; however, it is relatively unclear whether grazers can compensate for this elevated production. Small invertebrate mesograzers are important herbivores in these systems, grazing predominately on epiphytic algae, potentially increasing the survival of seagrass during periods of nutrient enrichment. Using a cage-free technique and catchment-based nutrient concentrations, we experimentally manipulated mesograzers abundance and nutrient concentrations simultaneously in a subtidal *Posidonia angustifolia* meadow to test for trophic compensation. We tested the hypotheses that nutrient enrichment not only stimulates mesograzers to increase consumption, but that this increase in rate of consumption also matches the rate of increase in the production of epiphytes. Consecutive increases in nutrient addition caused consecutive increases in epiphyte production in treatments of reduced herbivory, but not in treatments of natural herbivory. Mesograzers fully compensated for the effects of minor nutrient addition and nearly compensated for the effects of moderate nutrient addition by decreasing the accumulation of epiphytic algae on seagrass leaves. Although nutrient addition alters food availability and quality of epiphytes, the abundance of grazing amphipods did not increase in treatments of natural herbivory, suggesting that the greater consumption of epiphytes was due to an increase in *per capita* grazing. These results suggest that herbivores may not only counter the effects of resource enhancement but may fully compensate minor to moderate events of nutrient pollution by consuming the additional productivity of opportunistic species that can cause loss of community dominants.

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1. Introduction

The human domination of the abiotic environment can drive change to the structure and function of biological communities (Hooper et al., 2005), yet many systems that experience substantial human pressure can persist in a constant state without shifting to an alternate state. The ability of an ecosystem to absorb this pressure without undergoing major shifts in composition is a critical aspect of habitat stability (Ghedini et al., 2015). In systems that are resource limited, anthropogenic inputs of nutrients favour a shift in the competitive balance from slow-growing primary producers to faster-growing opportunistic algal species (Duarte, 1995; Gorman et al., 2009).

Herbivores may play a key role in mediating the effects of nutrient addition by consuming the additional productivity of opportunistic

species. The extent to which herbivory and nutrient addition interact to influence primary productivity may, however, vary across ecosystems. For example, a meta-analysis of herbivore abundance and nutrient addition studies showed limited interactions between herbivores and nutrients on producer biomass in terrestrial systems (Gruner et al., 2008). In contrast, a meta-analysis of similar studies across marine ecosystems (tropical and temperate) showed that nutrient addition and herbivore removal had a positive interaction on producer biomass (Burkepile and Hay, 2006).

In marine systems, herbivores have been shown to counter the effects of resource enhancement by preferentially consuming nutrient-enriched algae (Miller et al., 1999; Neckles et al., 1993; Russell and Connell, 2007). Herbivores can control the production of such algae through a population response (i.e. increasing abundance leads to increased consumption; Karez et al., 2004; Neckles et al., 1993; Roll et al., 2005), or by an increase in individual consumption rates (i.e. *per capita* consumption; Falkenberg et al., 2014; Nictori, 1980). The ability of herbivores to counter the effects of nutrient enhancement is, however, difficult to predict as some studies have shown herbivores to increase consumption under periods of nutrient enrichment (population

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response: Neckles et al., 1993; *per capita* response: McSkimming et al., 2015), while others have found no difference in feeding rates (Burnell et al., 2013; Worm and Lotze, 2006).

Seagrass meadows are highly susceptible to disturbance from natural and anthropogenic sources which can alter their distribution, productivity, and community composition (Duarte, 2002; Waycott et al., 2009). Of key importance are factors that influence the growth of epiphytes that overgrow and smother seagrass, significantly reducing the quantity of light available to the seagrasses themselves (Wear et al., 1999) and resulting in the decline of seagrass habitats (for review, see Burkholder et al., 2007). It is well established that the biomass and species composition of epiphytic algae can be altered by resource controls such as nutrients (Bryars et al., 2011; Prado et al., 2008; Wear et al., 1999).

Invertebrate mesograzers can exert strong trophic effects in seagrass systems by grazing epiphytic algae (Duffy and Harvilicz, 2001; Hughes et al., 2004; Orth and Van Montfrans, 1984). Yet, it remains unclear under what circumstances mesograzers may increase the survival of seagrass by consuming the additional productivity of epiphytic algae under nutrient enrichment in systems that are resource limited. Previous experiments that have manipulated mesograzers in the field have used exclusion cages which have associated artefacts, such as altered water flow and reduced light (see Connell, 1997; Miller and Gaylord, 2007). Fortunately, a cage-free technique has been developed to reduce the abundance of mesograzers *in situ* (Poore et al., 2009), which has been successfully used in seagrass systems (Myers & Heck 2013; Whalen et al., 2013; Reynolds et al., 2014). Using this cage-free technique, we manipulated nutrient levels and mesograzers simultaneously to test the hypotheses that nutrient enrichment not only stimulates mesograzers to increase consumption, but that this increase in rate of consumption also matches the rate of increase in the production of epiphytes.

2. Materials and methods

2.1. Study site

The effects of nutrient addition and grazer reduction were tested in a monospecific seagrass meadow in Lady Bay, Fleurieu Peninsula, South Australia (35°28.240'S, 138°17.295'E). The shallow bay consists of a rocky intertidal zone followed by a sandy subtidal zone dominated by the seagrass *Posidonia angustifolia*. The site has a strong tidal influence, typically semi-diurnal with a spring tidal range of 2.7 m, and water depth was ~ 0.3 m on spring low tides. The experiment was run for 162 days, from October 2012 to April 2013.

2.2. Experimental design

The effects of nutrients (ambient vs. minor vs. moderate) and grazers (present vs. reduced) on seagrass and associated epiphytic algae were evaluated in an orthogonal combination ($n = 4$ plots/treatment). Experimental plots were haphazardly located throughout the *P. angustifolia* meadow at a constant depth, separated by a minimum of 2 m to prevent any nutrient transfer. Plots were considered to be a circular area with a diameter of 20 cm and each plot was marked by a central steel tent peg.

2.3. Nutrient addition and grazer reduction

Elevated nutrient treatments were designed to replicate minor and moderate nutrient enrichment conditions experienced in oligotrophic waters off the coast of Adelaide, South Australia. Target dissolved inorganic nitrogen (DIN) for enriched treatments was based on the concentration adjacent to agricultural catchments (minor nutrient enrichment: $0.051 \pm 0.004 \text{ mg L}^{-1}$), and urban catchments under light rainfall (moderate nutrient enrichment: $0.091 \pm 0.021 \text{ mg L}^{-1}$) (Gorman, et al., 2009).

Nutrients were supplied to elevated treatments as 100 g (minor nutrient enrichment) or 200 g (moderate nutrient enrichment) of Osmocote® controlled release fertiliser (12 month release, 17, 4.3, 8.2 N-P-K). Fertiliser pellets were placed in a nylon mesh bag (1 mm mesh size), attached to the central tent peg and positioned ~ 10 cm above the substrate surface in appropriate plots. This method of elevating nutrient concentrations has been successfully used in previous subtidal experiments (e.g. Bryars, et al., 2011; Bulleri et al., 2012; Worm et al., 2000). To determine whether nutrients were supplied at elevated rates, water samples were collected from each plot ($n = 8$ per treatment) 4 months after commencing the experiment and prior to changing nutrient bags. Samples were taken directly adjacent to the nutrient bag in elevated treatments or the tent peg in ambient treatments using 25 ml sterile syringes. Water samples were also taken 200 m from the plots to assess background nutrient concentrations. Samples were immediately filtered (0.45 µm filter) and frozen to allow later analyses on a Lachat Quickchem 8200 Flow Injection Analyser (Hach, CO, USA) for DIN and phosphate.

Carbaryl (1-naphthyl-N-methylcarbamate) was used to reduce mesograzers in selected plots. Carbaryl is a degradable insecticide (half-life of approximately 5 h in seawater and light) that has previously been used for the reduction of mesograzers in field-based experiments (e.g. Cook et al., 2011; Poore et al., 2009; Whalen et al., 2013) as it has no known effect on epiphytic algae, gastropods, or fishes (Poore et al., 2009).

Carbaryl powder (80% wettable) was mixed with water and added to a slow-release dental plaster at 7.6% carbaryl by weight based on the methods described in Poore et al. (2009). The mix was then poured into 230 ml paper moulds and left to dry for 3 days. Once dry, the carbaryl blocks were wrapped in a single layer of nylon mesh (1 mm mesh size) to reduce abrasion from seagrass, and attached to the central tent peg by cable ties. Blocks were positioned ~ 10 cm above the substrate surface within the seagrass canopy.

Nutrient bags and carbaryl blocks were replaced at approximately monthly intervals. An earlier trial showed that carbaryl blocks wrapped in a layer of nylon mesh were continuing to dissolve after 1 month. In order to maintain a similar level of disturbance between control and treatment plots, the steel tent pegs in the control plots were also replaced at this time.

2.4. Response variables

The response of epiphytes and seagrass to the experimental conditions was assessed using four response variables: percentage cover and load of epiphytes, above-ground biomass and leaf density of seagrass. Percentage cover of epiphytes growing on the leaves of seagrass was visually estimated *in situ* at 4 months ($n = 10$ leaves per plot).

Five seagrass blades were removed from each plot 8 days prior to the completion of the experiment to quantify epiphyte load (ratio of epiphyte biomass to seagrass biomass; Bryars et al., 2011). Seagrass was placed on ice during transport to the laboratory and frozen until processing. Upon processing, all epiphytes were carefully scraped from the seagrass using a scalpel blade. Epiphytes and epiphyte-free seagrass were then placed in individual, pre-weighed aluminium trays and dried at 60 °C for 72 hours, and then re-weighed to quantify dry mass of both seagrass and epiphytes.

All above-ground seagrass biomass and associated fauna in the experimental plots was harvested upon completion of the experiment, 8 days after the replacement of nutrient bags and carbaryl blocks. Samples were collected using a 9.0 cm internal diameter (64 cm²) PVC corer with a fine mesh bag (mesh size 0.5 mm) attached to the outside. The corer was carefully placed over the seagrass, flush with the sediment surface, approximately 5 cm from the nutrient bag, carbaryl block or tent peg in control treatments. The seagrass was then cut at the substrate surface using a serrated knife, and the mesh bag was pulled over the corer and tied closed to prevent the escape of motile

fauna. Samples were placed on ice for return to the laboratory and stored in 10% formalin solution until sorting.

Harvested samples were then sorted under magnification in the laboratory and motile fauna were removed and two groups separated out: amphipods and gastropods. These taxa were counted and amphipods were identified to family. The mean wet weight (g) of amphipods was also quantified for each treatment. All amphipods within a plot were gently patted dry and weighed together to the nearest 0.0001 g using an electronic balance. To quantify the above-ground biomass of seagrass, all epiphytes were carefully scraped from the seagrass leaves, which were then placed in a pre-weighed aluminium tray and dried to a constant weight at 60 °C for 72 hours. Leaf density was also recorded at this time.

2.5. Data analysis

The responses of seagrass, epiphytes, and mesograzers to the experimental treatments were quantified using analysis of variances (ANOVAs). The response of amphipod assemblage to the experimental treatments was quantified using multivariate analysis of variance (MANOVA). Both factors (nutrient vs. grazer) were treated as fixed and orthogonal, with three levels of nutrients (ambient vs. minor vs. moderate) and two levels of grazers (present vs. reduced) for all analyses. For epiphyte percentage cover, the factor of plot (four levels) was nested within nutrients and grazing ($n = 10$ replicate measurements per plot). Epiphyte load was quantified as an average per plot ($n = 4$ plots per treatment), so plots were treated as replicates, not as a nested term, and nutrients and grazing were treated as above. Where significant treatment effects were detected ($p < 0.05$), pairwise tests were used to determine where differences occurred. All multivariate and univariate analyses were carried out in PRIMER (version 6) with the PERMANOVA + add-on (PRIMER-E Ltd, Plymouth).

3. Results

Elevated nutrients and grazer reduction had positive effects on the percentage cover of epiphytes and epiphyte load (Fig. 1 A, B). Under ambient nutrients, the percentage cover of epiphytes and epiphyte load did not differ between grazer-present and grazer-reduced treatments (Fig. 1 A, B, Table 1 A, B, respectively). When nutrients were elevated, however, the percentage cover of epiphytes and epiphyte load was greater when grazers were reduced than present and cover and load were greatest when elevated nutrients (moderate) and grazer reduction were experienced in combination (Fig. 1 A, B, Table 1 A, B, respectively).

Grazing increased with the increase in nutrients. Under minor nutrient enrichment, grazers removed 25% of the epiphyte cover, while under moderate nutrient enrichment, they removed 37% (Fig. 1 A). Additionally, the reduction of grazers resulted in a 156–251% increase in epiphyte load under minor and moderate nutrient enrichment, respectively (Fig. 1 B).

Carbaryl blocks decreased the abundance of amphipods by 91–95% in the grazer reduction treatments, while elevated nutrients had no detectable effect on abundance (Fig. 2 A, Table 2 A). As a result, amphipod assemblage differed between the grazer-present and grazer-reduction treatments but not between ambient and elevated nutrient treatments (Table 3 A). Amphipod wet weight differed between grazer-present and grazer-reduction treatments, while elevated nutrients had no detectable effect (Fig. 3, Table 3 B). In contrast, the presence of carbaryl did not affect the abundance of herbivorous gastropods. The addition of nutrients, however, appeared to lead to a decline in the abundance of herbivorous gastropods, but this decline was not significant (Fig. 2 B, Table 2 B).

The above-ground biomass of seagrass was greatest when minor elevated nutrients (100 g) and the presence of grazers were combined (Fig. 4 A, Table 4 A). However, neither elevated nutrients nor grazer

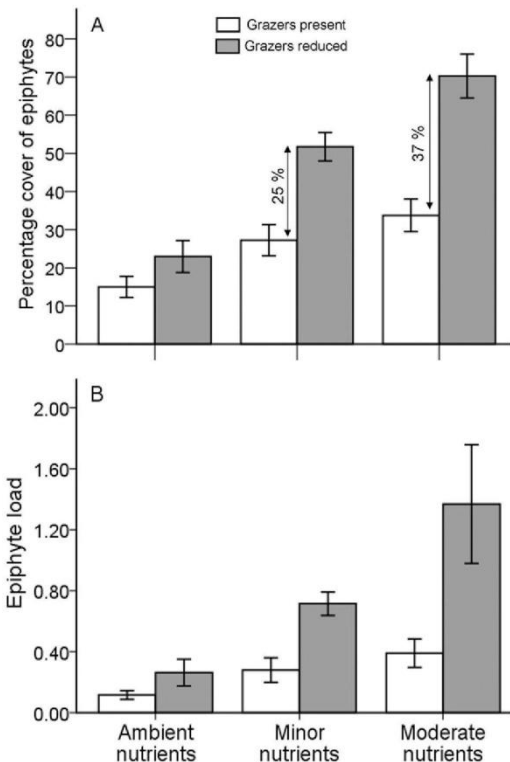


Fig. 1. The influence of nutrients and grazers on A. percentage cover of epiphytes and B. epiphyte load (ratio of epiphyte biomass to seagrass biomass). Values are mean \pm S.E. ($n = 4$).

Table 1

The combined effects of nutrients (ambient vs. minor vs. moderate) and grazers (present vs. reduced) on the A. percentage cover of epiphytes and B. epiphyte load, as determined by ANOVAs.

Source	df	MS	F	P
A. Percentage cover				
Nutrients	2	22207	13.17	0.001
Grazing	1	31740	44.55	0.001
Nutrients \times grazing	2	4095	5.47	0.01
Plot (nutrients \times grazing)	18	712.5	6.29	0.001
Residual	216	113.3		
B. Epiphyte load				
Nutrients	2	0.457	8.503	0.001
Grazing	1	0.564	10.481	0.001
Nutrients \times grazing	2	0.008	1.548	0.015
Residual	18	0.005		

Pairwise tests on significant nutrient \times grazing term in A:

Ambient nutrients: grazers present = grazers reduced;

Minor nutrients: grazers present < grazers reduced;

Moderate nutrients: grazers present < grazers reduced;

Grazers present: ambient nutrients = minor nutrients, ambient nutrients < moderate nutrients, minor nutrients = moderate nutrients;

Grazers reduced: ambient nutrients < minor nutrients < moderate nutrients.

Pairwise tests on significant nutrient \times grazing term in B:

Ambient nutrients: grazers present = grazers reduced;

Minor nutrients: grazers present < grazers reduced;

Moderate nutrients: grazers present = grazers reduced;

Grazers present: ambient nutrients = minor nutrients, ambient nutrients < moderate nutrients, minor nutrients = moderate nutrients;

Grazers reduced: ambient nutrients < minor nutrients, ambient nutrients < moderate nutrients, minor nutrients = moderate nutrients.

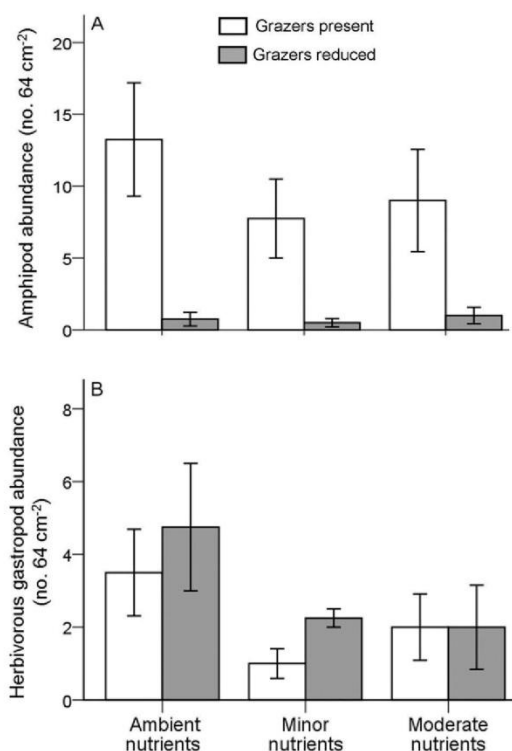


Fig. 2. The influence of nutrients and grazers on the abundance of A. amphipods and B. herbivorous gastropods in *P. angustifolia*. Values are mean \pm S.E. ($n = 4$).

Table 2

The combined effects of nutrients (ambient vs. minor vs. moderate) and grazers (present vs. reduced) on A. the abundance of amphipods and B. the abundance of herbivorous gastropods, as determined by ANOVAs.

Source	df	MS	F	p
A. Amphipod abundance				
Nutrients	2	19.35	0.739	0.527
Grazing	1	495.16	18.916	0.002
Nutrients \times grazing	2	20.4	0.78	0.487
Residual	17	26.18		
B. Herbivorous gastropod abundance				
Nutrients	2	14.34	3.272	0.063
Grazing	1	3.95	0.901	0.357
Nutrients \times grazing	2	0.94	0.214	0.817
Residual	17	4.38		

Table 3

The combined effects of nutrients (ambient vs. minor vs. moderate) and grazers (present vs. reduced) on A. the assemblage of amphipods and B. amphipod wet weight (g), as determined by MANOVA and ANOVA respectively.

Source	df	MS	F	p
A. Amphipod assemblage				
Nutrients	2	5149	1.681	0.06
Grazing	1	10768	3.515	0.004
Nutrients \times grazing	2	2216	0.72	0.783
Residual	17	3063		
B. Amphipod wet weight (g)				
Nutrients	2	0.0000002	0.166	0.872
Grazing	1	0.000018	16.664	0.001
Nutrients \times grazing	2	0.0000006	0.573	0.569
Residual	17	0.0000011		

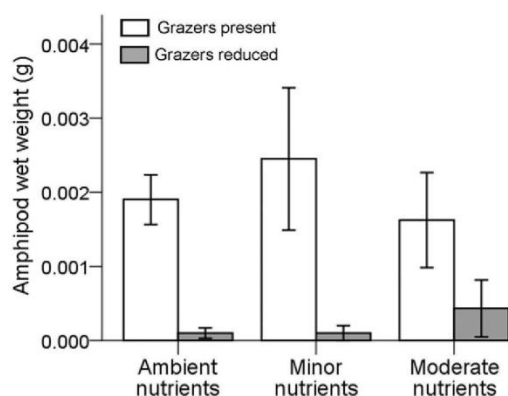


Fig. 3. The influence of nutrients and grazers on the wet weight of amphipods (g). Values are mean \pm S.E. ($n = 4$).

reduction had a detectable effect on leaf density over the experimental period (Fig. 4 B, Table 4 B).

The concentration of DIN was significantly greater in the moderate elevated (nutrient 200 g mean \pm SE; 0.079 ± 0.025 mg L⁻¹) than the minor elevated (nutrient 100 g 0.048 ± 0.008 mg L⁻¹) and ambient (control 0.041 ± 0.002 mg L⁻¹, background 0.041 ± 0.001 mg L⁻¹) nutrient treatments (ANOVA: $F_{2,25} = 3.835$, $p = 0.024$). The concentration of phosphate did not differ significantly between treatments (moderate 0.035 ± 0.0011 mg L⁻¹, minor 0.048 ± 0.0141 mg L⁻¹, control 0.033 ± 0.0002 mg L⁻¹, background 0.033 ± 0.0002 mg L⁻¹; ANOVA: $F_{2,24} = 1.605$, $p = 0.25$).

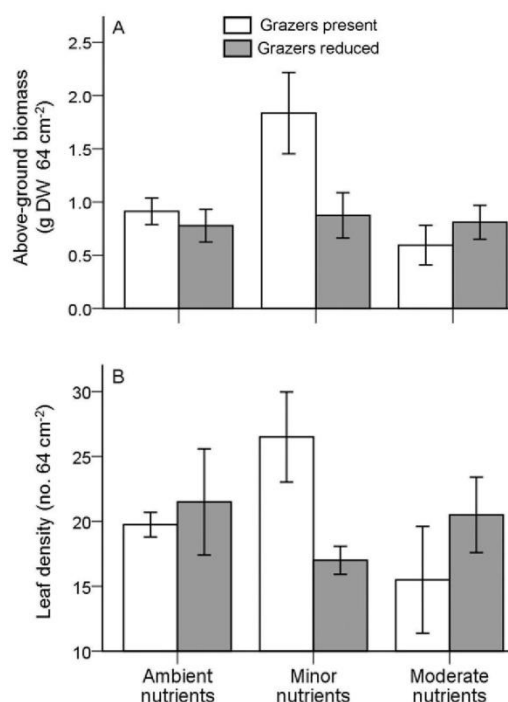


Fig. 4. The influence of nutrients and grazers on A. the above-ground biomass and B. leaf density of *P. angustifolia*. Values are mean \pm S.E. ($n = 4$).

Table 4

The combined effects of nutrients (ambient vs. minor vs. moderate) and grazers (present vs. reduced) on A. above-ground biomass and B. leaf density of *P. angustifolia*, as determined by ANOVAs.

Source	df	MS	F	P
A. Above-ground biomass (g)				
Nutrients	2	0.942	4.866	0.019
Grazing	1	0.516	2.668	0.139
Nutrients × grazing	2	0.728	3.76	0.033
Residual	18	0.194		
B. Leaf density				
Nutrients	2	19.625	0.791	0.479
Grazing	1	5.042	0.134	0.731
Nutrients × grazing	2	115.79	3.089	0.063
Residual	18	37.486		

Pairwise tests on significant nutrient × grazing term in A.:

Ambient nutrients: grazers present = grazers reduced;

Minor nutrients: grazers reduced < grazers present;

Moderate nutrients: grazers present = grazers reduced;

Grazers present: ambient nutrients = moderate nutrients < minor nutrients;

Grazers reduced: ambient nutrients = minor nutrients = moderate nutrient.

4. Discussion

In aquatic ecosystems, anthropogenic inputs of nutrients can drive profound change to habitat-forming perennials by favouring the growth of opportunistic algal species which then become competitively dominant (Bryars et al., 2011; Lotze et al., 2000). Herbivores that preferentially feed on nutrient-enriched algae have been shown to play a key role in mediating the effects of nutrient addition in coastal ecosystems such as coral reefs (Miller et al., 1999) and kelp forests (Russell and Connell, 2007). Here, we show that small invertebrate mesograzers can counter the effects of nutrient addition by decreasing the accumulation of epiphytic algae on seagrass; nutrient enrichment facilitated the growth of epiphytic algae, but only when herbivores were reduced. These results suggest that under some circumstances, herbivores may be efficient in offsetting the effects of small-scale resource enhancement in seagrass ecosystems by consuming the additional productivity of epiphytic algae that can cause the loss of community dominants.

Herbivores can control the proliferation of opportunistic algae under periods of nutrient enrichment by increasing in abundance (Heck et al., 2000; Karez et al., 2004; Neckles et al., 1993; Roll et al., 2005), or via an increase in *per capita* grazing (Falkenberg et al., 2014; McSkimming et al., 2015; Nicotri, 1980). Although nutrient enrichment enhanced food availability in this study, the abundance of amphipods did not increase in the grazed treatments with increasing nutrients. One explanation as to why an increase in amphipod abundance was not detected may be that an increase in abundance leads to greater rates of consumption; this would be a similar trophic compensatory response to that observed in other herbivores (Ghedini et al., 2015). The increase in epiphyte grazing under nutrient addition may therefore be due to greater abundances of amphipods attracted to these plots, which may lead to greater predation rates of amphipods, resulting in amphipod abundance being constant. If new arrivals eat more than established residents, which is likely if they move into the plot due to increased food availability (i.e., they move because they are hungry), this will then manifest as increased grazing pressure despite no apparent increase in abundance. Additionally, it is possible that a greater number of individuals are consuming the algae then were residing in the plots, given that samples represent a “single snapshot” of amphipod abundance which can vary over space and time (see Thom et al., 1995; Viejo and Åberg, 2003). However, we consider this unlikely given the close association between food and habitat, with plants providing amphipods with both food (epiphytic algae) and a refuge from predators (seagrass) (Duffy and Hay, 1991). If this is not the case and the number of individuals does not

increase, then it is possible that the greater consumption of epiphytes under nutrient addition was due to an increase in *per capita* grazing and not grazer abundance.

In systems that are resource limited, periods of nutrient enrichment can shift the species composition of epiphytic algae from encrusting calcareous assemblages to faster-growing filamentous brown, red and green assemblages (Bryars et al., 2011; Prado et al., 2008). Although epiphytic composition was not directly quantified during this study, filamentous algae appeared to be dominant in the nutrient addition treatments (C McSkimming, pers obs). Filamentous algae can rapidly take up available nitrogen (Hein et al., 1995), and grazers have been shown to actively select and consume greater amounts of such algae (Kraufvelin et al., 2006; Neckles et al., 1993; Williams and Ruckelshaus, 1993). If amphipod abundances at the time of sampling are representative of abundances present throughout the study, then the increased grazing pressure in the nutrient addition treatments may be due to an increase in *per capita* grazing. This suggests that individual herbivores may have consumed larger quantities of nutrient-enriched food than under more oligotrophic conditions. Additionally, there was a potential shift in the assemblage of amphipods with the addition of nutrients, suggesting that the increase in epiphyte load under nutrient addition may have attracted amphipods that consume larger quantities of food. Amphipod weight, however, did not differ between the ambient and elevated nutrient treatments, suggesting that any extra energy obtained is being used for metabolic processes other than growth, such as reproduction. Importantly, if an increase in *per capita* grazing is responsible for the decrease in epiphytic algae during small-scale resource enhancement, then grazing could potentially counter the effects of large-scale resource enhancement, as individuals are capable of increasing their consumption rates without having to increase in abundance.

The importance of herbivores in countering the effects of nutrient addition through either an increase in abundance or an increase in *per capita* consumption is becoming more commonly recognised in marine ecosystems (Burkepile and Hay, 2006; Gruner et al., 2008). A meta-analysis of studies that compare the effects of nutrients and grazing in marine systems found that herbivores had stronger effects on primary production than nutrients (Burkepile and Hay, 2006). Additionally, a meta-analysis of similar studies in seagrass systems (Hughes et al., 2004) supports our suggestion that herbivores may be efficient in offsetting the effects of nutrient addition by consuming the increased productivity of epiphytic algae. The ability of herbivores to counter the effects of nutrient addition, however, may be dependent on location and spatial extent as well as type of herbivores present as other studies have found no difference in herbivore feeding rates under nutrient enrichment (Worm and Lotze, 2006). Importantly, the net effect of grazing would depend on the level of nutrients entering the coastal zone, with recent work suggesting that grazers can compensate for increasing levels of resource enhancement up until a certain threshold, after which the productivity of opportunistic species escapes regulation by herbivores (Ghedini et al., 2015).

Nutrient enrichment favours the growth of epiphytic algae over seagrass, due to their position in the water column, and their advanced nutrient uptake kinetics (Pedersen and Borum, 1997; Wallentinus, 1984). Studies that assess the effects of nutrient enrichment in seagrass habitats generally report a decline in seagrass growth which usually corresponds with an increase in epiphytic algae cover (Bryars et al., 2011; Burkholder, et al., 2007). Although nutrient enrichment facilitated the growth of epiphytic algae in this study, seagrass above-ground biomass and leaf density did not decline. Indeed, seagrass in treatments with minor nutrient addition and grazers present had significantly greater above-ground biomass than seagrass in treatments with ambient or moderate nutrient addition. This suggests that low levels of nutrients may be beneficial to the growth of seagrass (Kelaheer et al., 2013), but only if epiphytic algae are maintained at low covers by grazers. Positive effects of minor nutrient enrichment on the growth of seagrass are not uncommon and have been shown by previous studies that have

manipulated nutrient addition and herbivore densities (Heck et al., 2000; Williams and Ruckelshaus, 1993). Although nutrient addition and grazing appeared to have a positive effect on the growth of seagrass under minor nutrient addition, seagrass biomass did not increase under moderate nutrient addition. We suggest that the observed differences in seagrass biomass was due to greater epiphyte cover and load under moderate nutrient addition, rather than other possible mechanisms such as direct grazing by mesograzers or toxic effects of nutrients. These mechanisms appear unlikely as both direct grazing and toxic effects cause a decline in seagrass biomass (grazing: Heck et al., 2000; nutrients: Burkholder et al., 2007; Ralph et al., 2006) which did not occur here. As epiphyte cover and load was greatest under moderate nutrient addition when grazers were reduced, we suggest that the epiphytic algae limited the amount of nutrients available to seagrass and hence an increase in seagrass growth was not observed. Importantly, if nutrient levels exceed minor elevations, then epiphytes overgrow seagrasses and cause their loss (Burkholder, et al., 2007); indeed, nutrient pollution is thought to be a major cause of the global decline in seagrasses (Orth et al., 2006; Short and Wyllie-Echeverria, 1996).

To conclude, invertebrate mesograzers may play a relatively understudied role in compensating for the growth of epiphytic algae during events of minor nutrient addition. This study shows that mesograzers can consume the additional production of epiphytic algae in seagrass ecosystems during short-term episodes of nutrient pollution. Indeed, we may have underestimated the importance of mesograzers in studies seeking to assist the management of coastal eutrophication, especially in systems where minor increases in nutrient concentrations have large effects on the growth of opportunistic algae.

Acknowledgements

We thank B. Florance and S. Langholz for their valuable assistance in the field and laboratory. Thanks to L. Falkenberg for providing laboratory assistance. Financial support for this research was provided by an ARC grant to S.D. Connell and B.D. Russell, including a Future Fellowship to S.D. Connell and partly funded by the Nature Foundation of South Australia Inc. and the Dr Paris Goodsell Marine Ecology Research Grant. [SS]

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CHAPTER 3

STATEMENT OF AUTHORSHIP

Title of Paper	A test of metabolic and consumptive responses to local and global perturbations: enhanced resources stimulate herbivores to counter expansion of weedy species
Publication Status	<input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Publication Style
Publication Details	McSkimming, C., Russell, B.D., Tanner, J.E. & Connell, S.D. (2015). A test of metabolic and consumptive responses to local and global perturbations: enhanced resources stimulate herbivores to counter expansion of weedy species. <i>Marine and Freshwater Research</i> . doi.org/10.1071/MF14266

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Contribution to the Paper	Designed and conducted the study, collected and analysed the data and drafted and revised the manuscript.		
Overall percentage (%)	80		
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By signing the Statement of Authorship, each author certifies that:

- iv. the candidate's stated contribution to the publication is accurate (as detailed above);
- v. permission is granted for the candidate to include the publication in the thesis; and
- vi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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A test of metabolic and consumptive responses to local and global perturbations: enhanced resources stimulate herbivores to counter expansion of weedy species

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Abstract. The capacity of natural systems to resist environmental change underpins ecosystem stability, e.g. the persistence of kelp-dominated states which are sometimes displaced by subordinates or weedy species (i.e. algal turfs). Perturbation by resource enhancement at global (e.g. CO₂ emissions) through local scales (e.g. nutrient pollution) increases the probability of turf domination, yet these same resources stimulate an increase in *per capita* consumption of turfs by herbivores. We test whether such resource perturbation can stimulate herbivores to absorb the additional productivity of turfs that cause kelp displacement. We tested the hypotheses that (1) elevated nitrogen (N) and carbon dioxide (CO₂) not only stimulate an increase in consumptive rates, but also stimulate an increase in underlying metabolic rates of gastropod herbivores, so that (2) enhanced primary productivity is countered by herbivory. We reveal that elevated nitrogen and CO₂ stimulated an elevation in rates of consumption in proportion to an increase in metabolic rate of grazers. Subsequently, grazers consumed proportionately greater cover of turfs to counter turf expansion. Resource enrichment, therefore, can stimulate metabolic and consumptive activity of herbivores to absorb the additional productivity of opportunistic species. Hence, the competitive potential of subordinates to displace community dominants may be checked by the very resources that otherwise drive instability.

Additional keywords: algal turfs, carbon dioxide, consumption, herbivory, nitrogen, nutrient, ocean acidification.

Received 3 September 2014, accepted 19 December 2014, published online 22 June 2015

Introduction

The ability of natural systems to absorb perturbations without undergoing major change in composition is a critical aspect of stability (Ghedini *et al.* 2015). An account of the mechanisms that enable natural systems to resist environmental change is needed if we are to understand the inherent stability of many systems facing human-driven environmental change. The liberation of resources into the environment tends to act as a perturbation that has unequal effects on competitors, often turning subordinates into dominants (Tilman and Lehman 2001; Diaz-Pulido *et al.* 2011; Connell *et al.* 2013). Although it is well known that the liberation of resources by human activities can drive profound change to habitat-forming perennials, often as an indirect effect on enhanced growth of opportunistic species or weedy species (Tilman and Lehman 2001; Connell and Russell 2010), less is known about the natural capacity for consumption to counter this excess production.

Change in resource availability drives shifts in marine ecosystems as a consequence of human activities on local (e.g. nitrogen: Vitousek *et al.* 1997) to global (e.g. carbon dioxide: Connell *et al.* 2013) scales. Locally, anthropogenic inputs of nutrients into coastal zones enhance resource availability (Gorman *et al.* 2009), favouring a shift in the competitive balance from slow-growing primary producers to weedy or faster-growing opportunistic algal species (Worm *et al.* 1999; Connell *et al.* 2008). Concurrent with local increases in nutrients is the global accumulation of carbon dioxide (CO₂), with marine waters currently absorbing ~30% of the CO₂ released into the atmosphere, resulting in the reduction of oceanic pH or ocean acidification (Feely *et al.* 2004). Although the negative effects of ocean acidification on calcifying organisms such as corals are well established (Leclercq *et al.* 2000; Feely *et al.* 2004), less is known about the potential for CO₂ to act as a resource to photosynthetic

species that are carbon limited (Falkenberg *et al.* 2013b; but see Beardall and Raven 2004).

Kelp forests form morphologically complex and highly productive coastal ecosystems in temperate marine waters (Steneck *et al.* 2002; Connell and Irving 2008). Under resource liberation, these kelp-dominated landscapes can switch to turf-dominated landscapes, which comprise low-lying mats of filamentous algae (approximate canopy height <5 cm: Connell *et al.* 2014). Although algal turfs are natural components of these systems, nutrient enrichment increases their productivity, allowing them to persist for longer periods (Gorman *et al.* 2009), inhibiting the recruitment of kelp (Connell and Russell 2010). In addition to nutrients, CO₂ can act as a resource to photosynthetic organisms that are carbon limited, by increasing carbon fixation rates (Hurd *et al.* 2009; Raven and Hurd 2012; Koch *et al.* 2013). An increased availability of CO₂ in coastal zones may therefore shift the competitive balance between subordinate species and their naturally dominant competitors (Connell *et al.* 2013).

The ability of herbivores to counter the elevated primary productivity of opportunistic species does not occur independently of resource availability because increased quality or availability of food can stimulate greater *per capita* feeding rates in herbivores under elevated nitrogen (Russell and Connell 2007) and carbon (Falkenberg *et al.* 2013c). Additionally, an increase in either food quality or quantity can stimulate an increase in metabolic rate (Lilly 1979), likely because of the increased energetic costs of processing the extra food (reviewed by Secor 2009). However, whether grazers can counter the increased growth of algal turf under the combined stress of elevated nitrogen and future CO₂ remains unclear.

Invertebrate mesograzers, such as gastropods, can exert strong trophic effects in temperate marine systems (Underwood 1980) by grazing opportunistic filamentous algae (Steneck and Watling 1982). If elevated resources can stimulate these herbivores into consuming this excess primary production then it should be possible to not only detect a proportional increase in consumption, but also a metabolic basis for the change in rate of consumption. We experimentally tested the hypotheses that (1) elevated nitrogen (N) and carbon dioxide (CO₂) not only stimulate an increase in consumptive rates, but also stimulate an increase in underlying metabolic rates of gastropod herbivores, so that (2) enhanced primary productivity is countered by herbivory.

Materials and methods

Experimental design

The effects of nitrogen and CO₂ on gastropod grazing and metabolic rate, and the expansion of algal turf were tested in a

laboratory microcosm experiment (SARDI Aquatic Sciences, Adelaide). Algae and grazers were subjected to nitrogen (ambient *v.* elevated) and CO₂ (current *v.* future) in an orthogonal design, with five replicate microcosms per treatment combination. Elevated nitrogen treatments were designed to replicate moderately enriched concentrations experienced in waters off the coast of Adelaide, South Australia. Target dissolved inorganic nitrogen (DIN) for enriched treatments was based on the concentration adjacent to agricultural catchments under heavy rainfall ($0.126 \pm 0.019 \text{ mg L}^{-1}$) (Gorman *et al.* 2009). Target concentrations for CO₂ were based on current conditions (~400 ppm) and RCP 8.5 model predictions for the year 2100 (scenario range 794–1142 ppm; Collins *et al.* 2013). Actual treatment *p*CO₂ in microcosms were respectively ~400 and ~850 with the corresponding pH of ~8.07 and ~7.81 (see Table 1). To estimate consumption in each replicate tank, all microcosms were divided into two equal halves, one side with, and the other without, a gastropod, so that the percentage cover of turfs consumed was calculated as the difference between the absence and presence of grazers. Although these estimates of consumption are independently measured among replicates, the individual estimates of responses of turf cover for each treatment of grazing and non-grazing are not independent (see *Data analysis* below). Each microcosm contained one gastropod in the grazing half, simulating natural herbivore densities (~110 individuals m⁻²), of the collection site (Russell and Connell 2005).

Each 20-L microcosm (L × W × H: 50 × 20 × 30 cm) was supplied with filtered natural seawater (three-stage particulate filter: 20-, 0.5- and 0.2-µm cartridges) in an open flow-through system. To ensure the quality of water conditions within the experimental microcosms, fresh seawater was constantly delivered at 8 L h⁻¹. Lighting was supplied in a 14:10 h light:dark cycle (to mimic summer conditions) by twelve 150-W metal halide flood lights (Pierlite), located 1 m above the experimental tanks (spectrum 14 000 K), giving an irradiance of ~150 µmol m⁻² s⁻¹ at the water surface. Temperature within the microcosms was set to fluctuate on a diurnal cycle between 21° and 23°C using heaters, to simulate summer conditions of shallow coastal water temperatures along the coast of South Australia. All microcosms were continually aerated at 10 L min⁻¹ with air.

Algal turfs (*Feldmannia* spp.) and associated algae-consuming gastropods (*Austrocochlea constricta*) were initially collected from the rocky intertidal zone (>1 m) at Encounter Bay, South Australia (35°34'S, 138°36'E). Algal specimens were placed in holding microcosms for 10 weeks before the experiment

Table 1. The measured and calculated seawater conditions within microcosms

Data shown are mean ± s.e. Measured: total alkalinity (A_T) and pH. Calculated: partial pressure CO₂ (*p*CO₂), carbonate (CO₃²⁻), bicarbonate (HCO₃⁻) and saturation states of calcite (Ω_{calc.}) and aragonite (Ω_{arag.}). Values were calculated from measured alkalinity (A_T) and pH (seawater scale) using constants from Mehrbach *et al.* (1973), as adjusted by Dickson and Millero (1987). EN, elevated nitrogen; FCO₂, future CO₂; EN + FCO₂, elevated nitrogen + future CO₂

Treatment	A _T	pH	<i>p</i> CO ₂	CO ₃ ²⁻	HCO ₃ ⁻	Ω _{calc.}	Ω _{arag.}
Ambient	2538 ± 5.6	8.07 ± 0.08	424 ± 85	257 ± 37	1906 ± 91	5.93 ± 0.85	3.89 ± 0.56
EN	2538 ± 3.5	8.07 ± 0.05	400 ± 50	257 ± 22	1905 ± 57	5.93 ± 0.52	3.89 ± 0.34
FCO ₂	2542 ± 1.4	7.81 ± 0.04	847 ± 57	156 ± 12	2161 ± 27	3.58 ± 0.27	2.35 ± 0.17
EN + FCO ₂	2541 ± 0.8	7.79 ± 0.03	854 ± 93	152 ± 13	2168 ± 32	3.51 ± 0.30	2.30 ± 0.20

commenced to acclimate to laboratory conditions and to allow algae to recruit to unoccupied PVC tiles (5 × 5 cm). Gastropods were placed in holding microcosms for 2 weeks before the commencement of the experiment to acclimate to laboratory conditions. Throughout acclimation, laboratory conditions corresponded to those at the collection site, at the time of algal collection (i.e. 18–19°C, ambient nitrogen and current atmospheric CO₂ conditions). After the acclimation period, tiles with approximately the same cover of algal turf were randomly reallocated to the experimental microcosms, to seed the experimental substrate. Experimental conditions were then gradually altered over a further week until predesignated levels were reached for elevated nitrogen and future CO₂.

Nitrogen and CO₂ addition

Nitrogen was supplied to elevated treatments as 4 g of ‘Osmocote’ controlled-release fertiliser (12-month release, 17:4.3:8.2 N:P:K). Fertiliser pellets were placed in a nylon mesh bag (1-mm mesh size) and positioned at the bottom of each elevated-treatment microcosm. This method of elevating nutrients has been successfully used in previous subtidal and laboratory experiments (see Worm *et al.* 2000; Russell and Connell 2007; Russell *et al.* 2009; Falkenberg *et al.* 2013a). To validate the concentration of the nitrogen addition water samples were drawn from each microcosm using 25-mL sterile syringes. Samples were then filtered (0.45-µm filter) and immediately frozen to allow analyses on a Lachat Quickchem 8200 Flow Injection Analyser (Hach, Loveland, CO, USA).

Experimental conditions in the future CO₂ treatments were maintained using pH electrodes (Aquatronica ACQ310N, Reggio Emilia, Italy) and automatic solenoid controllers (Dupla, Geldsdorf, Germany), which allowed CO₂ to be directly diffused into the water column of each individual microcosm. pH and temperature of individual tanks were recorded every 3 h using data loggers (Aquatronica ACQ110, Reggio Emilia, Italy). pH electrodes were calibrated using NBS calibration buffers and pH readings were corrected to the seawater scale using TRIS (2-amino-2-hydroxymethyl-1,3-propanediol, pH ~8.1) and AMP (2-aminopyridine, pH ~6.8) buffers twice per week. Total alkalinity (A_T) in each experimental treatment was measured on a fortnightly basis using a Titrand alkalinity titrator (Metroham, Switzerland). Concentrations of pCO₂, carbonate (CO₃²⁻), bicarbonate (HCO₃⁻) and saturation states of calcite and aragonite were then calculated from the A_T, pH (seawater scale), salinity and temperature measurements, using CO2SYS for Excel with constants from Mehrbach *et al.* (1973), as adjusted by Dickson and Millero (1987).

Response variables

Upon commencement of the experiment, additional preweighed PVC tiles (5 × 5 cm) were randomly allocated to the experimental microcosms to quantify either the response of grazers or the response of algae to the experimental conditions ($n = 4$ tiles per half of microcosm). These tiles were initially devoid of algae and used as unoccupied substrate to allow algae to recruit and expand in either the presence or absence of a grazer. Response variables were measured 36 days after the commencement of experimental treatments.

The response of grazing to the experimental conditions was assessed by measuring the consumption of algal turf (%), as well as oxygen consumption (mg O₂ min⁻¹ g⁻¹), as an approximation of resting metabolic rate. The consumption of turf in each microcosm was calculated from the change in percentage cover of turf in the absence and presence of grazers. The measurement of basal metabolic rate is difficult in motile organisms such as gastropods; however, the measurement of resting oxygen consumption is regarded as a good approximation of routine metabolic rate if movement of the individual is restricted (Sokolova and Pörtner 2003). Oxygen consumption of the gastropods was measured using an optical sensor system (Ocean Optics, Dunedin, FL, USA). Gastropods were allowed to consume algae until the commencement of measurements. Prior to determining metabolic rate the gastropod shells were cleaned with 95% ethanol to remove any potential microorganisms (Innes and Houlihan 1985; Sokolova and Pörtner 2003). Individual gastropods were then chased into their shells while submerged to remove excess water and placed in a 50-mL syringe containing an optical sensor and filled with seawater at the same temperature as experimental treatments. Syringes were then plunged to expel any air in the chamber and sealed using a luer lock 3-way valve. Gastropod movement was minimal during the measurement period. Blank chambers were also run to determine the respiration of any microorganisms in the seawater. Gastropods were then euthanased, extracted from their shells, dried and weighed in order to calculate mass-specific metabolic rate.

The response of algal turf to the experimental conditions was quantified as the percentage cover of algae on initially unoccupied substrate in both the absence and presence of a grazer. The percentage cover of algae was visually estimated by overlaying each tile with a 5 × 5-cm quadrat that was divided into a grid of 25 evenly spaced squares (Drummond and Connell 2005), and counting the number of squares that were occupied by algae.

Data analysis

First, a linear regression was used to test whether there was a relationship between oxygen consumption by herbivores and their rates of consumption of algae across all treatments. Second, two-factor analysis of variances (ANOVAs) were used to test for the independent and interactive effects of resource enhancement (nitrogen and CO₂) on (1) the consumption of algal turf by grazers (i.e. algae cover in the absence of a grazer – algae cover in the presence of a grazer) and (2) the percentage cover of algae turf in the absence of grazers. Both factors of resource enhancement (nitrogen and CO₂) were treated as fixed and orthogonal, with two levels in each factor (ambient *v.* elevated and current *v.* future respectively), with $n = 5$ replicate microcosms per treatment combination. Where a significant interaction was detected, Student–Newman–Keuls (SNK) *post hoc* comparison of means was used to determine which factors differed. Third, ANOVAs were used to test whether the concentration of DIN and phosphate differed between ambient and elevated nutrient treatments. Prior to analyses, homogeneity of variances was tested using Cochran’s *C*-test and data were appropriately transformed if required.

Results

An increase in oxygen consumption due to resource enhancement was associated with an increase in rates of consumption by herbivores (linear regression: $P < 0.001$, $R^2 = 0.536$) (Fig. 1). The effect of resource enhancement on herbivore metabolism and consumption was similar for both nitrogen and CO₂, but greater in combination (Fig. 1). Indeed, the consumption rates of algae increased from 33% under ambient conditions to 76% under resource enhancement (ANOVA: $F_{1,16} = 4.60$, $P = 0.047$) (Fig. 2).

There was a positive effect of resource enhancement on turfs when herbivores were absent, but this was eliminated by grazing. Both elevated nitrogen and future CO₂ independently

caused expansion of turfs (Fig. 2), but cover was greatest when both conditions were experienced in combination (40% greater than ambient conditions) (ANOVA: $F_{1,16} = 5.61$, $P = 0.03$) (Fig. 2). Grazers eliminated these effects of resource enhancement such that these treatments were not significantly different from ambient treatments when grazers were present (ANOVA: $F_{1,16} = 0.91$, $P = 0.32$ and $F_{1,16} = 0.46$, $P = 0.45$, for nitrogen and CO₂ respectively) (Fig. 2). The lack of differences amongst treatments with grazers underscores grazing as a process that counters the effects of enrichment (Fig. 2).

The concentration of DIN was greater in the elevated (0.129 ± 0.029 mg L⁻¹, mean \pm s.e.) than ambient (0.060 ± 0.008) nitrogen treatments (PERMANOVA: $F_{1,15} = 4.841$,

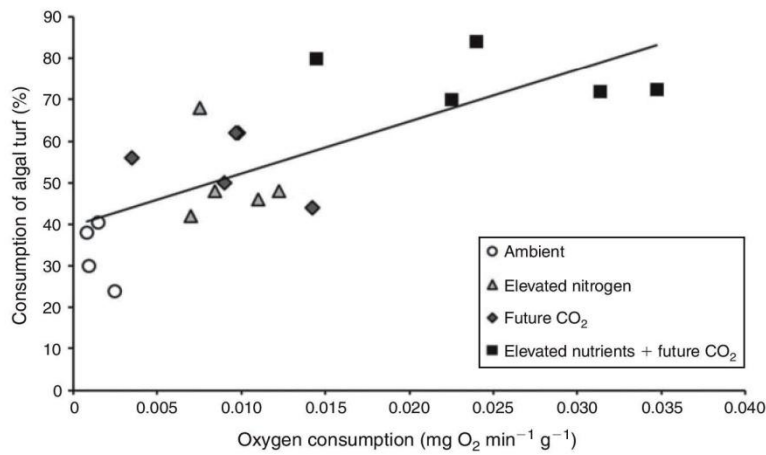


Fig. 1. Scatter plot showing the relationship between oxygen consumption by gastropods (x-axis) and consumption of turf (y-axis) when subjected to ambient, elevated nitrogen, future CO₂ and elevated nitrogen + future CO₂ conditions. Solid line: best-fit linear regression. There are only four values for the ambient treatment due to the loss of a replicate.

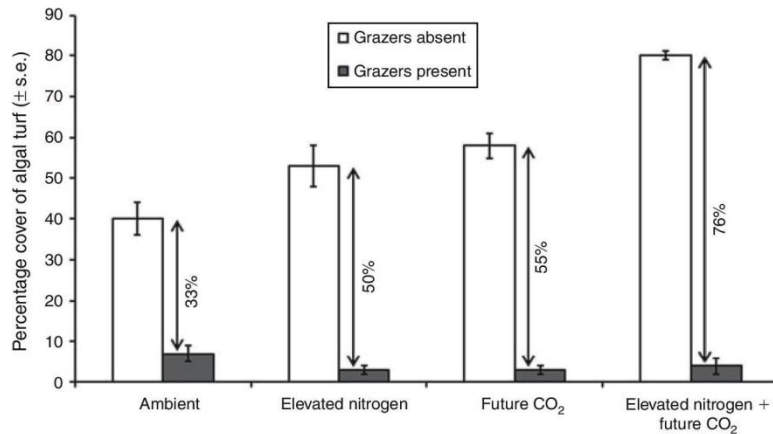


Fig. 2. The percentage cover of algal turf in the absence and presence of grazers when subjected to ambient, elevated nitrogen, future CO₂ and elevated nitrogen + future CO₂ conditions. Arrows represent grazer consumption, being the percentage of algal turf consumed by grazers. Values are mean \pm s.e.

$P=0.032$). The concentration of phosphate did not differ between treatments (elevated nitrogen = 0.029 ± 0.0006 , ambient nitrogen = 0.023 ± 0.003) (PERMANOVA: $F_{1,18} = 3.022$, $P=0.162$).

The pH, concentration of CO_3^{2-} and saturation state of calcite and aragonite were reduced in future CO_2 treatments compared with ambient CO_2 treatments (Table 1). In comparison, the concentration of $p\text{CO}_2$ and HCO_3^- were increased in the future CO_2 treatments compared with ambient CO_2 treatments (Table 1).

Discussion

Perturbation by resource enhancement can displace ecosystems based on large perennials to small ephemerals by favouring the growth and persistence of opportunistic species. Herbivores can mediate the effects of local nutrient enrichment by consuming faster-growing subordinates, which can subsequently dominate available space, and drive ecosystem shifts (Lotze *et al.* 2001; Worm and Lotze 2006; Russell and Connell 2007). Although appearing strong, however, the ability of herbivores to compensate for the increased productivity is an under-investigated component of marine systems (Ghedini *et al.* 2015). Here, we show that grazers countered the combined effects of elevated nitrogen and CO_2 by consuming larger quantities of algal turfs exposed to these conditions; the proportion of algae consumed by grazers was equal to the growth of algae in response to the increased availability of resources.

Importantly, the present study is one of the first to show that the increased feeding rates in elevated nitrogen and CO_2 treatments is associated with an increase in oxygen consumption and hence metabolic rate of herbivores. It appears that an increase in resource availability may not only stimulate elevated primary productivity, but also elevated consumption and metabolic rate. These results suggest that gastropods may dampen the effects of combined global and local resource enhancement by absorbing the additional productivity of opportunistic species. The net effect of grazing, however, would depend on the amount of resource enhancement. Recent work suggests that grazers can counter increasing levels of resource enhancement up until a point, after which productivity escapes regulation (Ghedini *et al.* 2015; N. L. Mertens, B. D. Russell and S. D. Connell, unpubl. data) and drives competitive shifts in dominance and ecosystem state (Connell *et al.* 2013).

Resource enhancement can alter the interactions between plants and herbivores by modifying the nutritional value of plants and influencing the physiology of herbivores (Cruz-Rivera and Hay 2000). Under periods of nutrient enrichment, algal turfs can rapidly take up available nitrogen (Hein *et al.* 1995), and grazers have been shown to actively select and preferentially consume greater amounts of such algae due to the increase in nutrient content (Neckles *et al.* 1993; Miller *et al.* 1999; Russell and Connell 2007). Additionally, elevated CO_2 can modify the nitrogen content of algal turfs, resulting in greater *per capita* feeding rates of gastropod grazers (Falkenberg *et al.* 2013c). Similarly, we found that gastropods consumed greater quantities of turf exposed to elevated nitrogen and future CO_2 conditions. Importantly, the proportion of algae consumed by grazers was equal to the response of algae to the increased availability of resources.

Altered environmental conditions can have direct effects on the physiology of herbivores (O'Connor 2009; Cummings *et al.* 2011; Russell *et al.* 2013). Future CO_2 conditions have been shown to reduce the feeding abilities of calcifying marine invertebrates (Siikavuopio *et al.* 2007), potentially limiting their ability to compensate for the increased productivity of algae under resource enhancement. In contrast, we found that gastropods consumed greater quantities of algae under the combination of future CO_2 and elevated nitrogen conditions, relative to ambient conditions. We propose that combined resource enhancement (elevated nitrogen and future CO_2) and subsequent increase in the quality and quantity of food (e.g. Falkenberg *et al.* 2014) directly affect the metabolic demands of gastropods. An increase in metabolism has been found in response to elevated CO_2 conditions (and thus ocean acidification), and is thought to be in response to the enhanced energy demand required to manage the change in external acidity and to aid in the maintenance and repair of physiological processes (Wood *et al.* 2008; Cummings *et al.* 2011; Li and Gao 2012). The present study is one of the first to show that the metabolic rate of herbivores increases under increasing local and global resource enhancement.

The notion that oxygen consumption increases due to the quality, availability and predictability of diet of endothermic organisms is well established, and is termed the 'food-habit hypothesis' (see review by Cruz-Neto and Bozinovic 2004). This hypothesis proposes that organisms subjected to low-quality diets will have low metabolic rates (McNab 1986; Bozinovic and Novoa 1997; Cruz-Neto and Bozinovic 2004). For example, the metabolism of juvenile sea lions was 16% lower when individuals were fed a low-energy diet of squid in comparison to those fed a high-energy diet of herring (Rosen and Trites 1999). It has further been suggested that the quantity (Wallace 1973) as well as the quality of available food influences the metabolic rate of ectothermic organisms (Lilly 1979). Indeed, a study assessing oxygen consumption rates of sea urchins found that oxygen uptake increased when the urchins consumed algae that had higher growth-supporting values than when they consumed seagrass thought to have low growth-supporting values (Lilly 1979). Metabolic response to change of diet may, however, vary between taxa. For example, fish fed on diets high in protein had significantly lower oxygen-consumption rates than fish fed lower-protein diets (Stadtlander *et al.* 2013). Algal turfs are known to rapidly absorb nitrogen under periods of resource availability and, in response, may have higher nutritional value than algal turfs not exposed to elevated nitrogen (Falkenberg *et al.* 2013b). Additionally, the quantity of algae available to grazers was greatest in the combined nitrogen and CO_2 treatments. This suggests that the increase in metabolic rate seen in this treatment may be in response to both the quality and quantity of food consumed by grazers, in addition to any direct effects of elevated CO_2 .

In conclusion, to improve models that account for ecosystem stability we need to be aware of the existence of mechanisms that resist environmental change. Perturbation by resource enhancement invariably has unequal effects on competitors, often turning subordinates into dominants (Connell *et al.* 2013). If, however, such resource perturbations also stimulate herbivores to consume the excess production of subordinates

(e.g. counter their expansion), then it should be possible to not only detect a proportional increase in consumption (Ghedini *et al.* 2015), but also observe a metabolic basis for this change in rate of consumption. We show that herbivores can increase *per capita* consumption and their underlying metabolic rate when exposed to global (i.e. future CO₂) and local (i.e. elevated nitrogen) perturbations. Resource enhancement, therefore, may stimulate herbivores to counter the direct positive effects of resources on subordinates and thereby hold competitive balances in check. Stability is enhanced because the specific perturbation that stimulates greater competitive potential in subordinates also stimulates resistance to this potential. The probability for displacement of the existing community dominants by weedy species, therefore, remains stable because resistance to change increases, which of its nature is difficult to detect in natural communities unless the ecosystem switches.

Acknowledgements

Submission of this manuscript was invited by the organisers of the Tenth International Temperate Reefs Symposium, Perth. We thank K. Wiltshire, E. Brock, N. Mertens and L. Falkenberg for their laboratory assistance. Our research was funded by Australian Research Council grants to S. D. Connell and B. D. Russell, an Australian Postgraduate Award scholarship to C. McSkimming and South Australian Research and Development Institute (SARDI) Aquatic Sciences. This paper will be included in C. McSkimming's Ph.D. thesis.

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CHAPTER 4

STATEMENT OF AUTHORSHIP

Title of Paper	Habitat restoration: Rates, early signs and extent of faunal recovery relative to seagrass recovery
Publication Status	<input type="checkbox"/> Published <input checked="" type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Publication Style
Publication Details	McSkimming, C., Connell, S.D., Russell, B.D. & Tanner, J.E. Habitat restoration: Rates, early signs and extent of faunal recovery relative to seagrass recovery. <i>Estuarine, Coastal and Shelf Science</i> . Accepted with revisions 30/06/2015.

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Contribution to the Paper	Decided on collection methods, assisted with sample collection, processed the samples, analysed and interpreted the data and drafted and revised the manuscript.		
Overall percentage (%)	80		
Signature		Date	22/07/2015

CO-AUTHOR CONTRIBUTIONS

By signing the Statement of Authorship, each author certifies that:

- vii. the candidate's stated contribution to the publication is accurate (as detailed above);
- viii. permission is granted for the candidate to include the publication in the thesis; and
- ix. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sean D Connell		
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Contribution to the Paper	Provided manuscript feedback and assisted with revisions. Provided funding.		
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Contribution to the Paper	The existing restoration plots that were sampled for this chapter were established and maintained by Jason Tanner, who also assisted with the experimental design for this chapter, collected the samples and assisted with interpretation and manuscript development. Acted as corresponding author and provided funding.		
Signature		Date	27/07/2015

CHAPTER 4

HABITAT RESTORATION: RATES, EARLY SIGNS AND EXTENT OF FAUNAL RECOVERY RELATIVE TO SEAGRASS RECOVERY

4.1 ABSTRACT

The overall intent of restoration is often not only to restore the habitat *per se*, but to restore the ecosystem services it supplies, and particularly to encourage the return of fauna.

Seagrass meadows act as habitat for some of the most diverse and abundant animal life, and as the global loss of seagrass continues, managers have sought to restore lost

meadows. We tested how quickly the faunal richness, abundance and community

composition of experimental restoration plots recovered to that in an adjacent natural

seagrass meadow relative to rates of recovery of seagrass *per se*. Seagrass structure in the

restoration plots took three years to become similar to a nearby natural meadow. The

recovery of overall faunal richness and abundance, however, occurred within one year.

These results suggest that although recovering habitats may not be structurally similar to

undisturbed habitats, they can support a similar richness and abundances of fauna, and thus

have greater economic and social value than otherwise might have been expected.

Nevertheless, whilst faunal richness and total abundance recovered prior to the recovery of

seagrass structure, full recovery of seagrass was required before the composition and

relative abundances of the faunal community matched that of the natural seagrass meadow.

4.2 INTRODUCTION

Habitat restoration can help to alleviate habitat loss or re-establish ecosystem structure and function (Elliott *et al.* 2007; Reynolds *et al.* 2013). Often, a primary motivation for habitat restoration is to restore the richness and abundance of fauna associated with the lost habitats (e.g. Muotka *et al.* 2002; Ruiz-Jaén & Aide 2005; Trexler & Goss 2009).

However, restoration success varies, due to the inherent difficulties involved in restoring complex environments (Elliott *et al.* 2007; Irving *et al.* 2010). Further, ecosystems are not stable through time, meaning the “baseline” that should be used for restoration targets is often uncertain. Therefore, setting goals for restoration success based solely on compositional or structural attributes that were characteristic of the system prior to disturbance can be problematic (Hobbs & Harris 2001).

Restoration success is often most reliably assessed by comparing structural and functional attributes of the restoration site to those of a neighbouring undegraded habitat or reference site (Hobbs & Harris 2001; Ruiz-Jaén & Aide 2005; Benayas *et al.* 2009). A general element of structural restoration is the replenishment of plant species which provide the physical structure of an ecosystem (McCay *et al.* 2003). Recovery of structure, however, does not necessarily lead to the return of ecosystem function (Zedler & Lindig-Cisneros 2000). For example, arthropod diversity in restored coastal sage scrub was lower than in undisturbed habitat after 15 years, even though vegetation was structurally similar (Longcore 2003).

In marine systems, seagrass meadows form ecologically and economically important coastal habitats (Short & Wyllie-Echeverria 1996; Beck *et al.* 2001; Duarte 2002; Orth *et al.* 2006). Due to their coastal location, seagrass meadows are highly susceptible to

disturbance from natural and anthropogenic sources (Short & Wyllie-Echeverria 1996; Ralph *et al.* 2006), and approximately 29 % of the world's seagrass habitat has been lost (Waycott *et al.* 2009). As a consequence, seagrass restoration has become an element of coastal management, with early research primarily focused on establishing the most effective techniques of transplantation (Van Keulen *et al.* 2003; Bell *et al.* 2008; Cunha *et al.* 2012).

The success of seagrass restoration projects has, however, been limited, with only 30 % of studies reporting success (Fonseca *et al.* 1998), which is thought to be primarily due to poor site selection (Fonseca 2011). Restoration success can be defined by a lack of detectable differences in structure (e.g. shoot density; Fonseca *et al.* 2000) between recovering treatments and undisturbed treatments. Studies that do report 'success', generally do so based on short-term monitoring (< 1 year), and hence long-term success is not known (Cunha *et al.* 2012). Increasing the length of restoration monitoring may increase the ability to identify successful restoration. For example, long-term monitoring of seagrass restoration near Tampa Bay Florida, showed the recovery of seagrass to be slow during the first 3 years, which was followed by the rapid recovery 4-7 years after restoration was implemented (Bell *et al.* 2014). Further, the recovery of ecosystem function, such as the recovery of fauna due to the provision of habitat, may be used to assess restoration success (e.g. Bell *et al.* 1993; Fonseca *et al.* 1996b; Sheridan *et al.* 2003). For restoration to be successful, restored seagrass patches should persist and recover similar ecosystem function to that of a natural undisturbed seagrass meadow (Fonseca *et al.* 1998).

Wear *et al.* (2010) developed a novel seagrass restoration technique, using biodegradable hessian (burlap) bags to stabilize the sediment and facilitate the natural recruitment of *Amphibolis antarctica* seedlings, with the overall intention of re-establishing an extensive continuous seagrass meadow, which was present in the area prior to substantial seagrass loss (> 5,200 ha) (Neverauskas 1987; Nayar *et al.* 2012). This technique has allowed *A. antarctica* seedlings to become established and create new patches (Irving *et al.* 2013), which have persisted for > 5 years (Tanner 2014). Here, we explicitly seek to estimate the rate of faunal recovery of these restoration patches. We define the rate and extent of faunal recovery as the time taken and the degree to which faunal abundance, richness and composition become similar to a naturally occurring seagrass meadow, respectively. To estimate this rate, we compare initially small and expanding patches of restored seagrass to an adjacent continuous natural seagrass meadow. We consider this to be the gold standard for recovery in this situation, as this meadow is well established (hundreds if not thousands of years), large, and not subject to fragmentation, and thus should provide the best possible habitat for fauna. Additionally, we tested whether the rate of recovery of faunal inhabitants in these restoration patches matched the rate of recovery of seagrass. If faunal recovery occurs before seagrass recovery, then demonstrating this may assist managers by showing early signs of achievement, thus justifying continued investment in restoration.

4.3 MATERIALS AND METHODS

4.3.1 RESTORATION SITE AND SAMPLING DESIGN

Structural recovery and faunal use were examined in an experimental seagrass restoration site located just inshore of a large, naturally occurring *A. antarctica* meadow, in approximately 8 m water depth, along the Adelaide metropolitan coast, South Australia

(35° 1' S, 138° 18' E). The natural *A. antarctica* meadow consists of a dense continuous canopy with the edge of the meadow being an abrupt change from dense seagrass to bare sand. The current edge of the natural seagrass meadow marks the margin of seaward retreat of inshore seagrass at this site due to eutrophication (Westphalen *et al.* 2004). In recent years, extensive effort has been invested in improving water quality, allowing a small amount of natural seagrass recovery in deeper waters (Bryars & Neverauskas 2004), and prompted initial studies on restoration.

Restoration trials at this site began in 2007 by deploying hessian bags to promote the recruitment of *A. antarctica* seedlings, which are released from the adjacent natural meadow. Hessian bags (area 0.35 m² per bag) were deployed approximately bimonthly, from September 2007 to October 2009 and again from January 2011 to March 2013. On each deployment, ten replicate bags, which represent a restoration plot, were filled with ~ 25 kg of clean play pit sand to anchor and deployed on sandy substrate, shoreward of and parallel to the natural meadow. Bags were placed end-to-end in a double row by divers ~ 0.5 – 1 m apart, making restoration plots rectangular in shape. Each bimonthly deployment was separated by ~ 2 to 3 m and there was a minimum distance of 10 m between restoration plots deployed in different years. All bags were deployed within 50 m of the natural meadow, and extended over a distance of ~ 100 m. The variation in recruitment of *A. antarctica* seedlings with distance from the natural meadow has previously been tested at this site, and bags located within ~ 80 m of the natural meadow effectively recruit *A. antarctica* seedlings (Irving *et al.* 2013). While not formally measured due to the small size of the entire site (~ 2 km between the edge of the seagrass and shoreline, Wear *et al.* 2010) there were no obvious environmental gradients present. Importantly, there was no measurable difference in water depth between the offshore and

inshore margin of the restoration site (~ 8 m water depth). In addition, previous measurements showed that seafloor light intensities at this site averaged 15 – 18 % of surface irradiance ($86.83 \pm 22.71 \mu\text{mol m}^2 \text{s}^{-1}$) (Irving *et al.* 2010).

We used a space-for-time substitution approach to establish the rate of recovery of the restoration site. Space-for-time substitution (SFT) has long been used in ecology, particularly as a standard method for looking at successional theory (Pickett 1989; Kratz *et al.* 2003), and involves comparing sites of different ages at a single point in time, rather than sites that were established at a single point in time and sampled at different ages. This technique has allowed us to assess the rate and extent of faunal recovery by taking a series of samples from restoration plots of known ages, representing a “single snapshot” of succession, instead of sampling the one site multiple times. *A. antarctica* samples with associated fauna were collected from three restoration plots of known ages (based on year and month of bag deployment), 1 year (July 2011 deployment), 3 years (February 2009 deployment) and 5 years (September 2007 deployment). The three restoration plots were pre-selected based on their relatively high recruitment rates and subsequently, high stem densities for year of deployment as observed in January 2012 (Tanner 2014). Seagrass within the 1 year old restoration plot was still constrained within the boundaries of the bags, whereas vegetative expansion of seagrass had occurred within the 3 year old (~ 10 cm from the bags) and 5 year old (seagrass coalescence) restoration plots (J Tanner, per obs). *A. antarctica* samples were also collected from two plots within the adjacent natural meadow, the edge (defined as within 0.5 m of the abrupt boundary that divides seagrass and bare sand) and the interior (~20 m into the natural meadow, $n = 5$ per site).

Restoration samples were collected from the centre of five randomly selected bags from each of the three restoration plots (1 year, 3 years and 5 years), while a 20 cm x 20 cm

quadrat was haphazardly thrown five times and a sample taken from the centre for the natural meadow (or the quadrat rethrown if it did not land within 0.5 m of the edge for edge samples). All samples were collected in July 2012, using a 9.0 cm internal diameter (area of 64 cm²) PVC corer attached to a fine mesh bag (mesh size 0.5 mm). The corer was carefully placed over the seagrass, flush with the sediment surface. The seagrass was then cut at the substrate surface using a serrated knife and the mesh bag was tied closed to prevent the escape of motile fauna. Samples were then drained into the mesh bag and preserved in 10 % formalin solution until sorted.

4.3.2 RESPONSE VARIABLES

All samples were sieved using a 1 mm mesh screen and sorted under magnification in the laboratory. Motile fauna were removed, counted and identified to the highest taxonomic resolution possible, for most taxa family, except for some rare or poorly known taxa which could only be reliably identified to phylum or class. In addition, the seagrass structure itself was quantified as aboveground seagrass biomass (g dry weight of stems, branches and leaves [DW] m⁻²), stem length (cm) and density (no. m⁻²), leaf cluster density (no. m⁻²) and stem and leaf epiphyte biomass (g dry weight [DW] m⁻²). *Amphibolis antarctica* has wirey stems ~15 cm long, and branches that are topped by clusters of 5-10 leaves ~5 cm long (Ducker *et al.* 1977; Verduin & Backhaus 2000). Stem length was measured from the base of the stem to the top of the most distal leaf cluster. All epiphytes were carefully scraped from the seagrass using a scalpel blade. Epiphytes and epiphyte-free seagrass were then placed in separate pre-weighed aluminium foil trays and dried to a constant weight at 60° C for 72 hours.

4.3.3 DATA ANALYSIS

To establish whether the physical structure and faunal composition of *A. antarctica* varied between the restoration plots of known ages and the natural meadow, one-way permutational multivariate analyses of variances (PERMANOVA), followed by pairwise tests, were used. Euclidean distance was used for the physical structure analysis, while the Bray-Curtis similarity measure was used with fourth root transformed data for the faunal composition. When the PERMANOVA was significant ($p < 0.05$), separate univariate analyses using Euclidean distance, followed by pairwise tests, were run on each of the individual seagrass structural variables. Univariate analyses were also used to determine whether faunal richness and abundance varied between the restoration plots and the natural meadow. Fauna were then grouped into the three most abundant classes (amphipods, gastropods and polychaetes) and analysed similarly. Bray-Curtis similarity measures for the multivariate analyses of seagrass structure and faunal composition were plotted to assess the rates of convergence of seagrass structure and faunal composition onto their natural counterpart (the interior). All multivariate and univariate analyses were carried out in PRIMER (version 6) with the PERMANOVA + add-on (PRIMER-E Ltd, Plymouth).

4.4 RESULTS

The physical structure of *A. antarctica* differed between the restoration plots and the natural meadow (PERMANOVA: $F_{4, 20} = 4.534$, $p = 0.005$), with pairwise tests showing the structure of the 1 year old restoration plot being different to the older restoration plots (3 and 5 years old) and the natural meadow (edge and interior). The older plots did not differ from the natural meadow. Patch age had a clear effect on above-ground biomass, leaf cluster density, stem epiphytic biomass and leaf epiphytic biomass (Table 4.1a, d, e

and f, Fig. 4.1a, d, e and f), with 1 year old plots having significantly lower values than all other restoration plots and the natural meadow. Stem length was significantly shorter in the restoration plots (1 year, 3 years and 5 years) than the natural meadow (edge and interior) (Table 4.1b and Fig. 4.1b). Stem length also differed significantly within the natural meadow; seagrass in the interior of the meadow was significantly shorter than at the edge of the natural meadow. There was no difference in stem density between the three restoration plots and the natural meadow (Table 4.1c and Fig.4.1c).

Faunal composition differed significantly between plots (PERMANOVA: $F_{4,20} = 1.70$, $p = 0.002$), with pairwise tests showing the 1 year old plot to be different to the older restoration plots (3 and 5 years old) and the natural meadow (edge and interior). Faunal composition in the 3 and 5 year old restoration plots did not differ from the natural meadow. There was no difference in faunal richness between the three restoration plots of known ages (1, 3 and 5 years old) and the natural meadow (Fig. 4.2a, PERMANOVA: $F_{4,20} = 2.509$, $p = 0.07$). Faunal abundance differed significantly between the restoration plots (Fig. 4.2b, PERMANOVA: $F_{4,20} = 3.09$, $p = 0.034$), which was due to a lower abundance in the 1 year old plot than the 5 year old plot. However, there was no difference in faunal abundance between any of the three restoration plots and the natural meadow (Fig. 4.2b). There was no difference in gastropod and amphipod abundance between the restoration plots and natural meadow (Fig. 4.3a, b, PERMANOVA: $F_{4,20} = 1.93$, $p = 0.139$ and $F_{4,20} = 1.30$, $p = 0.296$, respectively), however, polychaete abundance was lower in the 1 year old plot than the 5 year old plot and the interior of the natural meadow (Fig. 4.3c, PERMANOVA: $F_{4,20} = 2.175$, $p = 0.039$).

The scatter plot (Fig. 4.4) showed that samples from the 1 year old restoration plot are the most different from the natural meadow. Samples from the older restoration plots (3 and 5 years) are interspersed with samples from the natural meadow, showing that both seagrass structure and faunal composition in these plots are relatively similar to the natural meadow.

Table 4.1. The structural characteristics of *A. antarctica* (aboveground biomass, stem length, stem density, leaf clusters, stem epiphytic biomass and leaf epiphytic biomass) as a function of site, as determined by one-factor PERMANOVAs.

Source	df	MS	<i>F</i>	<i>P</i>
<i>(a) Aboveground biomass</i>				
Site	4	394600	3.732	0.026
Residual	20	105720		
<i>(b) Stem length</i>				
Site	4	878.180	37.224	0.001
Residual	20	23.592		
<i>(c) Stem density</i>				
Site	4	374510	1.013	0.437
Residual	20	369630		
<i>(d) Leaf clusters</i>				
Site	4	59688000	4.984	0.006
Residual	20	11976000		
<i>(e) Stem epiphytic biomass</i>				
Site	4	31558	2.711	0.041
Residual	20	11641		
<i>(f) Leaf epiphytic biomass</i>				
Site	4	9506.8	3.781	0.021
Residual	20	2514.6		

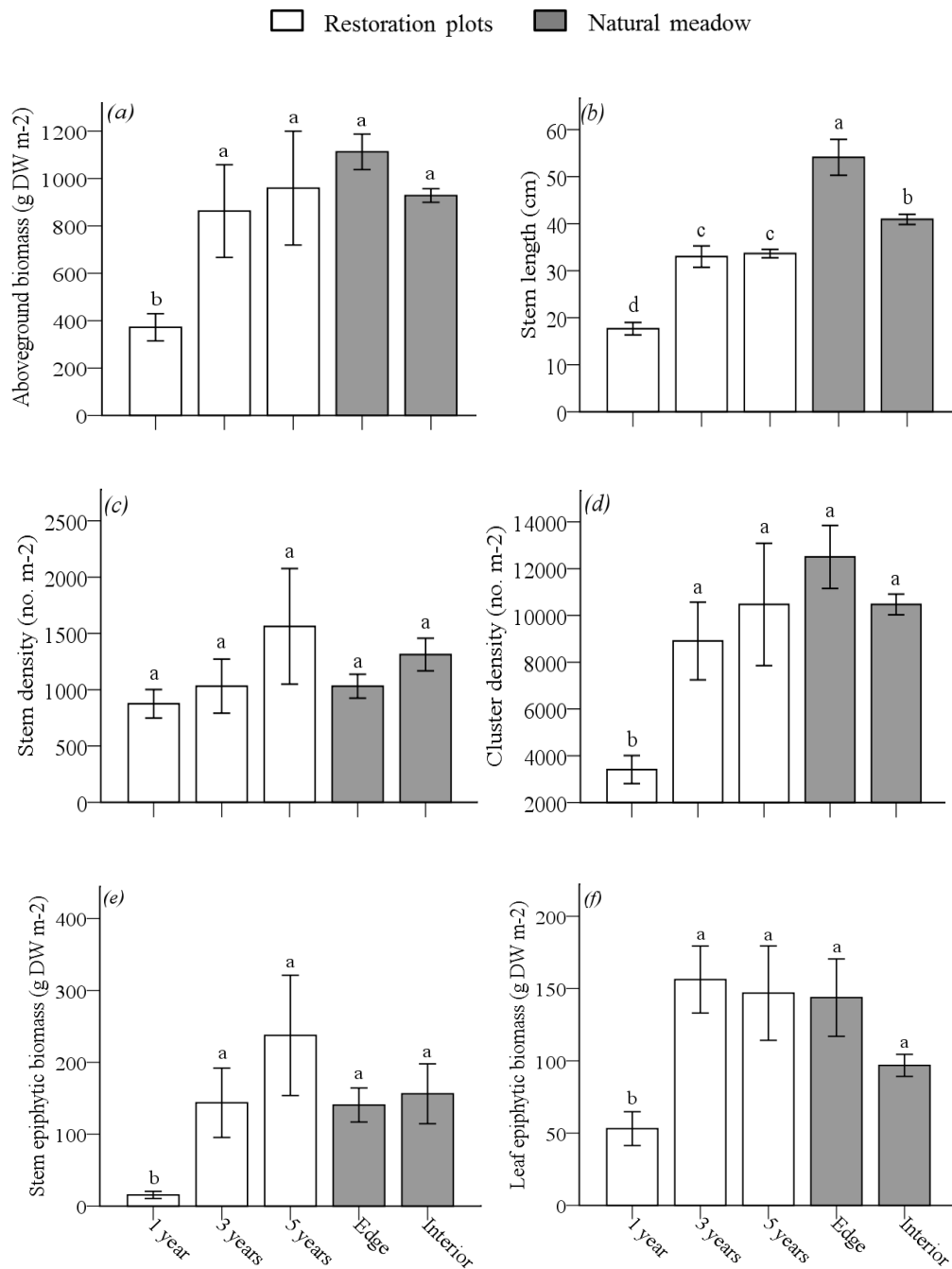


Fig. 4.1. Structural characteristics of *Amphibolis antarctica* at the three restoration plots of known ages: 1 year, 3 years, 5 years and the two natural meadow plots: edge and interior, including (a) aboveground biomass (g DW m⁻²), (b) stem length (cm), (c) stem density (no. m⁻²), (d) leaf cluster density (no. m⁻²), (e) stem epiphytic biomass (g DW m⁻²) and (f) leaf epiphytic biomass (g DW m⁻²). Values are mean ± S.E. ($n = 5$). Within each panel, plots with the same letter are not significantly different according to pairwise tests.

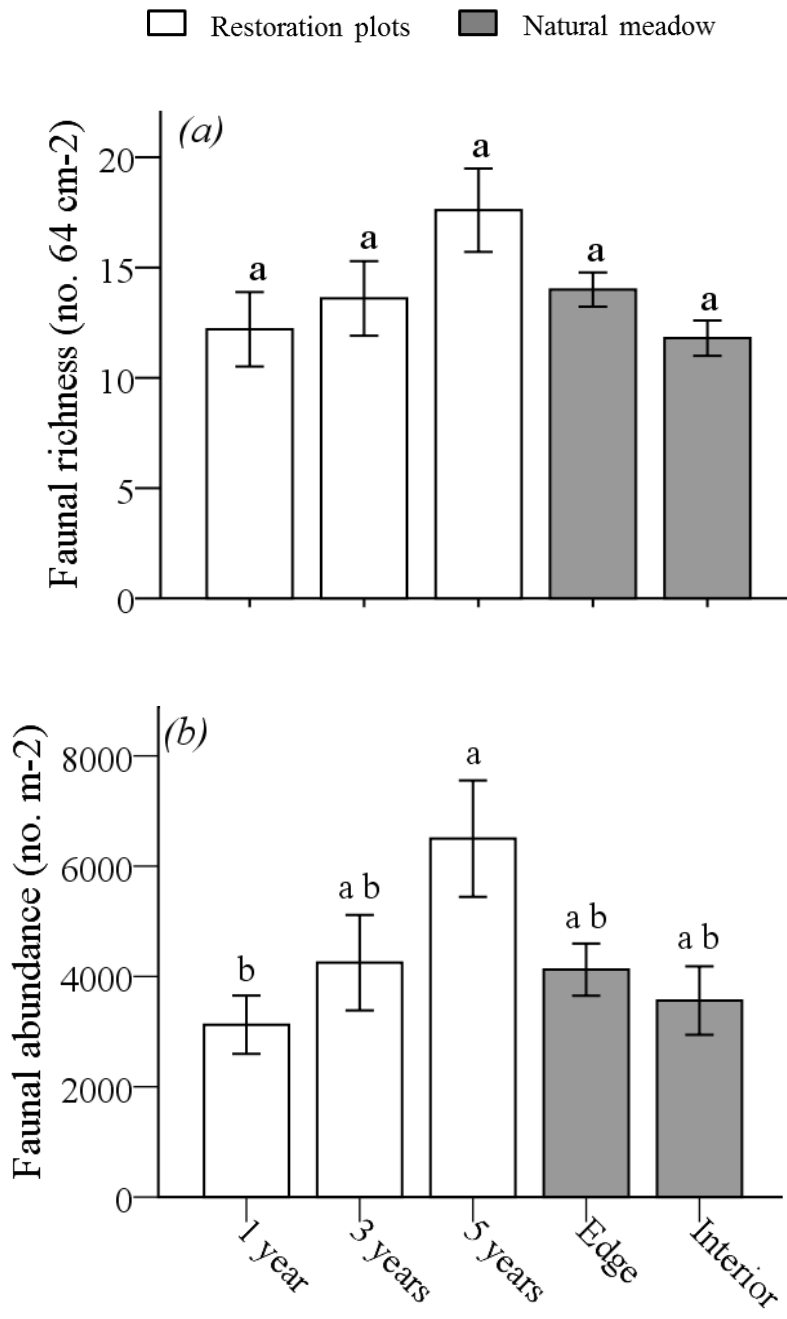


Fig. 4.2. Faunal richness (a) and faunal abundance (b) at the three restoration plots of known ages: 1 year, 3 years, 5 years and the two natural meadow plots: edge and interior. Values are mean \pm S.E. ($n = 5$). Within each panel, plots with the same letter are not significantly different according to pairwise tests.

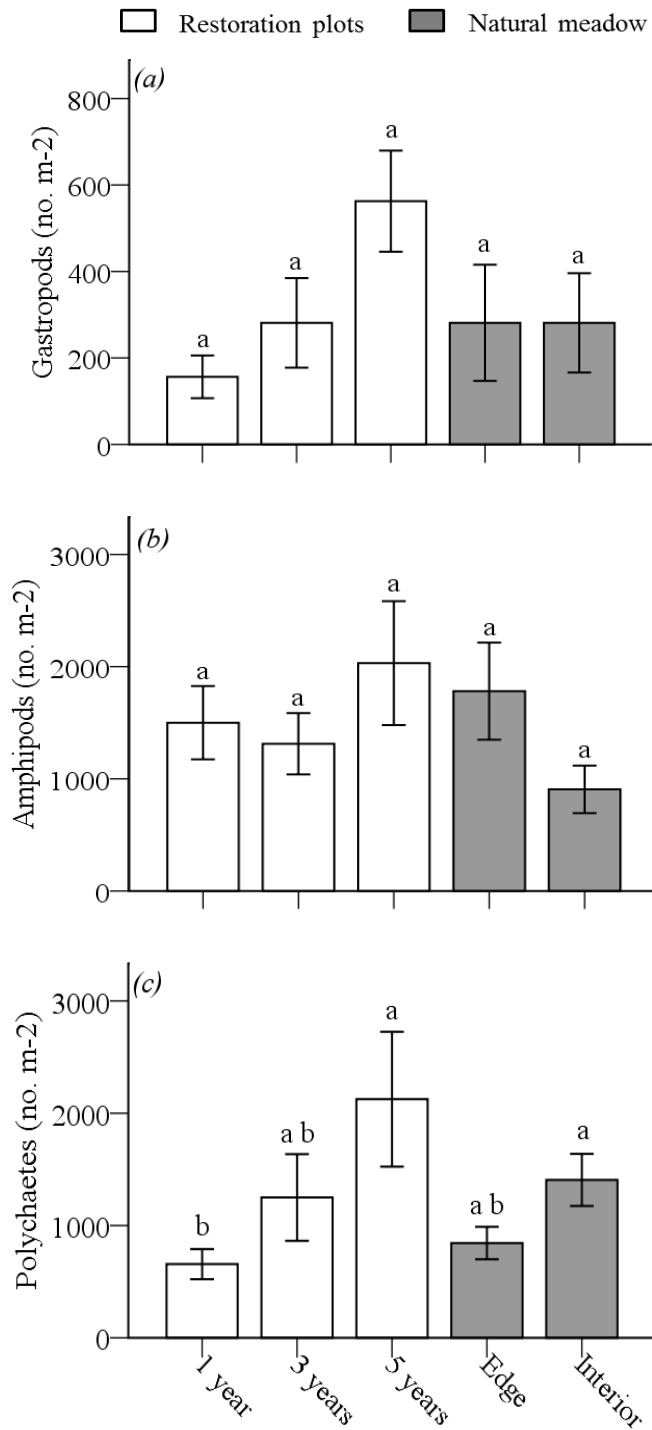


Fig. 4.3. Gastropod abundance (a), amphipod abundance (b) and polychaete abundance (c) at the three restoration plots of known ages: 1 year, 3 years, 5 years and the two natural meadow plots: edge and interior. Values are mean \pm S.E. ($n = 5$). Within each panel, plots with the same letter are not significantly different according to pairwise tests.

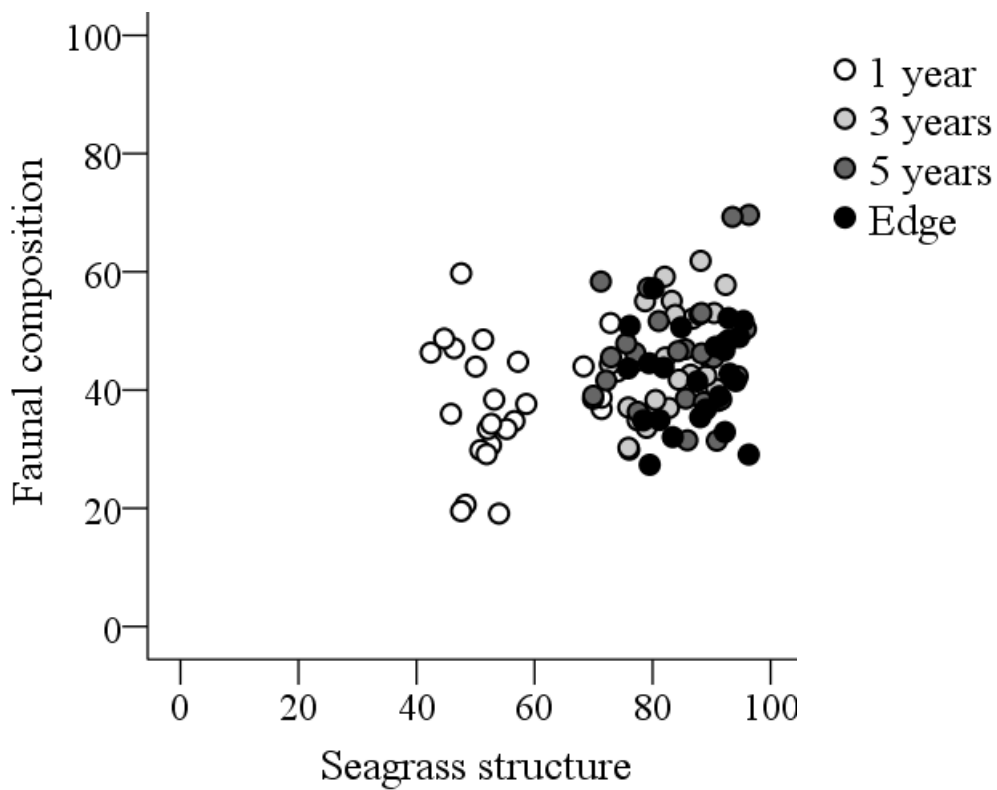


Fig. 4.4. Scatter plot of Bray-Curtis similarity measures seagrass structure (x-axis) and faunal composition (y-axis) showing the convergence between all replicate samples within the three restoration plots (1, 3 and 5 years) and natural meadow (edge), to the interior of the natural meadow. Similarity coefficient = 100 if two samples are completely similar, 0 if two samples are completely dissimilar.

4.5 DISCUSSION

The overall goal of restoration is often not only to restore the habitat *per se*, but to restore the ecosystem services it supplies, and particularly to encourage the return of fauna. Here, we show that faunal richness and abundance were comparable to a natural meadow after one year, even though the seagrass structure had not fully recovered. However, faunal composition did not recover until the seagrass had fully recovered after 3 to 5 years. These results show that although recovering habitats may not look structurally similar to undisturbed habitats, they can in at least some circumstances support a similar richness and abundance of fauna. Whilst this may be true for overall faunal richness and abundance, full recovery of seagrass was required before the taxonomic composition of the fauna matched that of the natural seagrass meadow.

The rapid recovery of small macroinvertebrate abundance before the recovery of seagrass structure in restored plots most likely reflects the greater proportional abundances of early successional species, which rapidly colonize new habitat patches due to the provision of physical structure. As restored seagrass patches are often isolated from natural meadows (Sheridan 2004), they provide structure which can attract actively dispersing fauna, such as amphipods, in what can be an otherwise un-vegetated environment. Such rapid colonization of fauna due to the provision of structure has also been observed with the transplantation of other seagrass species (e.g. Fonseca *et al.* 1996b; Sheridan *et al.* 2003; Sheridan 2004). For example, a 1.9 year old restored seagrass meadow in Galveston Bay, Texas had similar abundance and composition of fishes and shrimps to an adjacent natural seagrass meadow, and had greater faunal abundances than a nearby unvegetated habitat (Fonseca *et al.* 1990). Additionally, faunal abundance may respond to threshold values of seagrass structure, which was first suggested by (Fonseca *et al.* 1996b), who reported

similar faunal abundance between restored and natural seagrass beds, even though shoot density of the restored bed was one third that of the natural meadow.

Epifauna inhabiting *Amphibolis* meadows respond directly to changes in habitat complexity and can be divided into two groups: leaf-associated, being fauna that respond directly to the presence of seagrass leaves; and epiphyte-associated, being fauna that respond directly to epiphytic biomass (Edgar & Robertson 1992). In this study, the most prevalent taxa (amphipods, nereidid and nephtyid polychaetes) are known to be associated with the epiphytic algae that they consume, or consume faunal species that are associated with epiphytic algae (Fauchald & Jumars 1979; Duffy & Hay 2000; Caron *et al.* 2004). Although epiphytes were present throughout the restoration plots and natural meadow, epiphyte biomass was significantly lower in the 1 year old restoration plot. As expected based on this low epiphyte biomass, polychaetes were relatively less abundant in the 1 year old restoration plot. Unexpectedly, amphipod abundance was similar in the 1 year old restoration plot to that in the natural meadow, and so this group does not appear to be responding to total epiphyte biomass.

Patterns of colonization may also reflect the mobility of fauna (Virnstein & Curran 1986; Russell *et al.* 2005). Relatively motile fauna such as amphipods can actively select habitat that provides increased refuge from predators and food resources (Stoner 1980; Bell & Westoby 1986). Amphipod movement can be further enhanced through passive dispersal via tidal currents (Virnstein & Curran 1986), and they are therefore good dispersers with early opportunity for colonization of restoration plots, explaining their high relative abundance in the 1 year old plot.

The proximity of restored seagrass patches to the natural meadow may influence faunal abundance (Sheridan *et al.* 2003), with restored patches close to natural meadows having a greater probability of attracting or entraining dispersing fauna. Importantly, the closer habitats are to each other, the more likely, motile organisms are to encounter them in their daily movements (Russell *et al.* 2005), lowering the likelihood of dispersal related mortality, such as encountering a predator. Furthermore, in seagrass beds, hydrodynamic conditions change with distance from the habitat edge, with flow rate decreasing towards the habitat interior (Fonseca *et al.* 1982), resulting in the accumulation of fauna along the seagrass edge (Bologna & Heck Jr 1999; Tanner 2005). It is likely that the small sizes of the restoration plots sampled here mean that they are made up entirely of patch edge. These influences would actually bias our study away from finding recovery, as we would expect that at some stage as plots get smaller and more isolated, the faunal composition would change as a result of those factors alone. Although this study found no natural edge effects, small restoration plots may more rapidly accumulate fauna, as the increased amount of habitat edge relative to the plot size may increase the relative encounter rates of fauna dispersing passively (Boström *et al.* 2006).

In the majority of systems that we study, the life spans of the plants and animals exceed that of several generations of scientific careers, which means that progress in testing recovery theory is challenging. Seagrass systems, therefore, open an opportunity to test these ideas because the structure and function (in terms of recovery of composition and relative abundance of fauna) of these systems often return relatively quickly after restoration commences (e.g. Fonseca *et al.* 1996b; Sheridan 2004). Whilst epifauna recovered to similar levels as the natural patch, further work is required in order to determine the composition and abundance of fish species using the restoration plots as

habitat for shelter or foraging activity. Nevertheless, this study demonstrates that the rate of recovery of restored patches can be remarkably quick, with faunal richness and abundance taking as little as one year and seagrass structure and faunal composition taking three years to resemble adjacent natural systems. Further trials are now being undertaken in order to assess the potential use of hessian bags for the restoration of seagrass species that have different life history strategies to *Amphibolis*.

Restoration of this site began as a trial of new techniques to facilitate the natural recruitment of *A. antarctica* seedlings, with the overall intention of re-establishing an extensive continuous seagrass meadow (see Irving *et al.* 2010; Wear *et al.* 2010; Irving *et al.* 2013; Tanner 2014). As a result, restoration plots are replicated temporally rather than spatially. Although this design has enabled us to quantify the rates of recovery of faunal inhabitants and seagrass structure over time, it has resulted in a sampling design that was unavoidably pseudo-replicated. However, due to the small size of the entire restoration site, we consider that the sampling design had no influence on the interpretation of the results, as there were no obvious environmental gradients present that could affect the recruitment of seagrass or fauna. Furthermore, more detailed analysis of data on stem length and density on all 240 bags deployed during the study (Tanner 2014) confirms our results for these two variables. The seemingly logical progression in our results from 1 to 3 to 5 year old restoration plots also suggests that these results are robust against this pseudo-replication.

To conclude, recovering habitats may not be structurally similar to undisturbed habitats, but they can support similar components of composition (e.g. faunal richness and total faunal abundance), suggesting that whilst habitats may not appear fully recovered they can

act as equivalents for some aspects of richness and abundance. If such faunal recovery occurs before full recovery of the habitat, then the intention of restoring the function of the lost habitat may assist managers by showing early signs of achievement of pressing goals towards full habitat recovery *per se*.

CHAPTER 5

STATEMENT OF AUTHORSHIP

Title of Paper	Rapid recovery of belowground structure and function of seagrass following habitat restoration
Publication Status	<input type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input checked="" type="checkbox"/> Publication Style
Publication Details	Co-authored paper, with the intention to submit to an international peer-reviewed journal

PRINCIPAL AUTHOR

Name of Principal Author	Chloe McSkimming		
Contribution to the Paper	Decided on the collection methods, assisted with sample collection, processed the samples, analysed and interpreted the data and drafted and revised the manuscript.		
Overall percentage (%)	80		
Signature		Date	03/07/2015

CO-AUTHOR CONTRIBUTIONS

By signing the Statement of Authorship, each author certifies that:

- x. the candidate's stated contribution to the publication is accurate (as detailed above);
- xi. permission is granted for the candidate to include the publication in the thesis; and
- xii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Bayden D Russell		
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Contribution to the Paper	Provided manuscript feedback.		
Signature		Date	22/07/2015

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Contribution to the Paper	The existing restoration plots that were sampled for this chapter were established and maintained by Jason Tanner, who also assisted with the experimental design for this chapter, collected the samples and assisted with interpretation and manuscript development. Acted as corresponding author and provided funding.		
Signature		Date	27/07/2015

CHAPTER 5

RAPID RECOVERY OF BELOWGROUND STRUCTURE AND FUNCTION OF A SEAGRASS HABITAT FOLLOWING RESTORATION

5.1 ABSTRACT

Seagrass meadows function as habitat for a diverse array of fauna, and as the loss of seagrass continues globally, efforts are increasing to restore lost meadows. The aim of restoration is often not only to re-establish habitat structure, but to restore the ecosystem functions the habitat supplies, and in particular to promote faunal recolonisation. Success of seagrass restoration is typically measured by the recovery of aboveground structure, but this ignores the important role of the belowground component of seagrass ecosystems, which may not recover at the same rate as the aboveground component. We quantify infaunal abundance, richness and composition of experimental seagrass restoration plots and relate it to the rate of recovery of belowground biomass and sediment physical properties in expanding patches of restored seagrass, a natural seagrass meadow, and bare sediment. We found that infaunal abundance in restored seagrass converged on that in natural seagrass within two years, although unusually this was a result of a decline from high abundance in bare sediment to low in seagrass. Infaunal community composition also differed among plots, the bare sediment differing from both the restoration plots and the natural meadow. There was no difference in infaunal richness. Sediment carbon content recovered within two years, however, the recovery of belowground biomass and sediment grain size took four to six years. These results suggest that the belowground structure of

recovering seagrass habitats may not need to match that of a natural seagrass habitat in order to support similar infaunal abundance, richness and community composition.

5.2 INTRODUCTION

As human activities continue to modify the environment, causing profound changes to biological communities (Hooper *et al.* 2005), habitat restoration is increasingly being employed to re-establish the structure and function of lost ecosystems (Elliott *et al.* 2007; Reynolds *et al.* 2013). Often, the motivation for habitat restoration is not only to restore the habitat as such, but to restore the ecosystem functions it supplies. Restoration success is generally measured by comparing structural characteristics of the restoration site to those of an adjacent undegraded natural habitat or reference site (Hobbs & Harris 2001; Ruiz-Jaén & Aide 2005; Benayas *et al.* 2009). The success of restoration often varies, however, mainly due to the difficulties involved in re-establishing complex environments (Elliott *et al.* 2007; Irving *et al.* 2010).

Seagrass meadows are highly productive and play a fundamental role in the functioning of coastal ecosystems by forming extensive habitats for a large diversity of fauna, altering sediment characteristics and movement patterns, and sequestering and cycling nutrients (Short & Wyllie-Echeverria 1996; Beck *et al.* 2001; Duarte 2002; Orth *et al.* 2006). Due to their coastal location, seagrass meadows are subject to frequent disturbance from a range of natural and anthropogenic sources (Short & Wyllie-Echeverria 1996; Ralph *et al.* 2006) and approximately 29 % of the world's seagrass habitat has become degraded or lost (Waycott *et al.* 2009). The natural recovery of seagrass ecosystems after loss can be exceptionally slow (Thorhaug 1986; Reynolds *et al.* 2013), even after the cause of habitat loss has been ameliorated (Hobbs & Norton 1996; Van Katwijk *et al.* 2009).

Consequently, resource managers often seek to accelerate the recovery of seagrass ecosystems by applying various techniques of seagrass restoration (Van Keulen *et al.* 2003; Bell *et al.* 2008; Cunha *et al.* 2012).

Seagrass restoration projects have had limited success, with a recent review showing that 44 % of studies resulted in zero survival (Cunha *et al.* 2012), and hence restoration is yet to reverse the global loss of seagrass meadows (Orth *et al.* 2006; McGlathery *et al.* 2011). Restoration success is typically assessed using time efficient and low cost measures such as the recovery and persistence of aboveground seagrass structure; e.g. shoot density or area cover (Fonseca *et al.* 2000; Di Carlo & Kenworthy 2008). These measures of success may, however, tell us little about the recovery of belowground structure. For example, whilst the aboveground seagrass biomass at a disturbed site in the Florida Keys had recovered after 14 years, the recovery of belowground biomass had not occurred (Di Carlo & Kenworthy 2008). Furthermore, the recovery of belowground function is rarely evaluated, with only a few studies assessing the infaunal assemblages of restoration sites (but see Sheridan *et al.* 2003; Sheridan 2004; Bourque & Fourqurean 2014).

In areas that are hydrodynamically active, establishment of seagrass using traditional restoration methods may be limited; e.g. transplants and seedlings (Irving *et al.* 2010). In response to this limited success, Wear *et al.* (2010) developed a novel seagrass restoration technique, deploying biodegradable hessian (burlap) bags which stabilise the sediment whilst encouraging the natural recruitment of *Amphibolis antarctica* seedlings. To determine whether successful restoration of aboveground seagrass biomass equates to successful restoration of the belowground components of the seagrass ecosystem we take advantage of a series of regular experimental deployments of these bags. Deployments

commenced in 2007 and have resulted in successful re-establishment of seagrasses at a small experimental scale (Tanner 2014). Detailed examination of the aboveground structure and function of the restoration site showed seagrass structure in the restoration plots to be similar to an adjacent natural seagrass meadow after three years, and that the recovery of ecosystem function, as measured by epifaunal richness and abundance, occurred within one year (McSkimming *et al.* unpublished data). Here, we expand these findings to include infaunal use, and the rate of recovery of belowground seagrass biomass of the restoration plots. Additionally, we test the rate of recovery of sediment physical properties (grain size and carbon and nitrogen content). To establish the rate of recovery, we compare expanding patches of restored seagrass to a neighbouring un-degraded natural seagrass meadow, and bare sediment. If full recovery of structural and functional attributes has occurred at this site, then we expect to see no differences in belowground seagrass biomass, infaunal use and sediment characteristics between the restoration plots and the natural meadow.

5.3 MATERIALS AND METHODS

5.3.1 RESTORATION SITE AND SAMPLING DESIGN

Infaunal use, belowground biomass and sediment physical properties (grain size, carbon and nitrogen content) were examined in an experimental seagrass restoration site situated in approximately 8 m water depth along the Adelaide metropolitan coast, South Australia (35° 1' S, 138° 18' E). The restoration site is located immediately inshore of a naturally occurring *A. antarctica* meadow which consists of a dense continuous canopy with the edge of the meadow being an abrupt change from dense seagrass to bare sediment. The current edge of the seagrass meadow marks the extent of substantial seagrass loss

(> 5,200 ha) that has occurred since the 1930's due to eutrophication (Neverauskas 1987; Nayar *et al.* 2012); the restoration plots are therefore located where there was historically seagrass meadow. In recent years, substantial effort has been invested in improving water quality along the Adelaide coast, which has allowed a small amount of natural seagrass recovery in deeper waters where sediment movement is low (Bryars & Neverauskas 2004), suggesting restoration may be viable. However, natural recolonisation does not appear to be occurring along much of the seagrass margin, as sediment movement due to both hydrodynamic forces and infaunal activity prevents seagrasses from gaining a foothold (Irving *et al.* 2010).

Restoration trials at this site began in 2007 by deploying hessian bags to promote the recruitment of *A. antarctica* seedlings, which are released from the adjacent natural meadow. On each deployment, ten replicate bags (area 0.35 m² per bag) filled with ~ 25 kg of clean play pit sand, were deployed shoreward of the natural meadow, on sandy substrate, approximately bimonthly, from November 2007 to October 2009 and again from January 2011 to March 2013. Bags were placed in a double row by divers ~ 0.5 – 1 m apart. All bags were deployed within 50 m of the natural meadow, and extended over a distance of ~ 100 m along the seagrass edge. The small size of the entire site (~ 50 m wide compared to ~ 2 km between the edge of the seagrass and shoreline, Wear *et al.* 2010), and the constant depth, means that there are no obvious environmental gradients present.

As the original experimental deployments were designed to determine the optimal timing of bag deployment to promote seagrass recovery, no allowance was made for extra bags that could be destructively sub-sampled over time to assess belowground recovery and ecosystem function. As a consequence, the rate of recovery of these components was

established using a space-for-time substitution approach. Space-for-time substitution (SFT) is a standard method for evaluating ecological succession (Pickett 1989; Kratz *et al.* 2003), and involves comparing plots of different ages at a single point in time, rather than plots that were established at a single point in time and sampled at different ages. This technique allowed us to assess the rate of recovery of infaunal composition, richness and abundance by taking a series of samples from three restoration plots of known ages, representing a “single snapshot” of succession, instead of sampling the one restoration plot multiple times.

Sediment samples were collected ($n = 8$ per site) from three restoration plots of known ages, 2 years (2011 deployment), 4 years (2009 deployment) and 6 years (2007 deployment); from two plots within the adjacent natural meadow, the edge (defined as within 0.5 m of the abrupt boundary that divides seagrass and bare sand) and the interior (~ 20 m into the natural meadow); and from a bare sediment site, in order to establish whether infaunal use, belowground biomass and the physical properties of the sediment differed between plots. All samples were collected on the same day in June 2013.

Infaunal samples were collected using a 9.0 cm internal diameter (area of 64 cm²) PVC corer inserted to a depth of 10 cm, and samples were immediately preserved in 10 % formalin solution until sorted. Samples taken to assess the physical properties of the sediment (sediment grain size, carbon and nitrogen content) were collected using a 4.5 cm internal diameter (area of 16 cm²) PVC corer inserted to a depth of 4 cm, and were immediately placed on ice and then frozen on return to the laboratory.

5.3.2 RESPONSE VARIABLES

Infauna samples were sieved using a 0.5 mm mesh screen and sorted under magnification in the laboratory. All infauna were removed, counted and identified to the highest taxonomic resolution possible without specialist taxonomic expertise, for most taxa family, except for some rare or poorly known taxa which could only be reliably identified to phylum or class. Belowground seagrass biomass was also retained, and dried to a constant weight at 60° C for 72 hours.

Sediment samples were sieved using a 1 mm mesh screen before grain sizes were determined using a Mastersizer 3000 (Malvern Instruments, England). Sediment grain size distribution was analysed using the software package GRADISTAT (Blott & Pye 2001). The carbon (C) and nitrogen (N) content of the top 1 cm of sediment was assessed using an elemental analyser (LECO TruSpec). Sediment samples were freeze-dried and ground to a fine powder before analysis. To determine the organic carbon content, samples were treated with 1 N hydrochloric acid to remove carbonates, rinsed with Milli-Q water and oven dried at 60° C before analysis. Inorganic carbon content of the samples was then calculated as the difference between total carbon content and organic carbon content.

5.3.3 DATA ANALYSIS

To establish whether infaunal composition and sediment grain size differed between the restoration plots, the natural *A. antarctica* meadow and the bare sediment, one-way permutational multivariate analyses of variances (PERMANOVA), followed by pairwise tests, were used. The Bray-Curtis similarity measure was used with fourth- root transformed data for the infaunal composition, while Euclidean distance was used for the grain size analysis. Principal component analyses (PCA) were used to visually present the

differences in infaunal composition and sediment grain size. Univariate analyses were used to determine whether infaunal richness and abundance, belowground seagrass biomass and the total, inorganic and organic carbon content varied among the three restoration plots (2, 4 and 6 years old), the natural meadow (edge and interior) and the bare sediment ($n = 8$ per site). All multivariate and univariate analyses were carried out in PRIMER (version 6) with the PERMANOVA + add-on (PRIMER-E Ltd, Plymouth).

5.4 RESULTS

Infaunal composition differed significantly among plots (Table 5.1a), with pairwise tests showing the bare sediment to be different to the restoration plots (2, 4 and 6 years old) and the natural meadow (edge and interior), which did not differ from each other (PCA, Fig 5.1). Infaunal richness was consistently low, with a mean \pm S.E. of 2.02 ± 0.19 taxa per core, and did not differ among plots (Fig. 5.2a, Table 5.1b). Infaunal abundance differed significantly among plots (Fig. 5.2b, Table 5.1c), with abundance being greatest in the bare sediment plot. There was no difference in infaunal abundance among the three restoration plots and the natural meadow.

Unsurprisingly, belowground seagrass biomass was largely absent from the bare sediment samples, and increased from the youngest to the oldest plots, reaching similar levels to that in the natural meadow within 4 to 6 years (Fig. 5.3, Table 5.1d).

The sediment grain size distribution differed between the habitats (Table 5.1e), with pairwise tests showing the bare sediment to be different to the restoration plots (2, 4 and 6 years old) and the natural meadow (edge and interior). Sediment grain size in the oldest restoration plot (6 years) did not differ from the natural meadow. The PCA (Fig. 5.4)

shows that samples from the natural seagrass meadow and oldest restoration plot (6 years) are characterised by higher levels of medium sand. In contrast, samples taken from the bare sediment and younger restoration plots (2 and 4 years) are characterised by higher levels of fine sand. Samples from the bare sediment separate out into a distinct group with no overlap, whereas samples from the 2 and 4 year restoration plots are much more dispersed. Sediment grain size overall was characterised by fine to medium sand, with very little silt and no clay.

Total, inorganic and organic carbon content differed among plots (Fig. 5.5, Table 5.1f, g and h), being significantly lower in bare sediment than in plots with seagrass. Total and inorganic carbon was also lower in the interior of the natural plot than at the edge, with the restoration plots tending to be intermediate regardless of age. The nitrogen content of the sediment from all plots was below the detection limit of 0.03 mg.

Table 5.1. PERMANOVA results showing the influence of seagrass restoration on belowground components of the seagrass ecosystem.

Source	df	MS	<i>F</i>	<i>P</i>
<i>(a) Infaunal composition</i>				
Plot	5	3389	2.16	0.002
Residual	42	1566		
<i>(b) Infaunal richness</i>				
Plot	5	3.27	2.06	0.082
Residual	42	1.59		
<i>(c) Infaunal abundance</i>				
Plot	5	1.24	2.64	0.004
Residual	42	4.68		
<i>(d) Seagrass belowground biomass</i>				
Plot	5	207368	414.10	< 0.001
Residual	42	501		
<i>(e) Sediment grain size</i>				
Plot	5	438	10.27	0.001
Residual	42	42.7		
<i>(f) Total carbon content</i>				
Plot	5	0.097	9.27	0.001
Residual	42	0.010		
<i>(g) Inorganic carbon content</i>				
Plot	5	0.075	9.56	0.001
Residual	42	0.078		
<i>(h) Organic carbon content</i>				
Plot	5	0.009	4.34	0.003
Residual	42	0.0005		

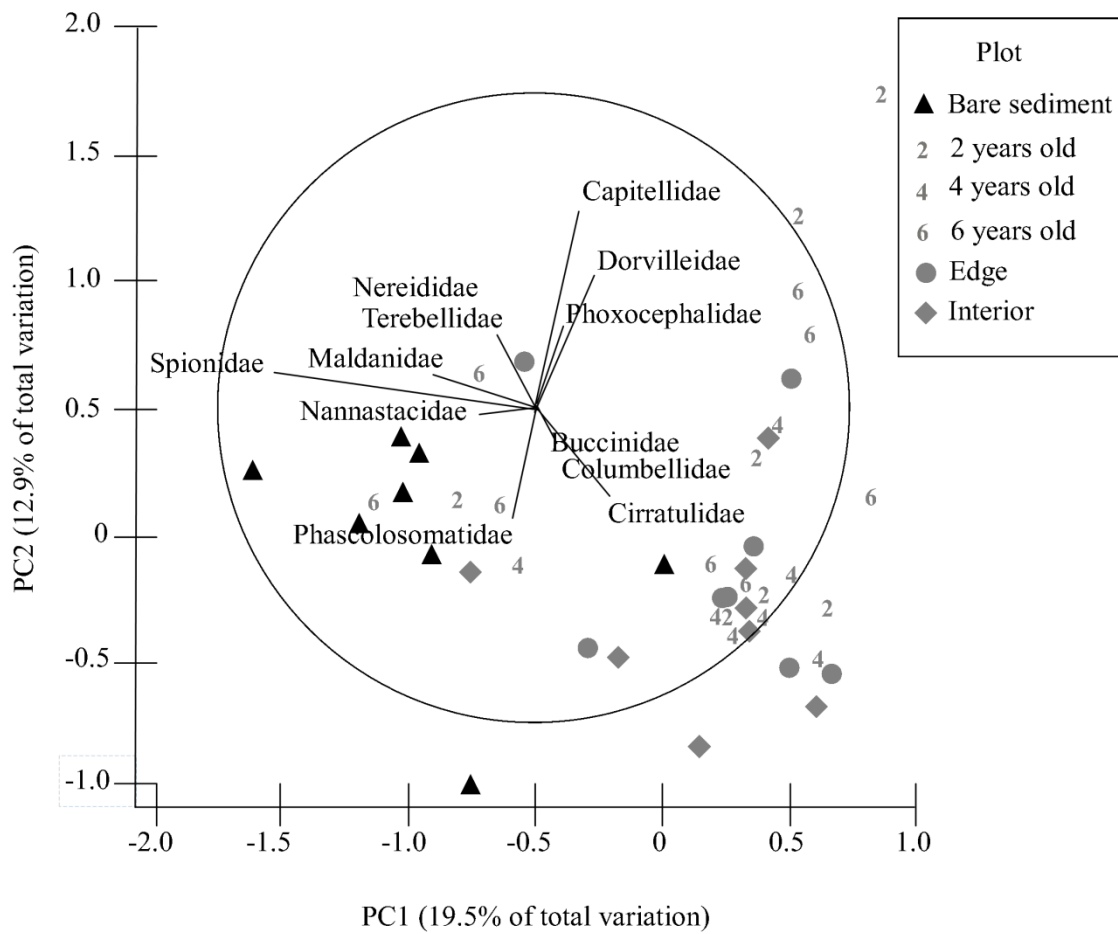


Fig. 5.1. PCA showing the effect of plot (three restoration plots of known age; the two natural meadow locations; and the bare sediment) on infaunal composition.

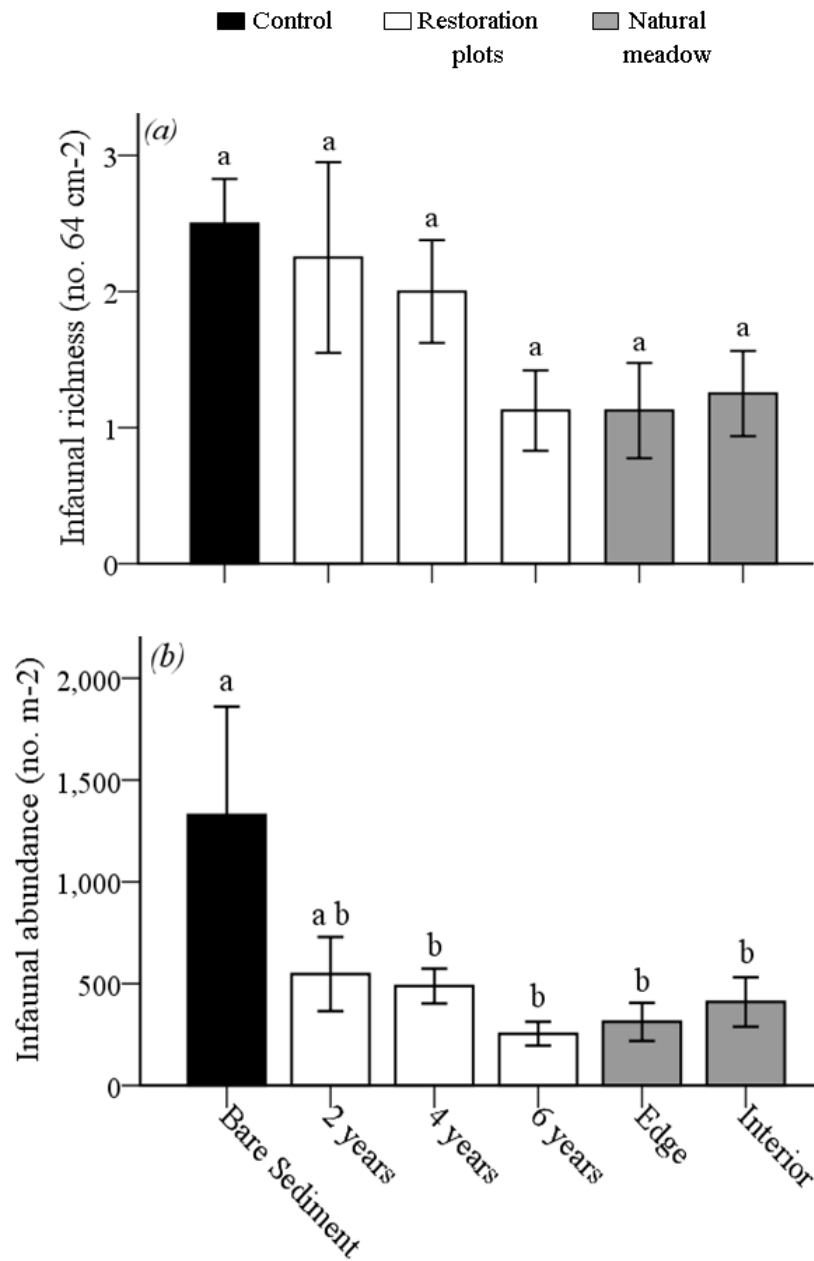


Fig. 5.2. Infaunal richness (a) and abundance (b) in the three restoration plots of known age: 2 years, 4 years, 6 years; the two natural meadow locations: edge and interior; and the bare sediment. Values are mean \pm S.E. ($n = 8$). Within each panel, plots with the same letter are not significantly different according to pairwise tests.

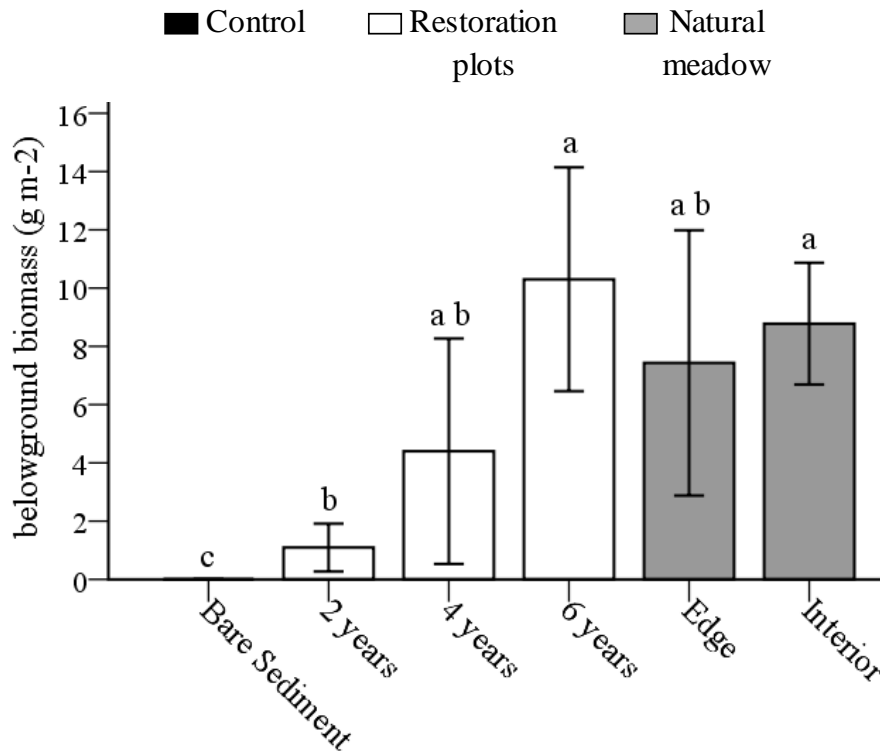


Fig. 5.3. Belowground *A. antarctica* biomass in the three restoration plots of known age: 2 years, 4 years and 6 years; the two natural meadow locations: edge and interior; and the bare sediment. Values are mean \pm S.E. ($n = 8$). Within each panel, plots with the same letter are not significantly different according to pairwise tests.

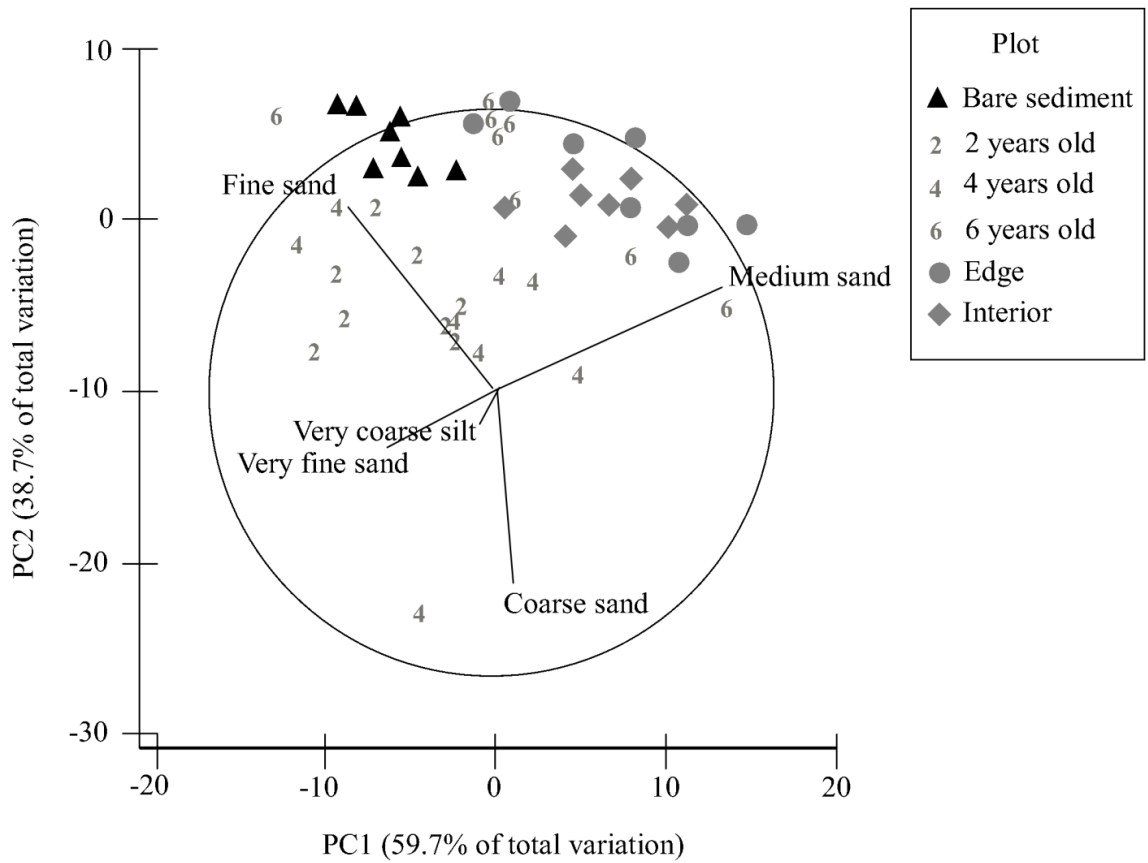


Fig. 5.4. PCA showing the effect of plot (three restoration plots of known age; the two natural meadow locations; and the bare sediment) on sediment properties.

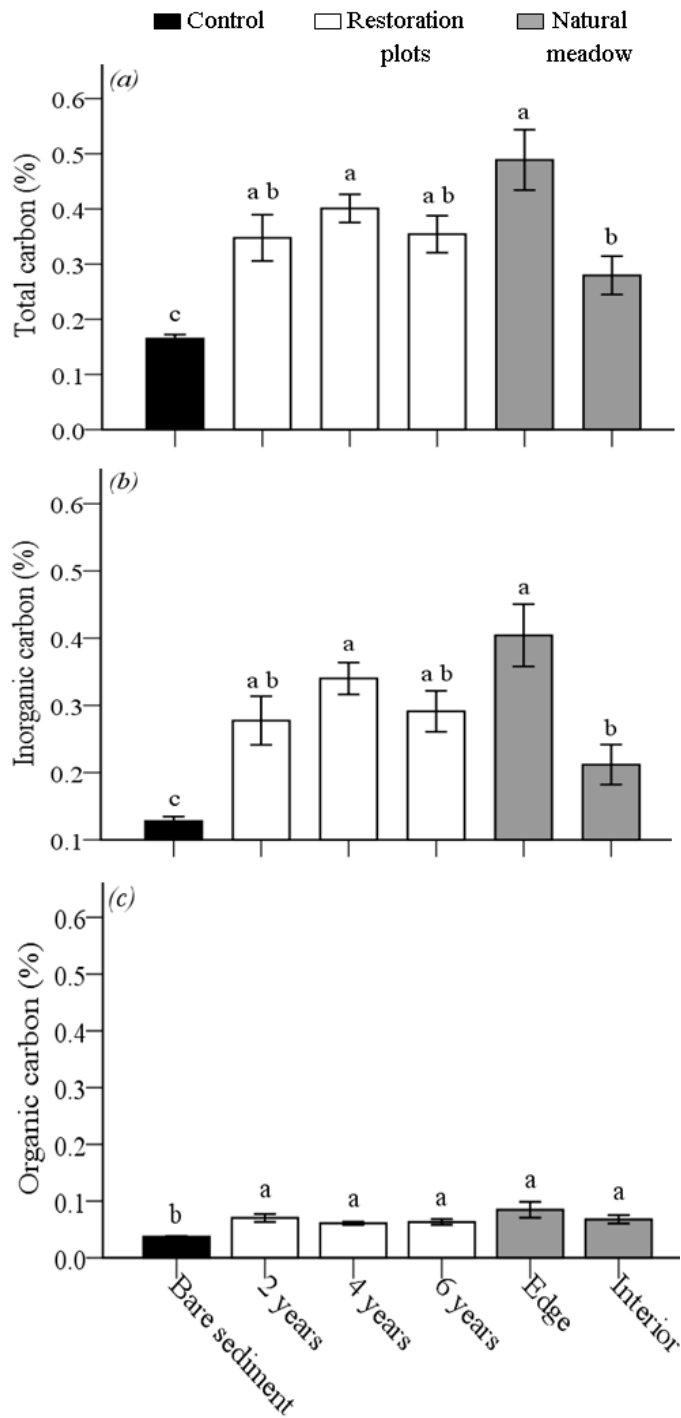


Fig. 5.5. Total carbon (a), inorganic carbon (b) and organic carbon (c) content in the three restoration plots of known age: 2 years, 4 years and 6 years; the two natural meadow locations: edge and interior; and the bare sediment. Values are mean \pm S.E. ($n = 8$). Within each panel, plots with the same letter are not significantly different according to pairwise tests.

5.5 DISCUSSION

We document a clear increase in belowground seagrass biomass in experimental restoration plots of *A. antarctica* over the first 6 years of restoration, with levels reaching those found in an adjacent natural meadow within 4 - 6 years. In contrast, infaunal abundance actually declined as plots got older, with most of this decrease occurring within the first two years. Sediment characteristics also changed rapidly with the initiation of restoration and were similar to the natural meadow within 6 years.

Recovery of the belowground seagrass biomass occurred within 4 - 6 years, with all seagrass sites exhibiting low belowground biomass (maximum biomass $\sim 10 \text{ g m}^{-2}$ in the 6 year old restoration plot). Many seagrass species (e.g. *Zostera*, *Posidonia* and *Thalassia*) have a greater proportion of living material belowground than aboveground, with belowground biomass contributing approximately 50 – 90 % of total biomass (Hillman *et al.* 1989; Paling & McComb 2000). Consequently, the recovery of belowground biomass of these species may lag the recovery of aboveground biomass (e.g. Di Carlo & Kenworthy 2008). In seagrass species where the rhizomes grow at faster rates vertically than they do horizontally (e.g. *Amphibolis*; Marba & Duarte 1998) only ~ 20 % of the total biomass is belowground (Hillman *et al.* 1989; Paling & McComb 2000), and hence the recovery of belowground biomass may be faster in these species. Indeed, this study shows that the belowground biomass of *A. antarctica* in the restoration plots recovered relatively quickly after restoration commenced, being similar to the natural meadow within 4 – 6 years. The recovery of aboveground seagrass biomass in the restoration plots also occurred relatively quickly at this site, converging with the natural meadow within 1 – 3 years (McSkimming *et al.* unpublished data).

Seagrass meadows generally support greater richness and abundance of infauna when compared to neighbouring non-vegetated habitats (Reise 1978; Orth *et al.* 1984; Sheridan 1997; Boström & Bonsdorff 1997), which is thought to be due an association between infauna and belowground seagrass biomass (Edgar 1990; Fredriksen *et al.* 2010).

Although infaunal richness did not vary among the habitats, infaunal abundance was significantly greater in the bare sediment habitat. The two most prevalent taxa in bare sediment (spionid polychaetes and phascolosomatid sipunculans) were surface and subsurface deposit feeders (Fauchald & Jumars 1979; Cutler 1994). These same deposit feeders had relatively sparse abundances in the seagrass and restoration plots, which may be due to seagrass roots preventing certain infaunal taxa from inhabiting the seagrass meadow (Orth *et al.* 1984). This is a similar pattern to that found by Stoner (1980), who reported a decrease in the relative abundance of deposit-feeding polychaetes with an increase in seagrass biomass. The greater abundance of polychaetes in the bare sediment may therefore be due to the lack of belowground biomass in this plot. Additionally, deposit feeding infauna tend to be numerically dominant in finer grained sediments (Sanders 1958; Rhoads & Young 1970). Although the sediments in all plots in this study contained no clay and very little silt, the bare sediment plot had more fine grained sand than the seagrass plots. Whether the difference in percentage of fine grain sand is sufficient to have an influence on infaunal abundance is, however, unclear.

The ability of seagrass to accumulate sediment is often strongly influenced by the hydrodynamic conditions at a location, as well as the particle size of the sediment (De Boer 2007), with coarse grained sediments occurring in areas of higher water-flow (Koch 2001; Madsen *et al.* 2001). Typically, the sediments in seagrass meadows are finer than in adjacent bare sediment areas because the structure of seagrass reduces water-flow, decreases sediment resuspension, and entrains finer particles (Kenworthy *et al.* 1982;

Gacia *et al.* 1999; Van Katwijk *et al.* 2010). Unexpectedly, we found the reverse here, with bare sediment having the greatest amount and natural seagrass the lowest (53 % and 41 – 42% respectively). One explanation for this counter-intuitive pattern may be related to the restoration method used. The hessian bags that were used to stabilise the sediment and encourage the natural recruitment of *A. antarctica* seedlings were filled with sand to anchor them, which may have influenced the sediment properties of these plots. For example, seagrass meadows that were disturbed via vessel groundings in the Florida peninsula, USA were restored using a carbonate sand fill, which altered the physical properties of the sediment (Bourque & Fourqurean 2014). We consider this unlikely as all restoration plots were dominated by fine to medium sand and not coarse sand, such as that used to fill the hessian bags. By the time of first sampling (2 years) the bags had also completely degraded and the restoration plots were exposed to naturally high levels of sediment movement, and there was no visible evidence of the filling (which had a different colour and texture to the natural sediments). Alternatively, infauna can influence sediment properties, with numerous studies reporting that deposit-feeding infauna selectively feed on smaller size particles resulting in a layer of fine grained sediment at the sediment surface, and coarse grained sediment at the bottom of the bioturbated zone (Rhoads & Stanley 1965; Cadée 1976; Baumfalk 1979). We found a high correlation between infaunal abundance and finer sediments, which may thus account for the pattern we see in sediment grain size distribution (although as noted above, the causative pathway may also be operating in the reverse direction).

Areas that have high water flow often have low organic matter content and are nutrient poor (Chambers *et al.* 1991; Madsen *et al.* 2001). Low nutrient availability in the sediment can limit seagrass growth and may lead to the uptake of nutrients via the leaves (Pedersen *et al.* 1997). *Amphibolis antarctica* is commonly found growing in small sand pockets on

rock, which limits the amount of available nutrients surrounding the root system (Paling & McComb 1994; Pedersen *et al.* 1997). This suggests that *A. antarctica* is efficient in taking up nutrients from the water column via the leaf clusters (Paling & McComb 1994). Although pore-water nutrient concentrations were not quantified, sediment nitrogen in all plots was below detection limits, suggesting low available nutrients in the sediments. Although all plots had low organic carbon content, inorganic carbon was higher in the restoration plots and natural meadow than the bare sediment. Seagrass uptake of inorganic carbon, however, is directly from the water-column only and not from the sediment (Nayar *et al.* 2009), and hence the low carbon content in the sediment would not influence the growth of seagrass.

To conclude, the recovery of the belowground characteristics of seagrass habitats (belowground seagrass biomass and sediment carbon content) occurred relatively quickly (within 4 – 6 years) once restoration had commenced at this site. Additionally, infaunal richness, abundance and composition were similar between the restored habitat and natural meadow. A previous detailed examination of the aboveground structure at this site showed that epifaunal richness and abundance took one year to become similar to the natural meadow even though the aboveground structure took between one to three years to recover (McSkimming *et al.* unpublished data). Results from these studies suggest that recovering seagrass habitats may not need to be structural similar to natural seagrass in order to support a similar abundance and richness of fauna. Additionally, these studies indicate that the recovery of belowground structure should be assessed in order to evaluate the full recovery of seagrass structure as it may not recover at the same rate as aboveground structure.

CHAPTER 6

CHAPTER 6

GENERAL DISCUSSION

An account of the mechanisms that enable ecosystems to resist major change when faced with intensive anthropogenic pressure is central for improving our understanding of ecosystem stability. Throughout this thesis, resource enhancement and herbivore abundance were experimentally manipulated to identify their likely effects on the stability of temperate marine ecosystems (seagrass and kelp forests). Additionally, I determined whether habitat restoration was successful in re-establishing the structure and function of a seagrass system. Specifically, I identified; 1) the role herbivores play in increasing the stability of coastal ecosystems during periods of resource enhancement and, 2) the rate of recovery of ecosystem function and structure after seagrass restoration has been implemented. When herbivores were absent, the growth of opportunistic algae was facilitated by resource enhancement (i.e. nutrients: Chapter 2, nutrients and CO₂: Chapter 3). Importantly, while resource enhancement facilitated the expansion of opportunistic algae, these same resources stimulated a compensatory response by herbivores when they were present. Herbivores were efficient in countering the effects of resource enhancement through an increase in consumption rate (Chapter 2 and 3), which was proportional to an increase in metabolic rate (Chapter 3). These results indicate that compensatory mechanisms may enable ecosystems to remain stable, resisting environmental change during periods of small-scale and/or low-level resource enhancement. While the relationship between resource enhancement and herbivore consumption appears strong, the net effect of grazing would, however, depend upon the level of resources entering the coastal zone, and at some point productivity of opportunistic algae may escape regulation

(Ghedini *et al.* 2015). In seagrass ecosystems, this can result in the accumulation of epiphytic algae and lead to seagrass decline (Burkholder *et al.* 2007). Habitat restoration can, however, be employed to reverse habitat loss and to re-establish ecosystem structure and function (Elliott *et al.* 2007; Reynolds *et al.* 2013). Unexpectedly, I found that the recovery of ecosystem function, as measured by faunal recolonisation, occurred before the recovery of physical structure (Chapter 4 and 5). These results have suggested that recovering habitats may not need to be structurally similar to undisturbed habitats to support similar ecosystem function, and hence, may have greater social and economic value than expected. This chapter (6) provides a discussion of the key findings and outlines possible directions for future research.

6.1 DISTURBANCE BY RESOURCE ENHANCEMENT

The discharge of resources into the coastal zone acts as a disturbance that can shift the competitive balance between primary producers, often turning subordinate species into dominant competitors (Tilman & Lehman 2001; Diaz-Pulido *et al.* 2011; Connell *et al.* 2013). My results from the experimental manipulation of nutrients (Chapter 2, and 3) compliment the literature indicating that elevated nutrients enhance the growth of opportunistic algae (Duarte 1995; Short & Wyllie-Echeverria 1996; Worm *et al.* 1999; Connell *et al.* 2008). In seagrass ecosystems, resource enhancement can facilitate the accumulation of epiphytic algae, reducing available light needed for processes such as photosynthesis (Wear *et al.* 1999), and ultimately leading to a shift from seagrass habitat to bare sediment (Walker & McComb 1992; Duarte 2002). Studies that measure the effects of nutrient enhancement in seagrass ecosystems usually report a decline in seagrass biomass, which is often associated with an increase in epiphytic algae cover (reviewed in Burkholder *et al.* 2007). My results suggest that although minor to moderate nutrient

enhancement increased the cover of epiphytic algae on seagrass leaves, seagrass biomass and leaf density remained stable (Chapter 2, Fig. 2.4). Indeed, under minor nutrient enhancement, seagrass had significantly greater aboveground biomass than seagrass under moderate nutrient enhancement and ambient conditions, but only when herbivores were present. Low level nutrient additions may therefore enhance the growth of seagrass (Kelaher *et al.* 2013), but only if epiphytic algae is held in check by herbivores. Studies that report positive effects of low level nutrient enhancement and herbivore presence on the growth of seagrass are not uncommon (Williams & Ruckelshaus 1993; Heck *et al.* 2000). It is important to acknowledge, however, that once nutrient levels surpass minor enhancement, epiphytic algae will outcompete seagrass, resulting in the loss of seagrass habitat (Burkholder *et al.* 2007).

The global accumulation of CO₂ in the atmosphere is resulting in the reduction of oceanic pH, or ocean acidification (Feely *et al.* 2004), which can act as a stressor to some species and as a resource to others (Connell *et al.* 2013). It is well established that CO₂ can act as a stressor to calcifying organisms such as corals (Leclercq *et al.* 2000; Feely *et al.* 2004), however, recent work suggests that CO₂ potentially acts as a resource for photosynthetic species that are carbon limited, by allowing an increase in carbon fixation rates (Beardall & Raven 2004; Hurd *et al.* 2009; Raven & Hurd 2012). The addition of CO₂ into coastal zones may therefore favour the growth of opportunistic species over their naturally dominant competitors (Connell *et al.* 2013). In kelp systems, resource enhancement increases the productivity of naturally occurring algal turfs, which inhibit the recruitment of kelp, driving a shift in ecosystem state from a kelp-dominated landscape to a turf-dominated landscape (Gorman *et al.* 2009; Connell & Russell 2010). Indeed, experimental manipulation of CO₂ resulted in the enhanced growth of algal turf, in the absence of

herbivores (~ 20 % greater than ambient conditions, Chapter 3, Fig. 3.2). Additionally, primary producers can be co-limited and hence their response to multiple resource enhancement will be greater than their response to resources enhanced independently (Davidson & Howarth 2007; Allgeier *et al.* 2011). For example, another study that considered resource co-limitation of algal turf found that enhanced nutrients and CO₂ independently increased the biomass of turf, however, turf biomass was greatest when both resources were enhanced in combination (Falkenberg *et al.* 2013b). My results further indicate that the cover of algal turf was greatest when nutrients and CO₂ were elevated in combination (40 % greater than ambient conditions, Chapter 3, Fig. 3.2). Nevertheless, many ecosystems that experience disturbance (or multiple disturbances) remain stable, continuing in a particular state without undergoing a major change in composition (Ghedini *et al.* 2015).

6.2 COMPENSATORY RESPONSES MAINTAIN STABILITY

Compensatory responses to resource enhancement are thought to be important in the maintenance of habitat stability (Ghedini *et al.* 2015). Herbivores have been shown to control the accumulation of opportunistic algae during periods of nutrient enhancement by increasing in abundance (Neckles *et al.* 1993; Heck *et al.* 2000; Karez *et al.* 2004; Roll *et al.* 2005). For example, Neckles *et al.* (1993) found grazing to have a strong negative effect on epiphytic biomass in enriched nutrient conditions, which was associated with an increase in herbivore abundance. Although an increase in herbivore abundance seems to be a simple example of a compensatory response, it is thought to only partly explain the ability of systems to remain stable when faced with altered environmental conditions (Loreau & Mazancourt 2013; Ghedini *et al.* 2015). Another explanation is that nutrient addition stimulates herbivores to increase their *per capita* feeding rates (Nicotri 1980;

Russell & Connell 2007; Falkenberg *et al.* 2014). Indeed, I found that although nutrient enhancement increased food availability, the abundance of herbivores did not increase (Chapter 2, Fig. 2.1 and 2.2, respectively). This suggests that the greater consumption of algae was due to an increase in *per capita* feeding and not grazer abundance. An alternate explanation as to why an increase in amphipod abundance was not found may be that an increase in abundance results in greater predation rates; that would be comparable to the compensatory response seen in herbivores (Ghedini *et al.* 2015). It is therefore difficult to tease apart which mechanisms enable herbivores to compensate for nutrient enhancement in field based experiments. Manipulation of nutrient enhancement and grazer abundance in microcosms did, however, support the suggestion that individual herbivores were capable of consuming greater quantities of food when nutrient conditions were enhanced, as gastropods increased their individual feeding rates when exposed to elevated nutrient conditions (Chapter 3).

The physiology of herbivores can be affected by altered abiotic conditions (O'Connor 2009; Cummings *et al.* 2011; Russell *et al.* 2013). Initial studies on the elevation of CO₂ and subsequent ocean acidification reported direct negative effects on the health, feeding abilities, and activity of calcifying marine organisms (Kurihara *et al.* 2004; Bibby *et al.* 2007; Miles *et al.* 2007; Siikavuopio *et al.* 2007). For example, adult green sea urchins had significantly reduced consumption rates when exposed to elevated CO₂ (corresponding pH of 6.98 ± 0.09) (Siikavuopio *et al.* 2007). Surprisingly, recent work has revealed that elevated CO₂ may actually stimulate an increase in invertebrate grazing (Cummings *et al.* 2011; Burnell *et al.* 2013; Falkenberg *et al.* 2013c). Similarly, my results indicate that gastropods consumed greater quantities of algal turf under elevated CO₂, relative to ambient conditions (i.e. consumption rates of algae increased from 33 % in ambient

conditions to 55 % under elevated CO₂, Chapter 3, Fig. 3.2). Additionally, the consumption of algal turf was greatest when elevated CO₂ and nutrients were experienced in combination (consumption rates of algae 76 %, Chapter 3, Fig. 3.2). Importantly, the quantity of algae consumed was proportional to the response of algae to the enhanced resource availability. This suggests that herbivores can compensate for the combined effects of local and global resource enhancement by consuming the additional productivity of opportunistic algae.

The ability of herbivores to increase their feeding rates is often explained by the effects elevated nutrients (Russell & Connell 2007) and carbon (Falkenberg *et al.* 2013c) have on food quality or quantity. Filamentous algae can rapidly absorb nitrogen during periods of nutrient addition (Hein *et al.* 1995), and studies have demonstrated that herbivores select and consume larger quantities of such algae (Williams & Ruckelshaus 1993; Miller *et al.* 1999; Kraufvelin *et al.* 2006). Further, the addition of carbon via CO₂ emissions can increase the nitrogen content of filamentous algae and has also resulted in increased consumption rates (Falkenberg *et al.* 2013c). Whilst it is relatively well established that resource enhancement can influence the feeding rates of herbivores through an increase in food quantity and quality, less consideration has been given to whether resource enhancement can directly influence the metabolic demands of herbivores. My study is one of the first to assess the effects of resource enhancement on oxygen consumption as a proxy for the metabolic rate of herbivores. I showed that enhanced feeding rates in combined elevated nutrient and CO₂ treatments correlated with an increase in herbivore metabolic rate (Chapter 3, Fig. 3.1). I proposed that local and global resource enhancement and subsequent improvement in the nutritional value and quantity of food (e.g. Falkenberg *et al.* 2014), influenced the metabolic demands of gastropods. The idea

that nutritional value and quantity of food can increase the metabolic rate of organisms is well established, and is termed the “food-habit hypothesis” (see review Cruz-Neto & Bozinovic 2004). This hypothesis suggests that animals exposed to diets of low nutritional value will have low metabolic rates (McNab 1986; Bozinovic & Novoa 1997; Cruz-Neto & Bozinovic 2004). Additionally, it has been suggested that the quantity of food influences the metabolic rate of animals (Wallace 1973), which is most likely due to the increased energetic costs of processing the additional food (reviewed in Secor 2009). Resource enhancement therefore not only stimulates elevated primary productivity, but also stimulates elevated consumption and metabolic rates to compensate.

6.3 RECOVERY OF ECOSYSTEM STRUCTURE AND FUNCTION

Despite showing that under some circumstances herbivores are capable of compensating for the effects of small-scale resource enhancement via an increase in epiphyte consumption (Chapter 2), seagrass meadows continue to decline globally (Waycott *et al.* 2009). Once seagrass habitats are lost, their natural recovery can be exceptionally slow, (Thorhaug 1986; Reynolds *et al.* 2013), even after the disturbance causing loss has been alleviated (Hobbs & Norton 1996; Van Katwijk *et al.* 2009). Consequently, restoration has become an element in the management of seagrass ecosystems (Van Keulen *et al.* 2003; Bell *et al.* 2008; Cunha *et al.* 2012). The success of seagrass restoration projects has, however, been limited (Fonseca *et al.* 1998). Additionally, studies that report success generally base this success on the recovery of aboveground structure, with only a few studies assessing ecosystem function (but see Bell *et al.* 1993; Fonseca *et al.* 1996b; Sheridan *et al.* 2003). For restoration to be successful, however, restored seagrass meadows should continue to persist and recover similar ecosystem functions to that of an undisturbed seagrass meadow (Fonseca *et al.* 1998). Monitoring should therefore include

measurements of ecosystem function in order to determine whether restoration has been successful. I showed that an experimental restoration site along the coast of Adelaide, South Australia, has been successful in re-establishing similar ecosystem structure and function to that of an adjacent natural meadow. More specifically, I showed that faunal richness and abundance were comparable to a natural seagrass meadow, even though seagrass structure had not fully recovered (epifauna and aboveground structure: Chapter 4, Fig. 4.1 and 4.2; and infauna and belowground structure: Chapter 5, Fig. 5.2 and 5.3). Together these results suggest that recovering habitats may not need to be structurally similar to natural habitats to support similar components of faunal composition (e.g. faunal richness and total faunal abundance). Importantly, studies that measure restoration success based solely on the recovery of an aspect of seagrass structure (e.g. stem length) may be underestimating the recovery of ecosystem function.

Epifauna that are relatively mobile can actively search for habitat that supplies food resources, as well as protection from predators (Stoner 1980; Bell & Westoby 1986). Epifauna residing in *A. antarctica* meadows have been shown to respond directly to seagrass structure and can be associated with epiphytic biomass (e.g. food resource) and the presence of leaves (e.g. refuge from predators) (Edgar & Robertson 1992). Although epiphytic algae and leaf clusters were present throughout all plots, epiphytic biomass and cluster density were lower in the one year old restoration plot. This suggests that epifaunal abundance may be influenced by threshold values of seagrass structure. Threshold values have previously been reported when monitoring the faunal recovery of restoration sites, for example, faunal abundance in restored seagrass beds were comparable to the natural meadow although shoot density of the restored site was only one third of the density of the natural meadow (Fonseca *et al.* 1996b). Additionally, epifauna can use tidal currents to

passively disperse (Virnstein & Curran 1986), and may settle when they encounter structure. Faunal abundance in the restoration plots (Chapter 4) may have, therefore, been influenced by the proximity to the adjacent natural meadow, which would have maximized dispersal potential. For example, a restored seagrass meadow in Galveston Bay, Texas had similar abundance and composition of fishes and shrimps to a natural seagrass meadow in close proximity after 1.9 years (Fonseca *et al.* 1990). Similarly, my results showed that epifaunal abundance was comparable to the natural meadow within one year, which may have been due to the close proximity with the adjacent natural meadow.

The distribution of infauna may vary in response to patch characteristics such as sediment stability and type (Zajac & Whitlatch 1982; Pinedo *et al.* 2000). I found that infaunal abundance, richness and composition were similar between the restoration plots and an adjacent natural seagrass meadow after two years (Chapter 5). Unexpectedly, however, the abundance of infauna was greater in the bare sediment plot when compared to the natural meadow and restoration plots (Chapter 5, Fig. 5.2). The greater abundance of infauna in the bare sediment plot may be due to sediment characteristics as this plot had finer sediment, with more fine grained sand than the restoration plots and natural meadow. Additionally, previous studies have shown that infauna may also respond to the belowground biomass of seagrass (Edgar 1990; Fredriksen *et al.* 2010). For example, infaunal abundance did not differ between a *Zostera* meadow and bare sediment in Barker Inlet, South Australia, which was explained by the substantial amount of belowground biomass in the bare sediment (Tanner 2005). Contrary to this, belowground seagrass biomass was largely absent from the bare sediment plot in my study, yet infaunal abundance was greatest in this plot. As *A. antarctica* has relatively low belowground biomass when compared to species such as *Zostera* and *Posidonia* (~ 20 % compared to

50 - 90 % of total biomass, respectively; Hillman *et al.* 1989; Paling & McComb 2000), the association between belowground biomass and infaunal abundance may not be as prevalent in *A. antarctica* meadows.

The rate and extent of ecosystem recovery following restoration may be influenced by the morphological characteristics of the seagrass species being restored. The morphology of *Amphibolis* is quite unique amongst seagrass, comprising a flexible wiry stem with leaf clusters and a wiry rhizome (Paling *et al.* 2001; Rivers *et al.* 2011). Additionally, *Amphibolis* species have a greater proportion of living material aboveground than belowground (~ 82 % and 18 %, respectively) and have fast vertical growth rates (7 – 32 cm yr⁻¹) (Marba & Duarte 1998; Paling & McComb 2000; Rivers *et al.* 2011). In comparison, seagrass such as *Posidonia* are characterised by strap-like leaves, fibrous rhizome, a greater proportion of living material belowground than aboveground (~ 41 and 59 %, respectively) and have slower vertical growth rates (0.2 – 8 cm yr⁻¹) (Marba & Duarte 1998; Paling & McComb 2000; Paling *et al.* 2001). Although I have shown that the structure of *A. antarctica* can recovery relatively quickly following restoration (3 – 5 years), species with different morphological characteristics such as *Posidonia* may take longer to recover, and hence monitoring for 3 to 5 years may not be adequate. Morphological characteristics of the seagrass being restored should therefore be taken into consideration when determining the length of monitoring programs.

6.4 FUTURE RESEARCH

Throughout the first half of this thesis, I focused on the independent and combined effects of nutrient and CO₂ enhancement on the growth of opportunistic algae and the consumption rates of herbivores. These anthropogenic pressures, however, do not act in

isolation, with the release of CO₂ in the atmosphere contributing to a pronounced increase in ocean temperature (Harley *et al.* 2006; Hoegh-Guldberg & Bruno 2010). Studies that assess the effects of increasing ocean temperature have reported an increase in herbivore consumption and metabolic rates (O'Connor 2009), as well as an increase in the growth of opportunistic algae (Mertens *et al.* in review). Additionally, the combination of multiple global disturbances (CO₂ and temperature) resulted in the doubling of algal turf biomass when compared to ambient conditions (Connell & Russell 2010). The magnitude of these global changes and the severity of their impacts will, however, vary regionally (Michener *et al.* 1997; Wernberg *et al.* 2011), depending upon the local drivers of an ecosystem (Falkenberg *et al.* 2010). Consequently, future research should focus on determining the influence multiple local and global disturbances have on the stability of coastal ecosystems. The disturbances considered in experiments should, however, reflect those experienced by the local study system.

Whilst the use of microcosms allowed the manipulation of multiple environmental conditions (nutrients and CO₂), the size of the microcosms limited the ecosystem complexity studied. Consequently, the conclusion that herbivores are able to counter the excess growth of algal turf when exposed to elevated CO₂ may be limited given the natural complexity of marine ecosystems. Larger mesocosms would allow a better understanding of complex community responses to elevated CO₂; however, most mesocosm experiments are short-term studies and hence are unable to address the long-term consequences that altered environmental conditions may have on marine systems (Fabricius *et al.* 2011). Field experiments allow ecosystem level responses to be determined, but, CO₂ is difficult and costly to manipulate *in situ* (Hall-Spencer *et al.* 2008). An alternative approach would be to study ecosystems that occur in naturally high CO₂ levels, as found around marine

CO₂ vents (Hall-Spencer *et al.* 2008; Fabricius *et al.* 2011; Porzio *et al.* 2011). CO₂ vents acidify seawater on large temporal and spatial scales, facilitating the study of ecosystem processes which are predicted to be altered under future CO₂ conditions, such as production, reproduction, predation and competition (Hall-Spencer *et al.* 2008; Fabricius *et al.* 2011). While this method will advance our understanding of ecosystem level responses to ocean acidification, it does have limitations, including temporal variability in pH, proximity to populations that are not exposed to acidified conditions and limited replication (Hall-Spencer *et al.* 2008; Calosi *et al.* 2013). Future research should therefore consider using a combination of field-based and mesocosm-based experiments to provide a better understanding of community responses to elevated CO₂.

This thesis demonstrates the compensatory responses of certain invertebrate mesograzers (amphipods and gastropods) to nutrient and CO₂ enhancement in seagrass and kelp ecosystems. It has been suggested, however, that the over-exploitation of top predatory fishes in coastal waters by humans, releases smaller predators that feed on such invertebrates. In turn, these smaller predators can reduce invertebrate abundance and subsequently, their ability to counter the growth of opportunistic algae (Heck *et al.* 2000; Hughes *et al.* 2004). In coral reef systems, herbivorous fish have been shown to negate the effects of nutrient enrichment by reducing the abundance of both algal turf and macroalgae (Miller *et al.* 1999). Future research should therefore focus on the role herbivorous fish may play in countering the growth of opportunistic species in temperate coastal ecosystems during periods of resource enhancement.

The second half of this thesis focused on the recovery of above- and belowground ecosystem structure and function of an experimental seagrass restoration site. Together the

results suggest that the recovery of ecosystem structure lagged the recovery of ecosystem function at this site. Ecosystem function was measured as the abundance, richness and composition of epifauna and infauna inhabiting the restoration plots in comparison to the natural meadow. I suggest that future monitoring should concentrate on quantifying further aspects of ecosystem function and processes of the restoration site. For example, monitoring could determine the use of the restoration plots by higher trophic levels such as quantifying the composition and abundance of fish species using the restoration plots as habitat for shelter or foraging activity.

6.5 CONCLUSION

Human activities continue to modify the abiotic environment (Hooper *et al.* 2005), yet many systems that experience intensive anthropogenic pressure remain stable, resisting environmental change (Ghedini *et al.* 2015). To understand the inherent stability of ecosystems facing altered environmental conditions, we need an account of the mechanisms that allow systems to persist in a certain state whilst withstanding major change. The data presented throughout this thesis demonstrates that compensatory mechanisms exist (i.e. the consumption of algae and herbivore metabolic rate) and may, to a certain extent, enable ecosystems to resist change (i.e. the shift in competitive balance between subordinate and dominant species) and remain stable during periods of local and global resource enhancement (i.e. nutrient and CO₂). Whilst resource enhancement stimulated the growth of opportunistic algae, it also stimulated an increase in *per capita* consumption and underlying metabolic rate of herbivores to compensate. Consequently, in our pursuit to manage resource enhancement, we may have underestimated the importance of herbivores, particularly in systems where minor increases in resources have considerable effects on the expansion of opportunistic algae. When resource enhancement is ongoing,

however, countering forces may be overwhelmed to the extent that production (i.e. growth of opportunistic algae) escapes regulation (i.e. herbivore consumption) (Ghedini *et al.* 2015), and perennial species are lost (Connell *et al.* 2013). As the global loss of seagrass continues (Waycott *et al.* 2009), habitat restoration can be implemented to re-establish the structure and function of lost systems (Elliott *et al.* 2007; Reynolds *et al.* 2013). This thesis further demonstrates that whilst recovering habitats may not be structurally similar to undegraded habitats, they can support similar ecosystem functions (e.g. faunal richness and abundance). Importantly, this suggests that although restored habitats may not appear fully recovered, they may have similar ecosystem functions to natural habitats. Recovering habitats may therefore have greater economic and social value than may have been expected.

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